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# 1

## Welcome to Biochemistry

### What Is Biochemistry?

- Biochemistry is the study of the molecular basis (chemistry) of life.
- It is a field of science that deals with chemical compounds and processes that occur in the cells of plants, animals and microorganisms etc.
- Deals with the cell's *composition, structure, and reactions (metabolism)*
- Involves quantitative determination and structural analyses of molecules (protein, carbohydrates, lipids etc.) that make up cells.
- Involves many complex and interrelated chemical changes (reactions) in cells.

### What Is Biochemistry?

- Biochemical reactions include those by which,
  - carbohydrates, lipids, proteins, nucleic acids and other biomolecules are synthesized,
  - food is converted to energy,
  - energy is stored and released,
  - all biological chemical reactions are catalyzed,
  - hereditary characteristics are transmitted.

### What Is Biochemistry?

- Biochemistry bridges the biological and physical sciences and employs techniques common to biomedical sciences as well as those of organic, analytical and physical chemistry.

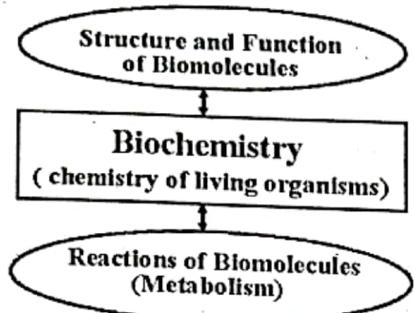
### What Is Biochemistry?

- Biochemistry has a great impact on life.
  - It provides a basic understanding of life's processes at the molecular level, and a profound influence on our understanding of medicine, nutrition, health etc.
  - It provides the molecular basis of diseases such as diabetes, sickle-cell anemia, cystic fibrosis, phenylketonuria, hypercholesterolemia, etc..
  - It provides a search for the cure for diseases (drug discovery and design), improvements in nutrition and a basis for the development of biotechnology to solve scientific problems confronting society in medicine, agriculture, etc.

### What Is Biochemistry?

- In addition to relationships between structure and function of biomolecules, biochemists are also interested in ***bioenergetics***.
- Bioenergetics is the study of energy flow (production and usage) in living systems.

## Concepts and Relationships in Biochemistry



## 2 Cell Structure and Function

### Elemental Composition of Cells

- Minerals usually serve as cofactors for enzyme reactions, oxidation-reduction centers, and as structural components of proteins and nucleic acids
- Six elements make up more than 99% of the human body. These are;
  - Oxygen 65%, carbon 18%, hydrogen 10%, nitrogen 3%, calcium 2%, phosphorus 1.1%
- K, S, Na, Cl, Mg make up < 0.5%
- Fe, Co, Zn, Cu, Mn occur in trace amounts < 0.05%
- Other elements present in certain organisms in trace amounts include: Al, As, B, Cr, F, Ga, I, Mo, Ni, Se, Si, W, V, Br

### Minerals

Mineral	Function	Source	Deficiency symptoms and disease
Calcium (Ca)	Muscle contraction, blood clotting, cell signaling	Milk, sardines	Fragile bones, muscle cramps
Chloride (Cl)	Osmotic pressure	Salt, smoked fish	
Chromium	Glucose metabolism	Meat, whole wheat flour	Non availability of glucose to cells
Cobalt (Co)	Component of vitamin B <sub>12</sub>	Meat, dairy products	Pernicious anemia
Copper (Cu)	Cofactor for enzymes	Sardines, liver	Loss of hair pigments
Fluorine (F)	Enamel production	Fluorinated water, toothpaste	Tooth decay
Iron (Fe)	Component of heme and nonheme proteins	Kidney, cocoa, beans, clams, smoked fish	Anemia

### Minerals

Mineral	Function	Source	Deficiency symptoms and disease
Magnesium (Mg)	Enzyme cofactor	Cocoa, chocolate, beans	Hypocalcemia
Manganese (Mn)	Bone formation	Fruits, vegetables, nuts	Hair and nail growth inhibited
Molybdenum (Mo)	Synthesis of proteins	Liver, kidney, beans, peas,	Growth retardation
Potassium (K)	Generates membrane potential	Banana, nuts, beans	Muscle weakness
Phosphorus (P)	Diet calcium balancing	Egg yolk, cocoa, nuts	Affects bone structure when in excess
Selenium (Se)	Fat Metabolism	Seafood, meat	Muscular disorders.
Zinc (Zn)	Cofactor in enzymes and insulin	Soybean, meat, poultry, corn	

### Biomolecules

- An organism is defined by a specific set of macromolecules.
- Macromolecules are formed from simple compounds (building blocks).
- Biological functions are determined by molecular structure.
- Biomolecular structures are defined by configuration and conformation.

# Biomolecules

- Major biomolecular types include:
  - carbohydrates
  - proteins
  - lipids
  - nucleic acids
- These macromolecules are composed of monomeric units and are essential components of every form of life.

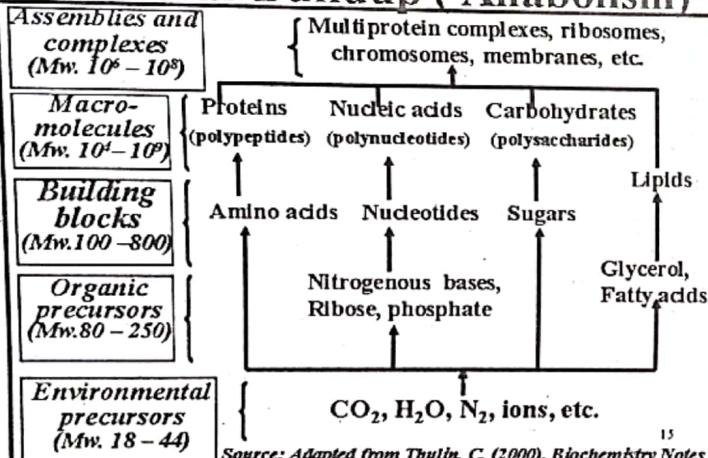
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## Composition of *E. coli* Cell

Component	% by weight	Number of Different species
Water	70	1
Protein	15	~3000
Carbohydrates	3	~5
Lipids	2	~20
DNA (nucleic acid)	1	1
RNA (nucleic acid)	6	~3000
Simple organic molecules	2	~500
Inorganic	1	~20

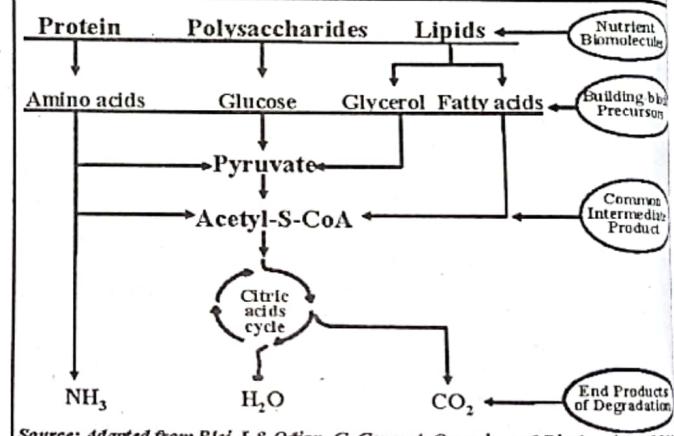
Source: Nelson, D. L. and Cox, M. M. (2000) Lehninger, Principles of Biochemistry,

## Molecular Buildup (Anabolism)

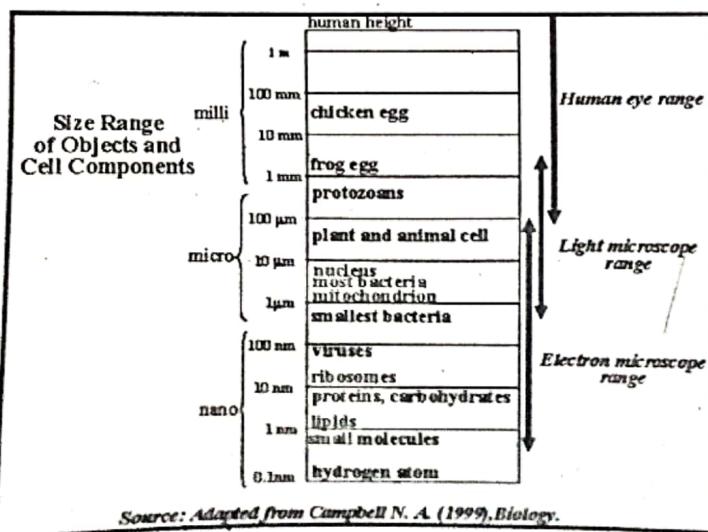


Source: Adapted from Thulin, C. (2000). Biochemistry Notes

## Molecular Breakdown (Catabolism)



Source: Adapted from Blei, J & Odian, G. General, Organic, and Biochemistry, 2005



Source: Adapted from Campbell N. A. (1999). Biology.

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## Cell Types

- Organisms are composed of different types of cells.
- Some organisms are unicellular (bacteria, protozoa, some fungi, and some algae).
- Other organisms are multicellular.
- An adult human has about 200 cell types and altogether about 10<sup>14</sup> cells.
- Cells are made up of subcellular structures referred to as organelles.
- Organelles are made up of complex assemblies of macromolecules.
- Organelles are found in eukaryotic cells.

## Cell Types

- Eukaryotes:** Their cells contain a membrane-bound nucleus and other membrane-bound components (*organelles*) of the cell. Eukaryotes include plants and animals.
- Prokaryotes:** Their cells don't have a membrane-bound nucleus nor other internal membrane-bound organelles. Prokaryotes include bacteria, blue-green algae etc.

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## Components of Eukaryotic Cell

- Nucleus:** Stores genetic information. It is the site of DNA replication and transcription and is surrounded by a double-membrane nuclear envelope with pores that allow passage of small molecules.
- Mitochondrion:** An oval double-membrane organelle where aerobic catabolism occurs. About 95% of cell's energy (ATP) is generated here.
- Golgi complex:** Also known as **Golgi apparatus**. Called **dictyosomes** in plants. A stack of flattened membrane sacs closely associated with the endoplasmic reticulum. Site of modification of proteins to glycoproteins and lipoproteins.

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## Components of Eukaryotic Cell

- Smooth endoplasmic reticulum (SER):** A continuous network of single-membrane-bound channels distributed throughout the cell. Site for lipid synthesis.
- Rough endoplasmic reticulum (RER):** Describes the endoplasmic reticulum coated with ribosomes (sites for protein synthesis).
- Lysosomes (in animals):** Digestive organelles surrounded by a single membrane. Contain granules of enzymes that are involved in the hydrolysis of ingested material (carbohydrates, proteins, lipids etc.,) following endocytosis.

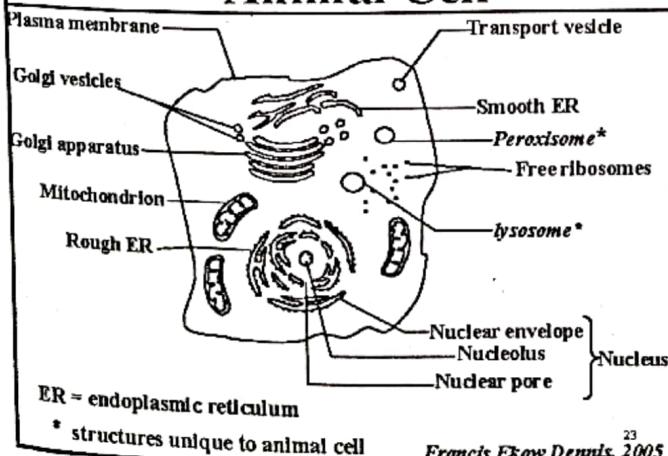
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## Components of Eukaryotic Cell

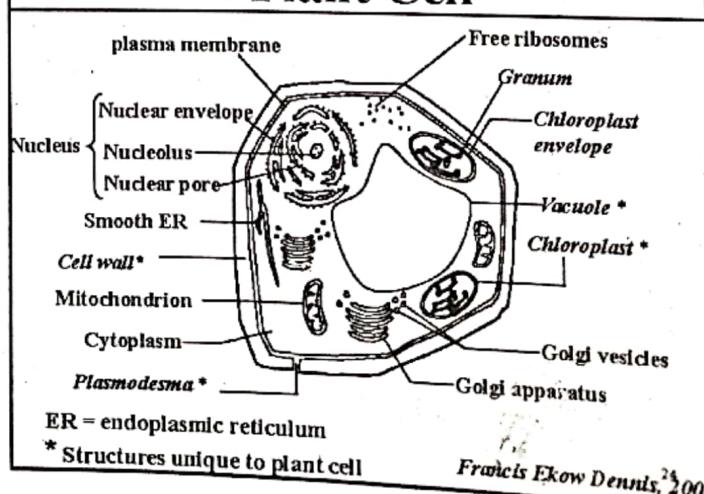
- Peroxisomes (in animals):** Are single-membrane vesicles. Contain oxidative enzymes (e.g. catalase for decompositon of hydrogen peroxide). Involved in oxidative metabolism of nutrients.
- Chloroplasts (in plants):** Are double-membraned organelles. Contain protein, lipid, chlorophyll, RNA, DNA and ribosomes. Site for photosynthesis.
- Glyoxysomes (in plants):** A modification of peroxisomes.
- Vacuoles:** For storage of nutrients and waste.

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### Animal Cell



### Plant Cell



## Major Structures of Prokaryotic and Eukaryotic Cells

Structure	Prokaryotes	Plants	Animals
Cell wall	+	+	-
Chloroplasts	-	+	-
Endoplasmic reticulum	-	+	+
Golgi complex	-	+	+
Microbodies	-	+	+
Microfilaments	-	+	+
Microtubules	-	+	+
Mitochondria	-	+	+
Nuclear envelope	-	+	+
Nucleoid	+	-	-
Nucleolus	-	+	+
Nucleus	-	+	+
Plasma membrane	+	+	+
Ribosomes	-	+	+

\* Absent; + Present

Source: Wolfe, S. L. (1993) Molecular and Cellular Biology.

## Prokaryotes: Cell Wall & Membrane Composition

CELL STRUCTURES	PROKARYOTES		EUBACTERIA	
	ARCHAEABACTERIA	GRAM-POSITIVE	GRAM-NEGATIVE	GRAM-POSITIVE
SURFACE LAYERS	Present	Wall-containing bacteria	Wall-less bacteria	Protein subunits
CELL WALL Thickness	Thick 15 nm to 30 nm	-	Thick 25 nm to 50 nm	Thin 2 nm to 10 nm
Composition	Polysaccharides Pseudomurein	-	Peptidoglycan Teichoic acids Teichuronic acids	Peptidoglycan
MEMBRANES Composition	CYTOPLASMIC Isoprenyl glycerol ethers Proteins	CYTOPLASMIC Isoprenyl glycerol ethers Proteins	CYTOPLASMIC Phospholipids Proteins	INNER CYTOPLASMIC Phospholipids Proteins
REPRESENTATIVE SPECIES	<i>Stethanosarcina barkeri</i> <i>Halococcus morrhuae</i>	<i>Thermoplasma acidophilum</i>	<i>Bacillus subtilis</i> <i>Staphylococcus aureus</i>	<i>Escherichia coli</i> <i>Acholeplasma sp.</i> <i>Mycoplasma sp.</i>

Source: Taken from Rogers, H.J., Perkins, H. R. & Ward, B.J. (1980) Microbial cell walls and membranes, Chapman and Hall.

## Cell Fractionation

- Biochemists usually work with subcellular components (cell extracts) instead of whole cells, or tissues.
- Cell extracts are obtained by **homogenization** through,
  - **grinding** (with abrasive material e.g. sand),
  - **shearing** (with pestle, beads, blade), and
  - **chemical, ultrasonic or pressure disintegration**,
 to obtain a cell/tissue **homogenate**

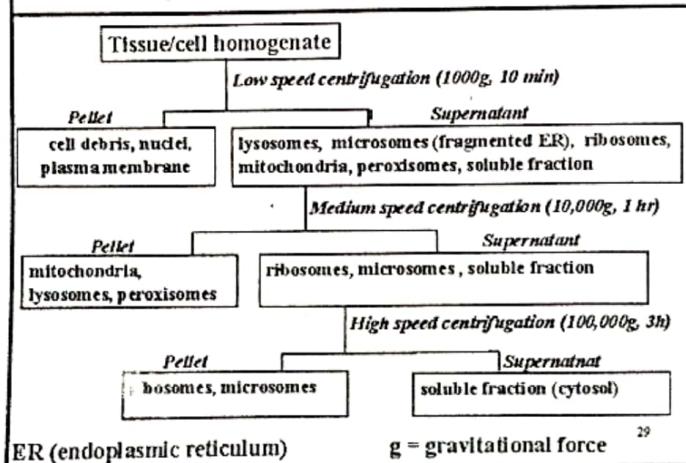
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## Cell Fractionation

- Subcellular components are separated from each other by differential centrifugation of the homogenate. Larger components sediment faster than smaller ones.
- By centrifuging at various speeds and times, different size organelles can be separated and collected from a mixture of subcellular components.
- To prepare say microsomes, the post-mitochondrial supernatant fraction can be treated with calcium chloride ( $\text{CaCl}_2$ ) to precipitate microsomal fractions. Calcium chloride precipitation is based on calcium dependent aggregation of ER fragments. The aggregated microsomes can be pelleted by low speed centrifugation.

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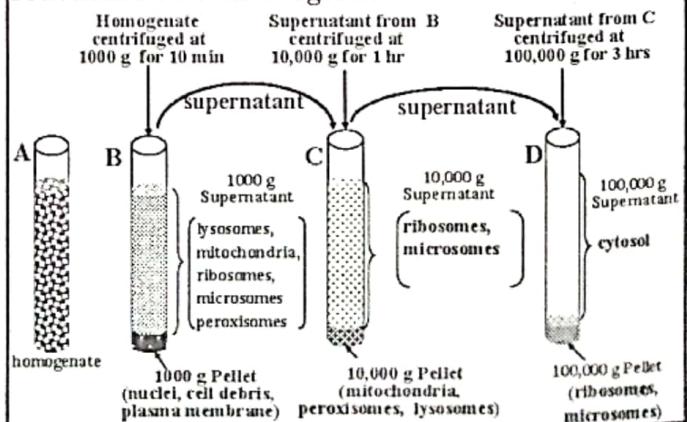
## Cell Fractionation



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## Cell Fractionation

### Fractionation of Homogenate



## Cell Fractionation

### Density Gradient Centrifugation

- Subcellular organelles which are similar in size and are hence difficult to separate by differential centrifugation can be separated using **density gradient centrifugation**.
- This technique separates components based on small differences in density.

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## Cell Fractionation

### Marker Enzymes

- Some enzymes are predominantly or exclusively present in or associated with a particular subcellular organelle or component.
- Such enzymes serve as marker enzymes to assess the purity and identity of subcellular fractions.

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## Marker Enzymes

Subcellular Fraction	Enzyme
Nuclei	Nicotinamide-nucleotide adenyltransferase
Mitochondria	Succinate dehydrogenase
Endoplasmic reticulum	Glucose-6-phosphatase
Lysosomes	Ribonuclease
Plasma membrane	Hormone-stimulated adenylyl cyclase, 5'-nucleotidase
Peroxisomes	Catalase
Cytosol	Lactate dehydrogenase
Golgi complex	Thiamine pyrophosphatase
Chloroplast	Ribulose bisphosphate carboxylase (Rubisco)

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## Self-Test Questions

- Describe the procedures that researchers use to separate and isolate the different organelles of a cell.
- Describe how you would centrifuge a homogenate of heart muscle cells in order to isolate mitochondria.
- Arrange the following in order of decreasing size. (a) Ribosome (b) Starch (c) Bacterial cell (d)  $\text{H}_2\text{O}$  (e) Sucrose (f) Mitochondrion

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## 3

## Stereochemistry

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## Stereochemistry

- Stereochemistry is the study of molecules in three dimension.
- Stereoisomers** are compounds that have the same chemical formula and connectivity but different *configurations* (that is the three-dimensional arrangement of atoms or group of atoms in space). Stereoisomers include:
  - Geometric isomers** (*cis-trans* stereoisomers), are isomers which vary in the arrangement of atoms or group of atoms about a double bond. They belong to the diasteromer type of stereoisomers.
  - Conformational stereoisomers**, vary in shape and how it (the shape) may change.

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# Stereochemistry

- Optical isomers, vary in the spatial arrangement of atoms or group of atoms around an asymmetric carbon atom. This results in molecules that are mirror images of each other like the left and right hands.
- Stereoisomerism is important for the specificity of biochemical reactions.
- For molecules that can exist as two or more stereoisomers, biological systems have preference for only one stereoisomer.
- Constitutional isomerism.** Whereas stereoisomerism results from differences in configuration (spatial orientation), constitutional isomerism results from differences in connectivity.

# Stereochemistry

## Isomerism

### Structural Isomers (Constitutional Isomers)

Different compounds with same molecular formula. Differ in molecular bonding arrangements. Distinguished by differences in 2-dimensional structural formulae e.g. glucose, fructose

### Isomeric Compounds

Have same molecular formula ( $C_2H_6O$ ) but different molecular structures  $CH_3CH_2OH$ ,  $CH_3OCH_3$  (ethanol) (dimethyl ether)

### Stereoisomers (Configurational isomers)

Same molecular bonding skeleton (atoms bonded in the same order or linkages) Differ in arrangement of atoms in space Distinguished only by 3-dimensional representation

### Enantiomers

Stereoisomers related as object and nonsuperimposable mirror image e.g. D-glucose and L-glucose

### Diastereomers

Stereoisomers not related as object and mirror image e.g. D-glucose and D-galactose (Non enantiomeric stereoisomers)

# Stereochemistry

## Chirality and Optical Activity

- All objects have mirror images.
- Molecular asymmetry results in non-superimposable mirror image structures.
- Asymmetric molecules are **optically active**, that is, they rotate the plane of polarized light.
- A molecule that is non-superimposable on its mirror image (an enantiomer) is said to be **chiral** (that is, possesses handedness)
- Chiral** molecules lack both points and planes of symmetry and exhibit optical activity
- Most biomolecules have chirality.

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# Stereochemistry

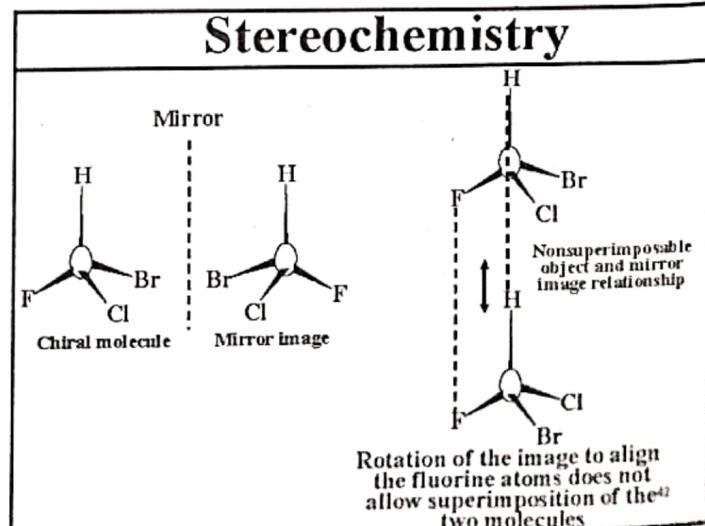
- A molecule is **chiral** if it has a **tetrahedral** carbon attached to four different atoms or group of atoms ( $C_{abcd}$ ) 
- Another element which has significance as a chiral center is the nitrogen atom. The quaternary nitrogen substituted with four different ligands is also chiral.
- A chiral carbon is called a **stereogenic center**, or **chiral center**.
- If an object or molecule has a plane of symmetry, it is **achiral** and is superimposable with its mirror image.

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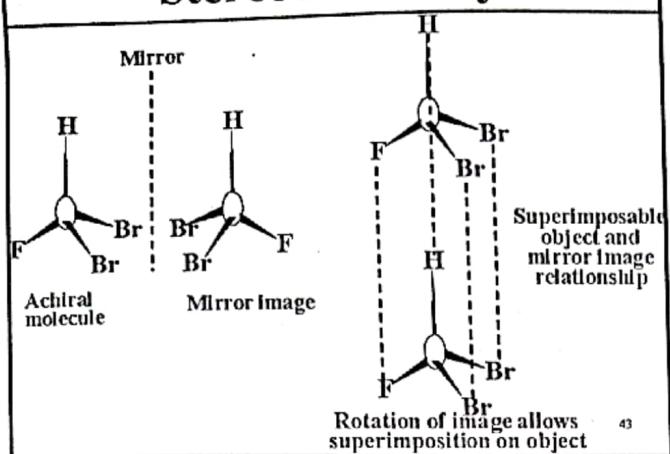
# Stereochemistry

- Achiral** molecules are optically inactive.
- If  $n$  is the number of nonequivalent chiral carbons in a molecule, then the number of optically active isomers is  $2^n$ .

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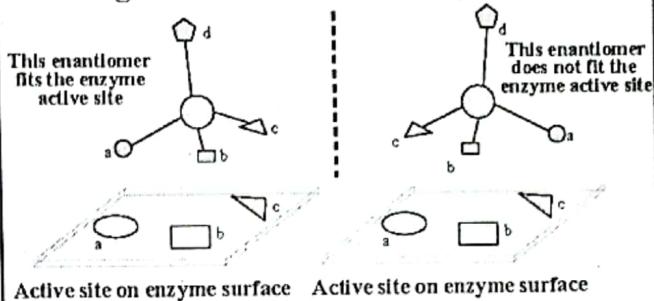


## Stereochemistry



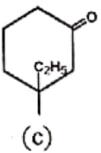
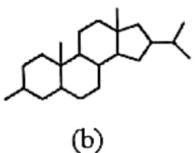
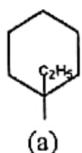
## Stereochemistry

### Binding of Enantiomers to Enzyme Surface



## Self-Test Questions

1. Which carbon atoms are chiral in the following compounds? Use an asterisk to indicate the chiral center(s) in each structure. How many stereoisomers are possible for each molecule?



2. Which of the following objects is (are) chiral?

- (a) a car (b) a pair of shoes (c) a pair of socks  
(d) a coke bottle (e) a tea cup with inscription UG

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## Measurement of Optical Activity

- Optical activity is measured using a polarimeter
- A polarimeter consists of a light source, a polarizer, a sample tube, an analyzer and an observer (the eye).
- Plane-polarized light: is light in which the direction of vibration of the electric vectors is in the same plane.
- Enantiomers are often characterized via their interaction with plane polarized light.
- A solution of a pure enantiomer rotates the plane of polarization of plane polarized light. A property referred to as optical rotation.



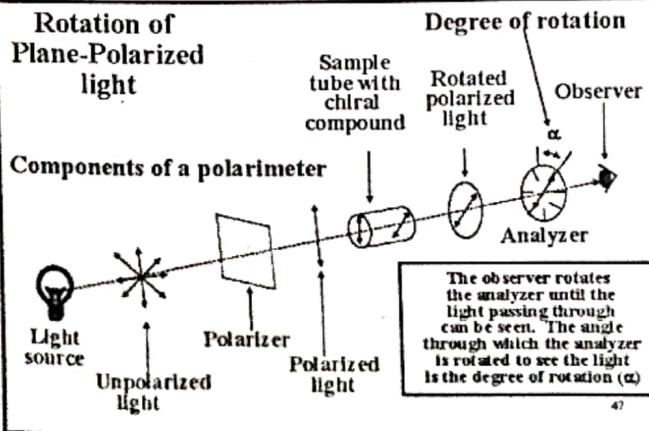
Ordinary light



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Plane polarized light

## Measurement of Optical Activity



## Measurement of Optical Activity

- Optical activity is the ability of a molecule to rotate the plane of polarized light.
- Enantiomers have optical rotations equal in magnitude but opposite in sign under equivalent conditions.
- In an enantiomeric pair, one isomer is **dextrorotatory** (rotates plane-polarized light in the clockwise direction) and is denoted by *d* or (+).
- The other is **levorotatory**, and rotates plane-polarized light in a counter-clockwise direction and is denoted by *l* or (-).

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## Measurement of Optical Activity

- Because of their optical activity, enantiomers are called **optical isomers**.
- Note: The direction of rotation which is indicated by using the prefixes (+) and (-) for dextrorotatory and levorotatory respectively, are **not** related to the prefixes D- and L- which indicate the absolute configuration about a stereocenter (chiral carbon).

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## Measurement of Optical Activity

- There is no general relationship between configuration and direction of optical rotation.
- The direction of optical rotation, indicated by (+) or (-), is determined using a polarimeter, whereas the configuration about a stereocenter indicated by D- or L-, is determined by X-ray crystallography.

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## Measurement of Optical Activity

- The direction of rotation depends on many factors but it is not related to the absolute configuration.
- Thus an L-enantiomer may rotate light in the clockwise direction (dextrorotatory), another L-enantiomer may rotate light in the counterclockwise direction (levorotatory).
- For example the levorotatory enantiomer of fructose is the D-enantiomer, and is represented as D-(-)-fructose.

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## Measurement of Optical Activity

### Specific Rotation

- The degree of rotation measured for an optically active compound depends on the number of chiral molecules encountered by the light as it passes through the sample.
- To standardize the observed rotation, a quantity called **specific rotation** is used.
- Quantitative measurements of optical activity are usually expressed in terms of the specific rotation.
- The value depends on the concentration of the sample, the length of the sample path, the solvent, temperature and wavelength ( $\lambda$ ) of the light source.

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## Measurement of Optical Activity

- Specific rotation is defined as the number of degrees through which plane-polarized light is rotated in traveling 1 decimeter through a sample of 1 g/mL concentration.
- Specific rotation,  $[\alpha]_D^{25}$ , is calculated from the equation  $[\alpha]_D^{25} = \alpha / (l \times c)$ .
- The symbol  $l$ , is the sample path length in dm,  $c$  is sample concentration in g/mL,  $\alpha$  is the observed (measured) optical rotation in degrees.
- For any measurement of optical rotation the wavelength of light and the temperature must be specified.
- "D" refers to the "D line" of sodium lamp at 589 nm and "25" refers to a measurement temperature of 25°C (both not part of the calculation).

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## Measurement of Optical Activity

- Specific rotation is an inherent physical property of a chiral molecule.
- It is unique to a compound and can be used for identification of an unknown compound.
- The value has been used in forensic studies to identify "hard" drugs.

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## Specific Rotation

Compound	$[\alpha]_D$
Azidothymidine (AZT)	.99
Cholesterol	-32
Cocaine	-16
Epinephrine (adrenaline)	-5
Heroin	-107
Morphine	-132
Progesterone	+172
Testosterone	+109
Sucrose (table sugar)	+67

Source: Taken from Ouellette, J. R. (1997) *Introduction to General, Organic and Biological Chemistry*. 4<sup>th</sup> Edition. Prentice Hall, New Jersey.

## Self-Test Questions

- The specific rotation of morphine is +132°. Calculate the observed rotation for a 10 g/L solution of morphine in a polarimeter tube of length 10 cm.
- A solution of L-fructose (4 g/100 mL) gives an optical rotation of +3.6° when measured in a 10 cm polarimeter tube. Calculate the specific rotation of L-fructose.
- Honey bees collect nectar which is approximately 10% sucrose and convert it into honey (a concentrated solution of about 40% each of glucose and fructose). They do this conversion by mixing the nectar with the salivary enzyme invertase then busily aerating and fanning the solution to drive off water. Given the specific rotation of sucrose (+66.5°), glucose (+52.7°) and fructose (-92°). Calculate the rotation that accomplishes honey production.

## Enantiomers and Diastereomers

- When a molecule has more than one chiral carbon, not all the optical isomers are enantiomers.
- It is **not** possible to interconvert distinct enantiomeric or diastereomeric isomers by free rotation about single bonds.
- A given stereoisomer may have only one enantiomeric isomer, but may have several diastereomeric isomers.
- Two stereoisomers cannot possess both enantiomeric and diastereomeric relationship with each other.

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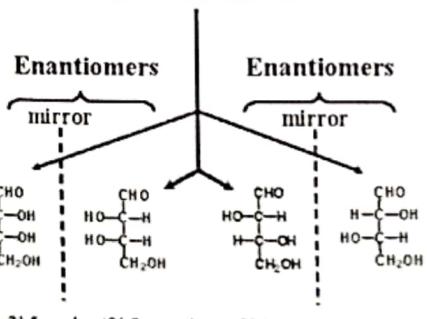
## Enantiomers and Diastereomers

- Geometric isomers (also called *cis-trans* isomers) are one of several subclasses of diastereomers.
- A pair of enantiomers:**
  - have the same physical (density, mp, bp, solubility) and chemical properties, but differ in:
    - how they rotate the plane of polarized light (optical activity), and
    - how they interact with other chiral molecules such as enzymes and receptors.

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## Enantiomers and Diastereomers

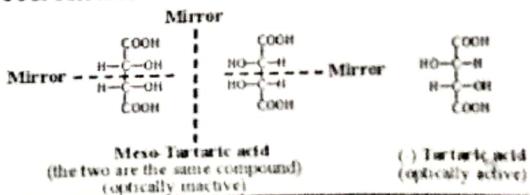
### Diastereomers



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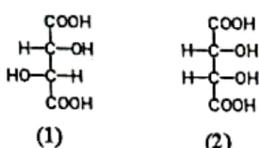
## Enantiomers and Diastereomers

- If a molecule with chiral carbons has a plane of symmetry, the stereogenic centres are said to be equivalent.
- Tartaric acid is a classical example. The two asymmetric carbons are identically substituted.
- The achiral optically inactive stereomer is referred to as the **meso-compound**. The mirror images are superimposable. Tartaric acid therefore has 3 and not 4 stereoisomers.



## Self-Test Question

1. There are three stereoisomers of tartaric acid. Two of the three structures are shown below.



Stereoisomer 1 has a specific rotation of +12.7° and a melting point of 173 °C. Draw the structure, and give the melting point and specific rotation of the third stereoisomer. Which of the stereoisomers is optically inactive?

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## Stereobiochemistry

- The study of molecular symmetry and biological stereospecificity are a vital part of biochemistry.
- Enzymatic catalysis has evolved to very high specificity. This places a tight control on the range of reactants which can be used.
- Biological systems can differentiate between stereoisomeric substrate molecules.
- Most stereoisomers that are active in biological systems consist of only one enantiomer. Rarely are both enantiomers of a biological molecule active.
- Biological stereospecific processes can also differentiate between chemically alike, but geometrically nonequivalent, paired groups of a single substrate molecule.

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## Stereobiochemistry

- Stereospecificity is possible because enzymes or receptors with which biological molecules interact are chiral.
- A chiral receptor or enzyme site fits one enantiomer but not the other.
- Biological stereospecificity between chemically alike paired groups are found in pathways of intermediary metabolism.
- Biochemical reactions use **chiral** or **achiral** starting materials and give chiral products because the enzymes that catalyze the reactions are chiral.

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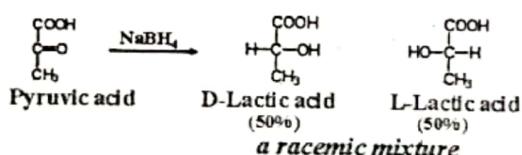
## Stereobiochemistry

- On the contrary, chemical reactions use **achiral** or **racemic** starting materials and give achiral or racemic products.
- Our senses of (or receptors for) smell and taste are sensitive to the chirality of molecules.
- Only one enantiomer of a drug is biologically active. Drug manufacturers (researchers) are using technology in which chiral catalysts are designed to produce only the active enantiomer of a drug and not the racemic mixture.
- This has the advantage of lower dosages, enhanced activity and minimization of undesirable drug interactions.

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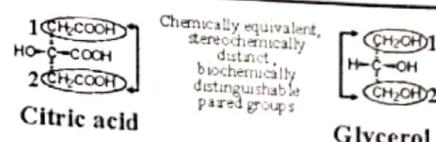
## Stereobiochemistry

- Enzymes will generally differentiate between enantiomers either by reacting with one of the two enantiomers as a substrate or by creating one enantiomer as a product.
- A chemical reaction, however, leads to a racemic mixture (50% to 50% mixture of enantiomers) unless the reactants are chiral.

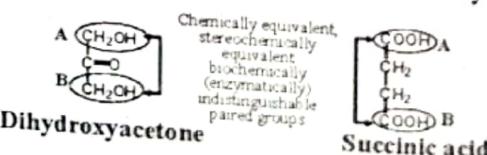


Only L-lactic acid is produced when the reaction is catalyzed by an enzyme.

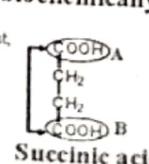
## Stereobiochemistry



The two groups (1 and 2) are biochemically nonequivalent



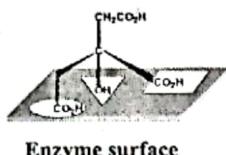
The two groups (A and B) are biochemically equivalent



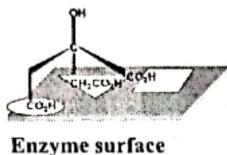
## Stereobiochemistry

### Enzyme-substrate interaction

Schematic representation of the binding of citric acid to an enzyme surface in a three-point attachment concept.



Enzyme surface

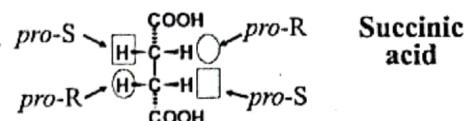


Enzyme surface

Citric acid lacks rotational symmetry and all paired groups are stereochemically distinct and biochemically distinguishable. There are stringent substrate-enzyme interaction geometries.

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## Stereobiochemistry

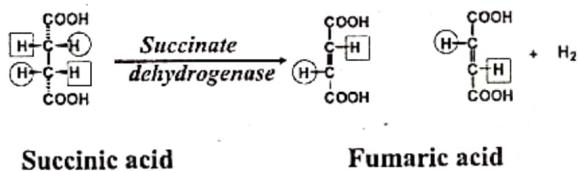


Succinic acid

Enzymes even though are asymmetric cannot distinguish between paired groups in a molecule that are chemically and geometrically equivalent. Although the carboxyl groups of succinic acid are biochemically indistinguishable, an enzyme is capable of differentiating between the pairs of enantiotopic hydrogen atoms. Although the paired methylene ( $-CH_2-$ ) groups are superimposable, the individual hydrogen atoms are not superimposable. The pair in the circle can be enzymatically differentiated from those in the square.

## Stereobiochemistry

- The four methylene hydrogens of succinate occur as two enantiotopically related pairs.
- The enzymatic oxidation of succinate by succinate dehydrogenase stereospecifically removes one circled (*pro-R*) hydrogen and one in the square (*pro-S*) from adjacent methylene groups.

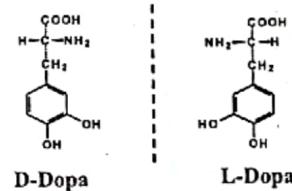


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## Stereobiochemistry

### Pharmacological Importance of enantiomers

mirror



D-Dopa

D-Dopa is biologically ineffective against Parkinson's disease, whereas L-Dopa is effective against the disease.

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## Stereobiochemistry

- Senses are sensitive to the configuration of molecules.
- Both the sense of smell and the sense of taste result from changes induced in a sensory receptor when it binds to a specific molecule.
- Binding causes a conformational change that triggers a sequence of events that lead to the transmission of a nerve impulse to the brain by sensory neurons.
- The brain interprets the input from sensory neurons as the smell or taste of a given compound.
- Enantiomeric and diastereomeric molecules interact differently with sensory receptors and enzymes.
- Because enzymes and receptors are chiral they interact stereospecifically with only one pair of an enantiomer.

## Self-Test Questions

- Suggest a reason for the stereochemical basis of the observation that L-Dopa is effective against Parkinson's disease whereas D-Dopa is inactive against the disease.
- What is the stereochemical basis of the observation that D-aspartyl-D-phenylalanine has a bitter taste whereas L-aspartyl-L-phenylalanine is significantly sweeter than sugar?

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# 4 Carbohydrates

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## Carbohydrates

- Once thought to be “hydrates of carbon” hence the name carbohydrates.
  - Carbohydrates are polyhydroxy aldehydes or polyhydroxy ketones or their derivatives.
  - They form the most abundant class of compounds and have the general formula  $(C_n(H_2O)_n$
  - For most naturally occurring carbohydrates,  $n = 3-7$ .
  - Some contain nitrogen, sulphur and phosphorus
  - They are formed by photosynthesis
- $$n CO_2 + n H_2O + hv \rightarrow (CH_2O)_n + n O_2$$
- Are stored in animals as glycogen and in plants as starch and used to generate energy:
- $$(CH_2O)_n + n O_2 \rightarrow n CO_2 + n H_2O + Energy$$

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## Carbohydrates

- Are found in all life forms and serve many functions including:
  - Energy**, (glucose and starch)
  - Structural**, cellulose (plant), chitin (fungi, exoskeleton shell of arthropods), peptidoglycan (bacteria cell wall)
  - Components of RNA and DNA**, (ribose and deoxyribose).
  - Molecular recognition**, when combined with proteins and lipids (glycoproteins, glycolipids) on cell surfaces.

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## Classification of Carbohydrates

- Monosaccharides**: Simple sugars like glucose and fructose.
- Monosaccharides can be bonded together through glycosidic linkages to form **disaccharides**, **trisaccharides**, etc.
- Carbohydrates made up of 2 to 10 monosaccharide units are called **oligosaccharides** (*oligo*: Greek for “a few”)
- Polysaccharides** are larger polymeric carbohydrates, some with molecular weights of several millions. Examples of polysaccharides are starch, cellulose etc.

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## Classification of Carbohydrates

Sugar	Class	Source	Uses
Ribose	monosaccharide	Nucleic acid	RNA synthesis
Deoxyribose	monosaccharide	Nucleic acid	DNA synthesis
Glucose (dextrose)	monosaccharide	Fruits, honey	Energy, sweetner
Galactose (milk sugar)	monosaccharide	Mammary glands	Component of lactose in milk
Fructose (levulose)	monosaccharide	Fruit, honey	Sweetner
Maltose	disaccharide	Starch	Energy
Lactose (milk sugar)	disaccharide	Milk, whey	5% of cow milk, 7% of human milk (by weight)
Sucrose (table sugar)	Disaccharide	Fruits, flowers, plant Juices	Sweetner

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## Classification of Carbohydrates

- Homopolysaccharides**: polysaccharides composed of the same monosaccharide (e.g. starch and cellulose).
- Heteropolysaccharides**: polysaccharides with two or more different types of monosaccharides (e.g. hyaluronic acid).
- Glycosidic linkages**: acetal or ketal bonds that join monosaccharides together to form di-, oligo-, or polysaccharides.

78

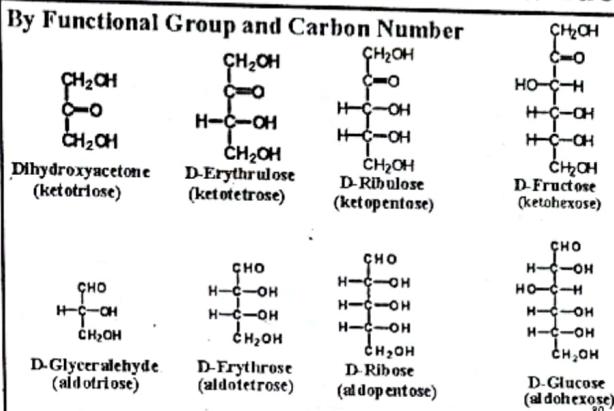
## Classification of Carbohydrates

- Monosaccharides are classified by functional group. Those with an aldehyde group are called **aldoses** and those with a ketone group are called **ketoses**.
- Monosaccharides are also classified by the number of carbon atoms as *triose*, *tetrose*, *pentose*, *hexose*, *heptose*.

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## Classification of Monosaccharides

### By Functional Group and Carbon Number



## Configuration of Monosaccharides

- The D and L system is used for designating relative configuration of sugars.
- In a Fischer projection of a sugar, a D or L assignment is based on the asymmetry at the *penultimate* (last but one) carbon atom.
- A monosaccharide is assigned the D-configuration if the OH group on the penultimate chiral carbon is written to the right and L- if it is written to the left.
- To change from the D to the L configuration and vice versa, the chirality of all carbons also changes.

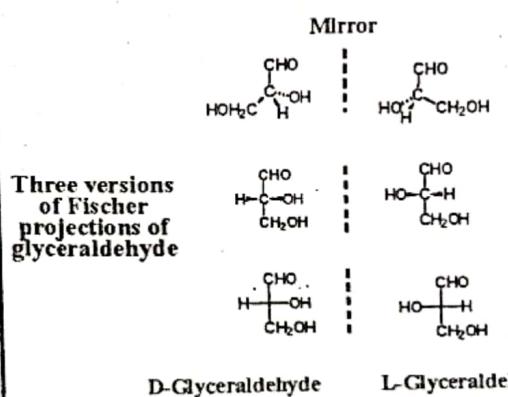
81

## Configuration of Monosaccharides

- Almost all naturally occurring monosaccharides have a D-configuration.
- The D and L configuration is not to be confused with *d* and *l*, sometimes used to refer to direction of rotation of plane-polarized light.
- Glyceraldehyde has two stereoisomers, the D- and L-forms. Stereochemically all monosaccharides are related to one of the two isomers of glyceraldehyde.

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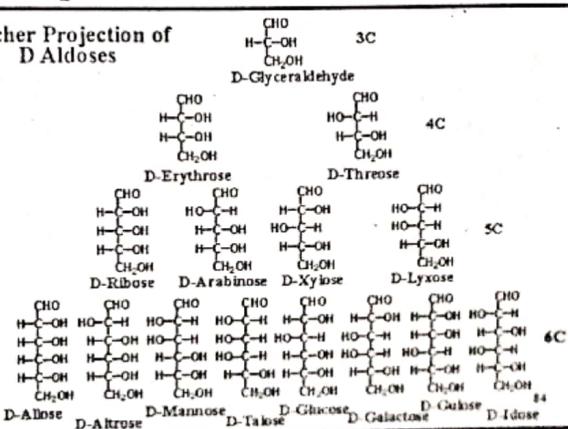
## Configuration of Monosaccharides



83

## Configuration of Monosaccharides

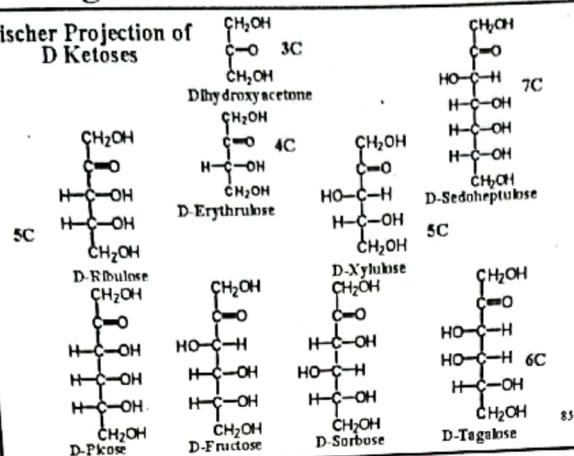
### Fischer Projection of D Aldoses



14

## Configuration of Monosaccharides

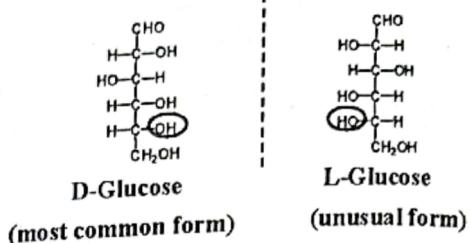
Fischer Projection of D Ketoses



## Configuration of Monosaccharides

### Enantiomers

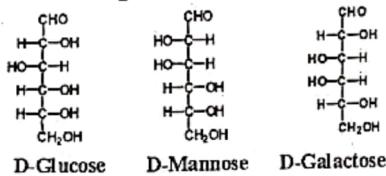
Mirror



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## Configuration of Monosaccharides

### Epimers



**Mannose and galactose are epimers of glucose**

D-Galactose and D-mannose, are diastereomers of D-glucose. Diastereomers which differ from each other in configuration at only one of their chiral centers are called epimers. Mannose is an epimer of glucose at C-2 and galactose an epimer at C-4. D-Galactose and D-mannose are not epimers since they differ in the asymmetry at both C-2 and C-4.

## Cyclic Derivatives of Monosaccharides

### Haworth Projections

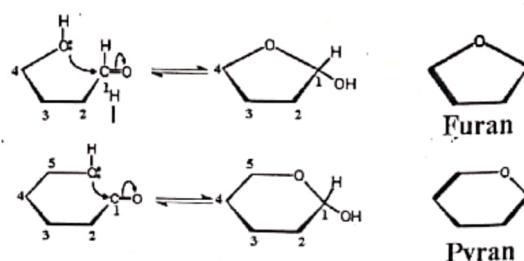
- In solution monosaccharides with five or more carbons occur mostly as cyclic structures.
- Although the Fischer projection is adequate to illustrate the configurations about the chiral carbons in the open chain form, it is a poor representation of cyclic structures.
- Cyclic structures of monosaccharides were first suggested by Walter Haworth in 1925, and called Haworth projections.
- Haworth projections are a close approximation of the predominant chair-form structures of hexose sugars in solution.

## Cyclic Derivatives of Monosaccharides

- Hydroxyl and aldehyde groups of **aldohexoses** can interact to form **hemiacetals** and **hemiketals**.
- Addition of the carbon 4-hydroxyl oxygen to the carbon of the aldehyde of an aldopentose, converts an acyclic structure into a cyclic hemiacetal structure with a five-membered ring similar to **furan** and are called **furanoses**.
- Addition of the carbon 5-hydroxyl oxygen to the carbon of the aldehyde of an aldohexose, generates a hemiacetal with a six-membered ring structure similar to **pyran** and are called **pyranoses**.
- Cyclic hemiacetals are favored over the open chain aldehyde forms.

## Cyclic Derivatives of Monosaccharides

### The mechanism of ring closure



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## Cyclic Derivatives of Monosaccharides

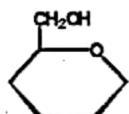
- Hexoses commonly form six-membered rings whereas pentoses form five-membered rings.
- Formation of the hemiacetal linkage creates an additional chiral center and doubles the number of stereoisomers.
- The new pair of stereoisomers created by the conversion of an acyclic sugar into a cyclic pyranose or furanose form are called **anomers**.
- Anomers differ only in the configuration at carbon 1 for pyranoses and carbon 2 for furanoses.
- The carbon which acquires chirality after ring formation, is called the **anomeric carbon**.<sup>91</sup>

## Cyclic Derivatives of Monosaccharides

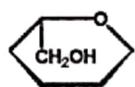
- In a Haworth structure, an  $\alpha$ -form of the sugar has the OH on C-1 written below the anomeric carbon whereas the  $\beta$ -form has the OH written above.
- Other OH groups written below the carbon correspond to OH groups written to the right in a Fischer projection. Conversely an OH written above corresponds to an OH to the left in a Fischer projection.
- For the asymmetric carbon (C-5), the  $-\text{CH}_2\text{OH}$  group written above C-5 designates the monosaccharide as a D-isomer, conversely a  $-\text{CH}_2\text{OH}$  written below C-5 represents the L-isomer of the sugar.<sup>92</sup>

## Configuration of Monosaccharides

### D and L in Pyranose Form



D-isomer



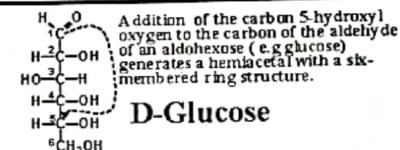
L-isomer

The terminal  $\text{CH}_2\text{OH}$  group is positioned above the plane of the ring.

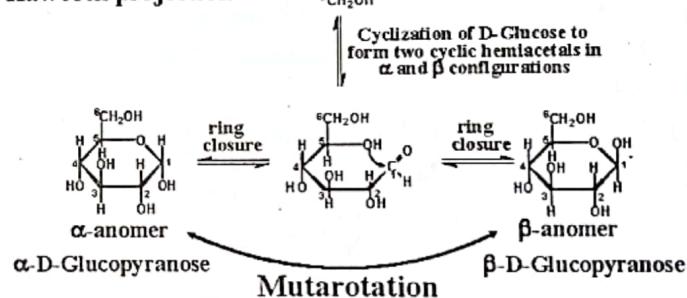
The terminal  $\text{CH}_2\text{OH}$  group is positioned below the plane of the ring.

To change from D to L configuration or vice versa the chirality of other carbons also change.<sup>93</sup>

### Conversion of Fischer projection of glucose to the Haworth projection



D-Glucose

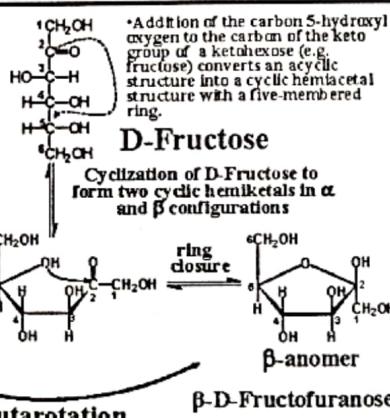


Mutarotation

$[\alpha]_D = +19^\circ$

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### Conversion of Fischer projection of fructose to the Haworth projection



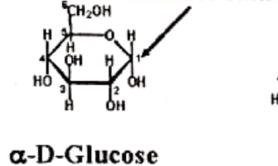
$\alpha$ -D-Fructofuranose

D-Fructose

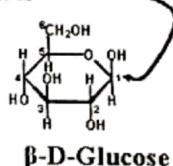
## Configuration of Monosaccharides

### Anomers

anomeric centres



$\alpha$ -D-Glucose

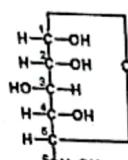


$\beta$ -D-Glucose

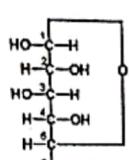
The  $\alpha$  and  $\beta$  designations refer only to the anomeric center and not to other chiral carbons. In solution, glucose is largely present as a mixture of  $\alpha$  and  $\beta$  anomers. Steric considerations however make the  $\beta$  anomer the more predominant species in solution.

## Configuration of Monosaccharides

### Fischer Projections of Anomers



$\alpha$ -D-Glucose

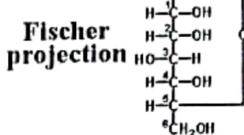


$\beta$ -D-Glucose

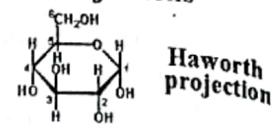
In an  $\alpha$ -configuration, the OH of the anomeric carbon (C-1) is on the same side as the OH of the highest numbered asymmetric carbon (C-5). In a  $\beta$ -configuration, the OH of anomeric carbon (C-1) is on the opposite side of the OH of the highest numbered asymmetric carbon (C-5).

## Configuration of Monosaccharides

### Fischer and Haworth Projections

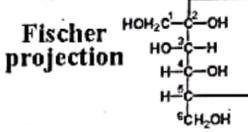


Fischer projection

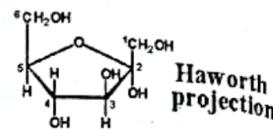


Haworth projection

$\alpha$ -D-Glucopyranose



Fischer projection

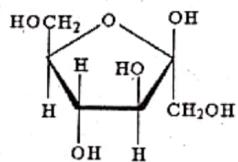


Haworth projection

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## Self-Test Questions

1. Draw the structure of the epimer of  $\alpha$ -D-galactopyranose.
2. Draw the enantiomer of  $\beta$ -D-glucopyranose.
3. Draw the structure of the anomer of  $\alpha$ -D-mannopyranose.
4. Name the sugar below and draw its Fischer projection formula.

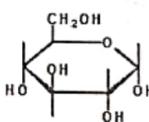


99

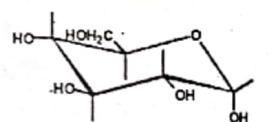
## Configuration of Monosaccharides

### Haworth and Conformational Formulae

- The Haworth projection (formula) is not entirely the correct representation of the pyranose ring. It is a fairly correct approximation for the furanose ring.
- A pyranose exists primarily in the chair conformation



Haworth formula for glucose

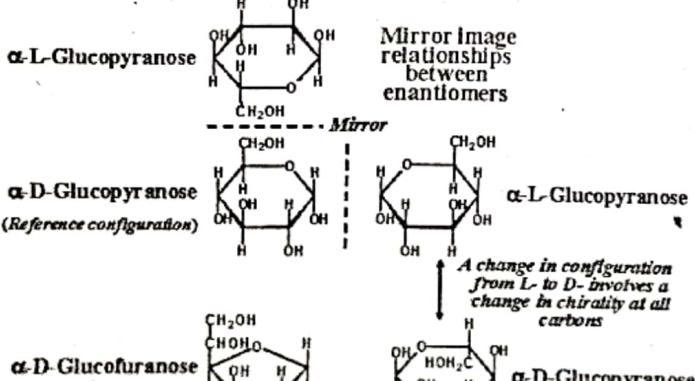


Conformational formula for glucose (chair conformation)

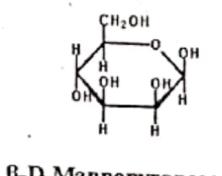
100

## Configuration of Monosaccharides

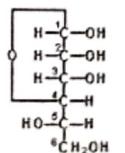
### D to L interconversions of glucose



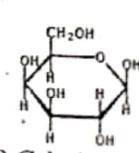
## Configuration of Monosaccharides



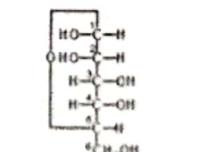
β-D-Mannopyranose



β-L-Mannofuranose



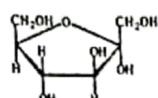
β-D-Galactopyranose



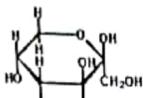
α-L-Galactopyranose

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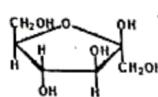
## Configuration of Monosaccharides



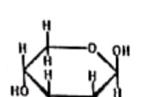
$\alpha$ -D-Fructofuranose



$\beta$ -D-Fructopyranose



$\beta$ -D-Fructofuranose

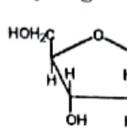


$\beta$ -D-Ribopyranose

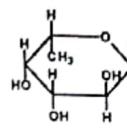
103

## Configuration of Monosaccharides

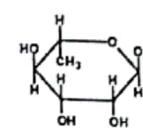
### Deoxy sugars



2-Deoxy- $\alpha$ -D-Ribose



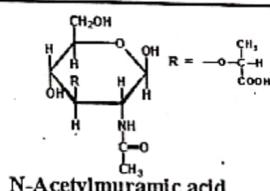
$\beta$ -L-Fucose  
(6-deoxy- $\beta$ -L-galactose)



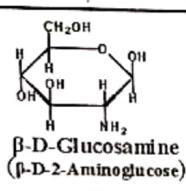
$\alpha$ -L-Rhamnose  
(6-deoxy- $\alpha$ -L-mannose)

- Deoxy sugars are monosaccharides in which some hydroxyl groups are replaced with hydrogens. They are usually found in glycoproteins and certain polysaccharides. **2-deoxy- $\alpha$ -D-ribose** is found in DNA. **Rhamnose** is found in ouabain, a very toxic cardiac glycoside present in the bark and roots of the ouabain plant. Ouabain is used as an arrow poison. **Fucose** is found in some cell walls.

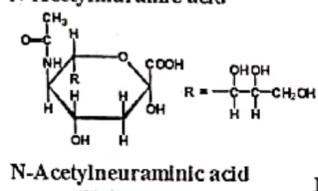
## Derivatives of Monosaccharides



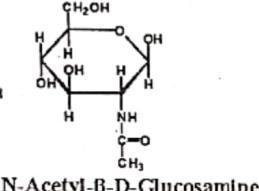
N-Acetylmuramic acid



$\beta$ -D-Glucosamine  
( $\beta$ -D-2-Aminoglucose)



N-Acetylneurameric acid  
(Sialic acid)



N-Acetyl- $\beta$ -D-Glucosamine

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## Self-Test Questions

1. What is the stereochemical relationship between each pair of compounds listed below?

- D-Glyceraldehyde:Dihydroxyacetone
- D-Glucose:D-Fructose
- D-Glucose:D-Galactose
- $\alpha$ -D-Glucose: $\beta$ -D-Glucose
- D-Glucose:L-Glucose

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## Mutarotation

- The spontaneous change in *optical rotation* due to the interconvertibility of the two anomeric forms ( $\alpha$  and  $\beta$ ) of a sugar is referred to as **mutarotation**.
- It occurs because either the  $\alpha$ - or  $\beta$ -D-sugar, undergoes a slow equilibration with the open-chain form to produce the other anomer.
- Glucose can exist in two crystalline forms ( $\alpha$ -D-glucose and  $\beta$ -D-glucose).
- The two forms of glucose are interconvertible in solution.

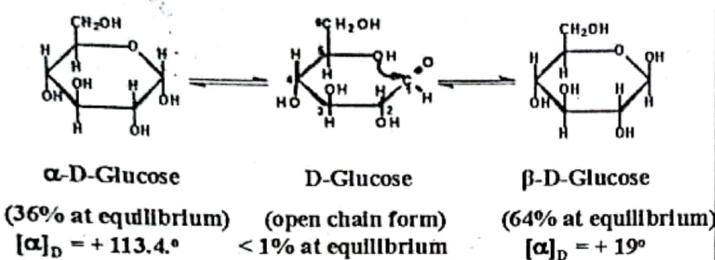
107

## Mutarotation

- The specific rotation of a freshly prepared solution of pure  $\alpha$ -D-glucose is  $+113.4^\circ$ , and that of  $\beta$ -D-glucose is  $+19.0^\circ$ .
- Specific rotation of a solution of either  $\alpha$ -D-glucose or  $\beta$ -D-glucose changes slowly until it reaches an equilibrium value of  $+52.2^\circ$ .
- The final specific rotation is due to an equilibrium mixture of 64%  $\beta$ -D-glucose and 36%  $\alpha$ -D-glucose.

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## Mutarotation



There are three forms of D-glucose. The two ring forms ( $\alpha$  and  $\beta$ ) and an open chain structure.

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## Specific Rotation of Sugars

Sugar	$\alpha$ Form	Equilibrium mixture	$\beta$ Form
D-Ribose	-23.1	-23.7	-
L-Arabinose	+54.0	+104.5	+175.0
D-Xylose	+92.0	+19.0	-20.0
D-Glucose	+113.4	+52.2	+19.0
D-Galactose	+144.0	+80.5	+52.0
D-Fructose	-21.0	-92.0	-133.5
D-Mannose	+34.0	+14.6	-17.0
L-Rhamnose	-7.7	+8.9	+54.0
L-Sorbose	-	-43.4	-
Lactose	+90.0	+53.3	+35.0
Maltose	+168.0	+136.0	+118.0
Sucrose	-	+66.5	-
Raffinose	-	+105.2	-
Trehalose	-	+178.3	-

Taken from Clark, J. M. Jr., Switzer, R. L. (1964) Experimental Biochemistry

## Reactions of Sugars

- The hydroxyl, aldehyde and ketone functional groups in sugars undergo diverse reactions.
- Reactivity of alcohol (hydroxyl) group.**
  - esterification by carboxylic acids, phosphoric acids or subjected to ether formation.
  - reaction with simple aldehydes or ketones to form cyclic acetals or ketals.
- Reactivity of carbonyl group.**
  - A typical monosaccharide behaves like a mixture of the cyclic anomer and open-chain aldehyde or ketone. The open chain form can undergo many simple carbonyl reactions.

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## Reactions of Sugars

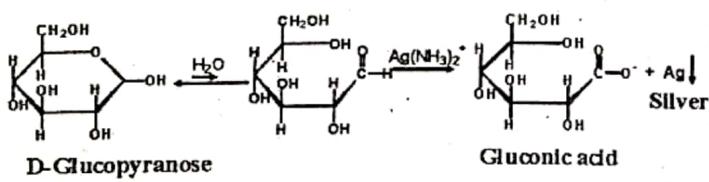
### Oxidation-Reduction Reactions

- Carbonyl and alcohol groups of sugars can be oxidized or reduced.
- The ease of oxidation of aldehydes allows certain mild oxidizing agents to oxidize the aldehyde functional group and not others such as alcohols or ethers.
- Sugars which can be oxidized by mild reagents such as silver ammonia complex,  $\text{Ag}(\text{NH}_3)_2^+$ , (in Tollens' reagent) and cupric ion,  $\text{Cu}^{2+}$ , (in Benedict's and Fehlings' reagents) are called reducing sugars (because the oxidizing reagent is reduced by the sugar in the process).

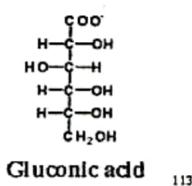
112

## Reactions of Sugars

### Oxidation of glucose



The cyclic hemiacetal forms of all aldoses are readily oxidized because a small amount of the open chain aldehyde form exists in equilibrium with the cyclic form.



113

## Reactions of Sugars

### Gluconates

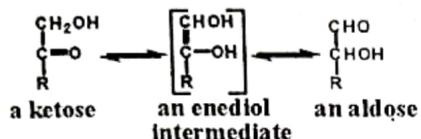
- Minerals form complexes called gluconates with gluconic acid.
- The mineral ion reacts with the negatively charged carboxyl function of gluconic acid.
- Most mineral supplements (Mg, Zn, Ca, and Fe) are often supplied as gluconate salts.
- In general, gluconates are usually preferred for use in vitamin and mineral supplements due to their good bioavailability and compressibility characteristics, neutral taste and high solubility.

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## Reactions of Sugars

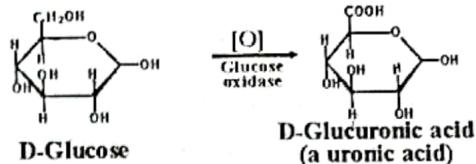
### Ketoses as reducing sugars.

Although fructose is a keto sugar, it is also a reducing sugar. Ketoses give a positive test with oxidizing agents because in alkaline solution, they are in equilibrium with the aldehyde form through an enediol tautomeric intermediate.



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### Enzymatic Oxidation of Glucose



Glucuronic acid is important in animals as many toxic substances are excreted as **glucuronides** (derivatives of glucuronic acid). In plants and some animal systems, D-glucuronic acid may be converted to **L-gulonic acid**, which is used to synthesize L-ascorbic acid (vitamin C). The last step in the synthesis of the vitamin does not take place in primates. They therefore require an outside source of the vitamin. <sup>116</sup>

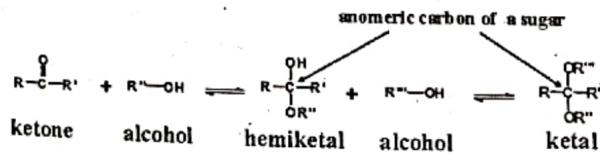
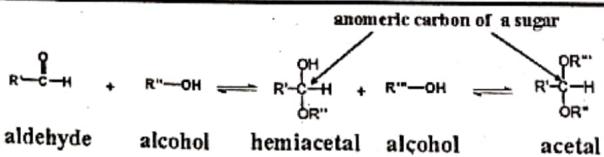
## Reduction of Monosaccharides

- Both aldoses and ketoses can be reduced by carbonyl reducing agents to form polyalcohols called **alditols**.
- The product of reduction of D-glucose is called D-glucitol or sorbitol. Galactose is reduced to galactitol, mannose to mannitol and ribose to ribitol.
- Sorbitol is a sugar alcohol used as a sugar-free food sweetener in diabetic diets because it is transported relatively slowly across membranes, and absorbed slowly from intestines. It contributes fewer calories than other monosaccharides.
- Sugar alcohols are found naturally in fruits. Sorbitol has a sweetness value of approximately 50% of sucrose. <sup>117</sup>

## Acetal and Ketal Sugars

- The product of adding one molecule of an alcohol to an aldehyde is called a **hemiacetal**.
- The product of addition of two molecules of alcohol (with loss of water) is called an **acetal**.
- Hemiketals** and **ketals** are corresponding terms used for ketone products.
- Acetals and ketals are effective blocking groups for aldehydes and ketones.
- Blocking groups prevent the functional group of the anomeric carbon from reacting.
- Acetal and ketal sugars are more stable and not in equilibrium with their open chain in solution and are therefore **non-reducing**. <sup>118</sup>

## Acetals and Ketals



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## Acetals and Ketals

- Hemiacetals and hemiketals of a monosaccharide are in equilibrium with their open chains in solution and are hence reducing.

## Glycosides

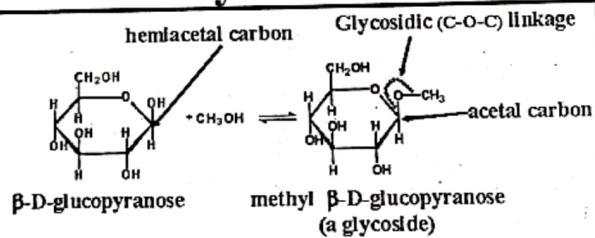
- Glycosides are formed when anomeric carbons of sugars form glycosidic linkages with hydroxyl groups of other sugar molecules or with organic non sugar molecules.
- Acetals and ketals of monosaccharides are called glycosides.
- Disaccharides and polysaccharides are also glycosides.
- The carbon-oxygen-carbon linkage that joins the anomeric carbon to another sugar or non sugar component is called an O-glycosidic bond (linkage). A carbon-nitrogen-carbon linkage at the anomeric carbon as found in nucleotides is called an N-glycosidic bond.<sup>121</sup>

## Glycosides

- Glycosidic (acetals and ketals) ends of sugars cannot mutarotate and are non reducing since the sugar ring in such a linkage cannot open. Mutarotation and reducing properties of sugars require chain opening
- Oligosaccharides with a non-glycosidic end are reducing and can mutarotate.
- Polysaccharides though have a reducing end, are overall nonreducing as the single reducing end is masked by the long nonreducing chain.

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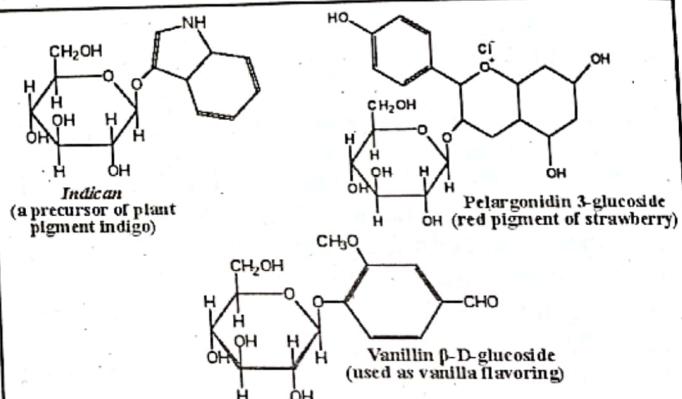
## Glycosides



Glycosides are carbohydrates containing an acetal or ketal group. The carbon-oxygen-carbon linkage is called a glycosidic linkage.

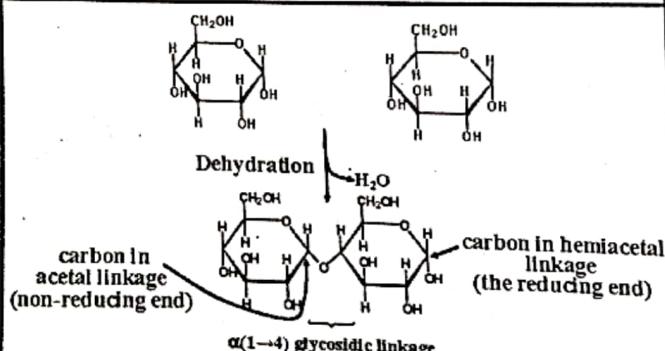
123

## Naturally Occurring Glycosides



The non sugar portion of a glycoside is called an *aglycone*<sup>124</sup>

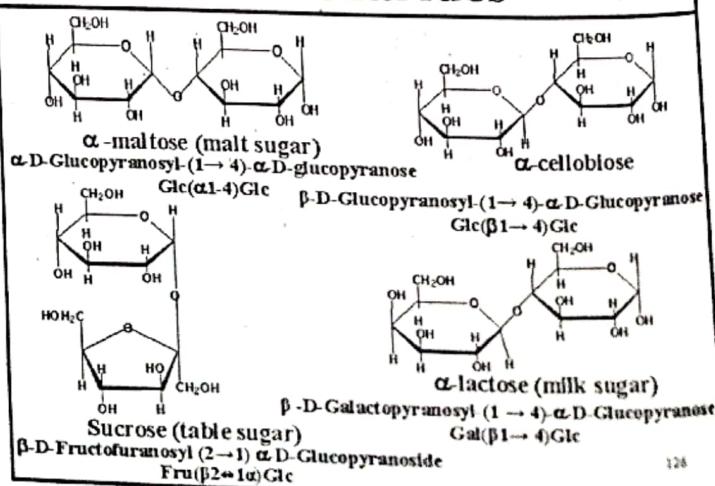
## Disaccharides



The most important disaccharides nutritionally are maltose, lactose and sucrose.

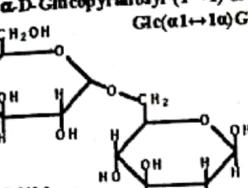
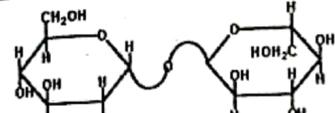
125

## Disaccharides

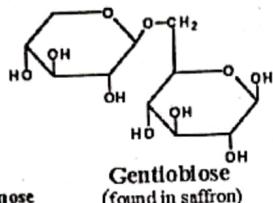


126

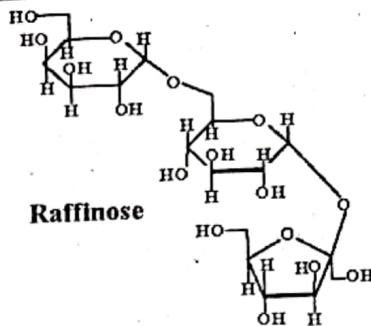
## Disaccharides



(prepared from raffinose by fermentation with yeast which removes the fructose)



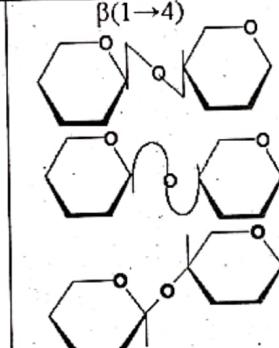
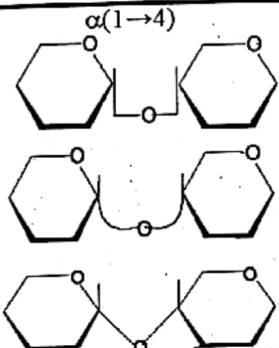
## A Trisaccharide



$\alpha\text{-D-Galactopyranosyl(1→6)-}\alpha\text{-D-Glucopyranosyl(1→2)-}\beta\text{-D-Fructofuranoside}$   
 (A naturally occurring trisaccharide found in sugar beet)

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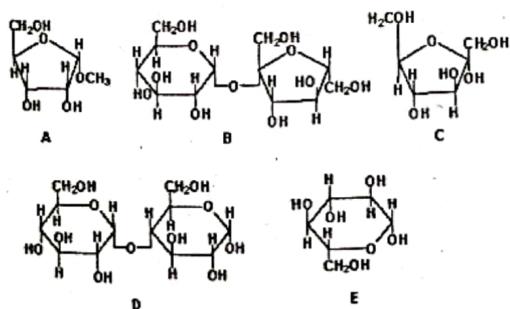
## Different Representations of Linkages



Depending on the sugar, the direction of the bond linking the oxygen of carbon 1 and carbon 4 of the next sugar may be different from what is shown here. The linkages to carbon 4 shown here are for sugars in which the OH group on carbon 4 is below the ring in a Haworth projection (e.g. glucose, allose, mannose and altrose). 129

## Self-Test Questions

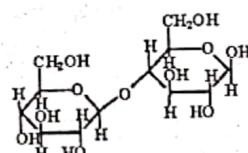
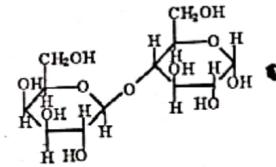
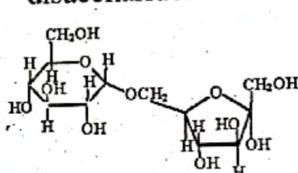
2. Indicate which of the following are reducing sugars? Explain your answer.



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## Self-Test Questions

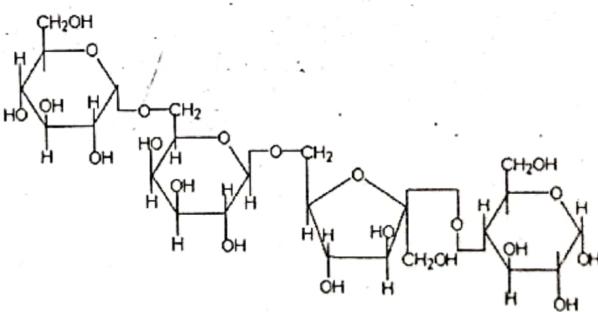
1. Give the systematic name for the following disaccharides.



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## Self-Test Questions

3. What is the systematic name of the oligosaccharide shown below? Can the sugar mutarotate?



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## Self-Test Questions

4. A disaccharide lactose contains D-galactose linked by a  $\beta$  (1 $\rightarrow$ 4) glycosidic linkage to D-glucose. Draw the structure of lactose.
5. Although lactose exists in two anomeric forms, no anomeric forms of sucrose have been reported. Why?

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## Sweetness of Sugars

- Conformation and configuration underlie all properties of sugars.
- Minor structural alterations in sugars cause chemical and physical differences.
- Intrinsic differences in stereochemistry within sugars also affect their sensory differences, nutritional and metabolic functions.
- For sugars, sweetness depends on molecular structure, size, and stereoisomerism.
- Relative sweetness is also affected by temperature and concentration.

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## Relative Sweetness of Sugars

Sugar	Sweetness	
	In Solution	In Crystalline Material
$\beta$ -D-Fructose	100-175	180
Sucrose	100	100
$\alpha$ -D-Glucose	40-79	74
$\beta$ -D-Glucose	-	82
$\alpha$ -D-Galactose	22-27	32
$\beta$ -D-Galactose	-	21
$\alpha$ -D-Mannose	59	32
$\beta$ -D-Mannose	Bitter	Bitter
$\alpha$ -D-Lactose	16-38	16
$\beta$ -D-Lactose	48	32
$\beta$ -D-Maltose	32-46	32.5
Raffinose	23	1

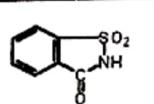
Source: Adapted from Birch, T. S & Green, A. R. (1971) *Chem. & Physical Development of Fruits*.

## Non Carbohydrate Sweeteners

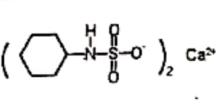
- Saccharin has a structure distinct from that of carbohydrates. It is about 500 times sweeter than sucrose.
- Aspartame is a dipeptide sweetener of considerable commercial importance. It is about 160 times sweeter than sucrose. It is marketed under the trade name "NutraSweet"
- Acesulfame K is 200 times sweeter than sucrose. It is useful in foods that require cooking.

These are among the non carbohydrate compounds useful for diabetics who have to restrict their sugar intake. They have no significant calorific value.<sup>135</sup>

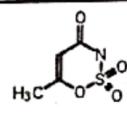
## Non Carbohydrate Sweeteners



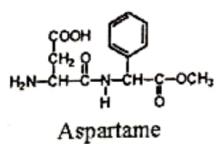
Saccharin



Calcium cyclamate



Acesulfame K



Aspartame

Artificial sweeteners are structurally very different and much sweeter than natural sugars. This is an indication of the complex relationship between taste receptors and the structures they respond to. Saccharin and cyclamates have been implicated in certain cancers.<sup>137</sup>

## Polysaccharides

- Polysaccharides are formed by the condensation of several monosaccharide units.
- They are usually not water soluble.
- Some can be hydrated to form colloidal solutions when heated (e.g. gelling of starch).

## Homopolysaccharides

- These are polysaccharides with the same sugar.

### Storage homopolysaccharides

- Starch:** A polymer of glucose produced by plants. It is a mixture of amylopectin (which is branched and contain up to 1 million monosaccharide units) and amylose (unbranched, has 50- 5,000 glucose units)
- Dextrins (starch gum):** Refer to various products obtained from the partial breakdown of starch by heating, enzymatic or chemical hydrolysis. Used in industry for sizing fabrics and paper, thickening dye pastes and mordants, as glues, added as modifiers to milk and milk products in infant formula.<sup>139</sup>

## Homopolysaccharides

- Dextran:** A storage polysaccharide in yeast and bacteria.
- Glycogen (animal starch):** A branched polymer of glucose produced by animals. Stored in cytoplasmic packages called granules as energy reserve.
- May contain up to about 50,000 glucose units.

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## Homopolysaccharides

### Structural homopolysaccharides

- Polysaccharides such as cellulose, chitin, and mucopolysaccharides are synthesized inside cells but secreted to the outside where they provide a protective wall or a lubricative coating to cells.

**Cellulose:** An unbranched polymer of glucose in  $\beta$  (1-4) linkage. Has about 10,000 to 15,000 glucose residues per molecule.

- most abundant organic substance in the biosphere (forms greater than 50 % of organic matter).
- small amounts found in cell walls of fungi and in algae.

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## Homopolysaccharides

- chains associate to form bundles of rigid fibrils suitable for making clothes, (cotton, linen, rayon) paper, and building material (wood).
- constitutes a dietary fiber and provides bulk through absorption of water.
- cellulose energy cannot be extracted for use by humans because the fibrous structure makes it insoluble. Also humans lack the enzyme to digest the  $\beta$ (1-4) linkage. Cellulose has no nutritional value to humans.
- bacteria in the stomach of ruminants (cattle, sheep, goats, etc) produce cellulases that are able to digest cellulose. Termites also harbor<sup>142</sup> bacteria that produce cellulases.

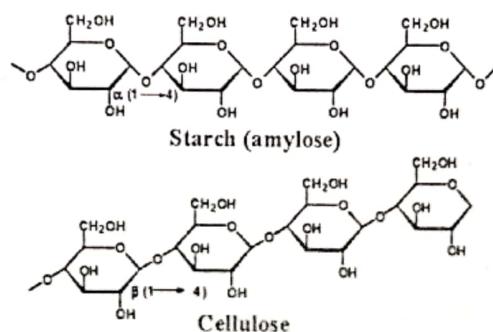
## Homopolysaccharides

### Structural homopolysaccharides

- Pectin:** A polymer of D-galacturonic acid with methylated C-6 carboxyl groups in an  $\alpha$ (1→4) linkage. Forms a network of fibers within which water can be trapped. This gives pectin its gelling property.
- Chitin:** A polymer of N-acetylglucosamine in a  $\beta$ (1-4) linkage. Forms the protective covering of arthropods, insects and crabs. Also a component of fungal cell walls. Second most abundant polysaccharide after cellulose. Forms bundles of fibers more rigid than cellulose due to the additional intermolecular H-bonding involving the acetamido group.<sup>143</sup>

## Homopolysaccharides

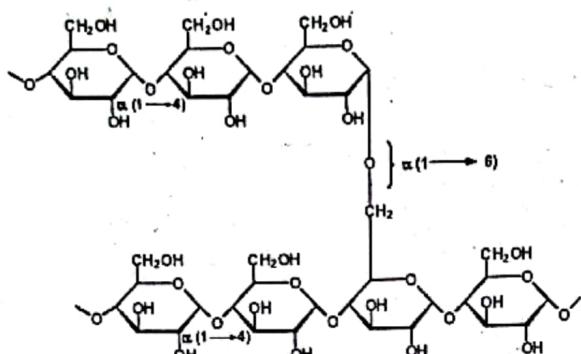
### Unbranched homopolysaccharides



Polysaccharides are overall nonreducing as the single reducing end is masked by the long nonreducing end<sup>144</sup>

## Homopolysaccharides

### Branched homopolysaccharide



Linkages found in amylopectin or glycogen

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## Homopolysaccharides

### Amylopectin and Glycogen

- Both are homopolysaccharides with glucose units.
- Both have  $\alpha(1 \rightarrow 4)$  glycosidic linkages with  $\alpha(1 \rightarrow 6)$  branch points.
- They function as energy storage molecules.
- Amylopectin is a component of plant starch.
- Glycogen is animal starch, stored in the muscle and liver.
- Glycogen has a branch at every 8-12 glucose units.
- Amylopectin has on the average a branch at every 12-30 glucose units.

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## Homopolysaccharides

Name	Source	Monosaccharide	Linkage	Branching	Molecular weight
Glycogen	animals	D-glucose	$\alpha(1 \rightarrow 4)$ and $\alpha(1 \rightarrow 6)$	branched (~ 9 %)	$\sim 5 \times 10^6$
Amylose	plants (starch)	D-glucose	$\alpha(1 \rightarrow 4)$	linear	$4 \times 10^3 - 1.5 \times 10^5$
Amylopectin	plants (starch)	D-glucose	$\alpha(1 \rightarrow 4)$ and $\alpha(1 \rightarrow 6)$	branched (~ 4 %)	$5 \times 10^4 - 1 \times 10^6$
Cellulose	plants	D-glucose	$\beta(1 \rightarrow 4)$	linear	$2 \times 10^6 - 2 \times 10^6$
Dextran	bacteria	D-glucose	$\alpha(1 \rightarrow 6)$ and $\alpha(1 \rightarrow 3)$	branched	
Pectin	plants	D-galacturonic acid	$\alpha(1 \rightarrow 4)$	linear	
Laminaran	seaweeds	D-glucose	$\alpha(1 \rightarrow 4)$	linear	
Inulin	Jerusalem artichoke	D-fructose	$\alpha(1 \rightarrow 4)$	linear	
Chitin	fungi, arthropods	N-acetyl-glucosamine	$\beta(1 \rightarrow 4)$	linear	147

## Test for Starch

### Iodine test

- An important test for the presence of starch is the reaction of amylose with iodine.
- Amylose forms helices or coils around  $I_2$ .
- A deep blue colour results from an electronic interaction between amylose and iodine

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## Self-Test Questions

1. Identify each of the following:

- The anomer of  $\beta$ -D-glucopyranose \_\_\_\_\_
- The enantiomer of D-galactose \_\_\_\_\_
- An epimer of D-galactose that is also an epimer of D-mannose \_\_\_\_\_
- A ketose that has no chiral centers \_\_\_\_\_
- A ketose that has only one chiral centre \_\_\_\_\_
- An epimer of  $\alpha$ -lactose \_\_\_\_\_
- Monosaccharide residues of cellulose \_\_\_\_\_
- Monosaccharide residues of chitin \_\_\_\_\_

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## Self-Test Questions

- Suppose you had three polysaccharides, amylose, amylopectin, and glycogen, each with the same number of monosaccharide subunits. Which would be degraded the fastest, assuming that the enzymes acting to degrade the polysaccharides all worked at the same rate?

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## Heteropolysaccharides

- These are polysaccharides constructed from different sugar units.
- Heteropolysaccharides are usually referred to as **glycosaminoglycans**.
- They are composed not only of simple sugars but also their derivatives.
- They typically consist of repeating disaccharides of amino sugars (e.g. N-acetylglucosamine, N-acetylgalactosamine, etc.) and usually uronic acids (e.g. glucuronic acid, iduronic acid, etc.), hence the term glycosaminoglycans.

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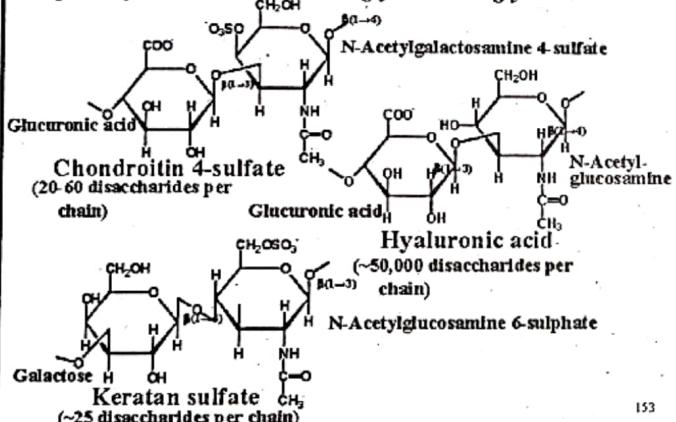
## Heteropolysaccharides

- At least six glycosaminoglycans (mucopolysaccharides) have been isolated. These are: **hyaluronic acid, chondroitin sulfate, dermatan sulfate, keratan sulfate, heparan sulfate, and heparin**
- They are found in cartilage, tendons, ligaments, synovial fluid, mucous secretions, etc., of mammals.

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## Heteropolysaccharides

*Repeating disaccharide units in glycosaminoglycans*



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## Heteropolysaccharides

### Hyaluronic acid

- The least complicated member of the series.
- Consists of up to 50,000 repeats of the basic disaccharide building unit, which is made up of **glucuronic acid** and **N-acetylglucosamine**.
- The principal component of mucous substance or connective tissue.
- Found in synovial fluid of the joint (where it acts as a shock absorber), vitreous humor of the eyeball (where it serves to hold the retina in place), and in the skin usually in combination with protein.

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## Heteropolysaccharides

### Chondroitin sulfate

- There are several types and designations.
- Chondroitin sulfate C is composed of glucuronic acid and N-acetylgalactosamine 6-sulfate
- Along with hyaluronic acid form part of the structure of connective tissue.
- Contributes to the tensile strength of cartilage, tendons, ligaments.
- Often associated with collagen and other proteins.
- The anionic groups attract water and because of their large sizes and the tendency to repel, chondroitin sulphate like other heteropolysaccharides can resist shear forces and are viscous and serve as bone-joint lubricants.

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## Heteropolysaccharides

### Heparin

- A polysaccharide composed of a sulfonylaminoglucose (glucosamine N-sulfate) and the sulfate esters of glucuronic acid.
- Molecular weight 17,000-20,000
- Has the highest negative charge of any known biomacromolecule.
- Functions as an anticoagulant.
- Prevents blood clotting by inhibiting the conversion of prothrombin to thrombin.

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## Heteropolysaccharides

Polysaccharide	Function	Composition	Linkage
Hyaluronic acid	Lubricant in synovial fluid	D-glucuronic acid N-acetylglucosamine	$\beta(1-3)$ $\beta(1-4)$
Chondroitin sulfate	Lubricant in synovial fluid	N-Acetylgalactosamine D-glucuronic acid (GalNAc is sulfated on C6)	$\beta(1-3)$ $\beta(1-4)$
Heparin	Anticoagulant	D-glucuronic acid (sulfated on C2) N-acetylglycosamine (sulfated on N and C6)	$\alpha(1-4)$ $\alpha(1-4)$

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## Heteropolysaccharides

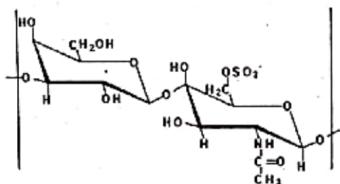
### • Agarose

- Consists of alternating D-Galactose and 3,6-anhydro-L-galactose chains with 6-methyl-D-galactose side chains.

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## Self-Test Question

1. Keratan sulfate is an important component of the cornea of the eye. The following is the repeating unit of this acidic polysaccharide.



From what monosaccharides or derivatives of monosaccharides is keratan sulfate made?

Describe the glycosidic bond in the repeating disaccharide unit.

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## Glycoconjugates

- These are carbohydrates with protein or lipid conjugates.
- Include *peptidoglycans*, *proteoglycans*, *glycolipids* and *glycoproteins*. The term *glycan* is used to denote a carbohydrate unit.
- **Peptidoglycan** (a protein-carbohydrate complex)
  - Heteropolymer consisting of alternating N-acetylglucosamine and N-acetylmuramic acid in  $\beta(1-4)$  glycosidic linkage.
  - Has peptide crosslinks between strands of linear polysaccharides.
  - A major component of bacterial cell wall. Important for maintaining the structural and functional integrity of gram-positive bacteria.

## Glycoconjugates

### • Proteoglycans

- Proteoglycans are carbohydrate protein complexes.
- The distinction between *glycoproteins* and *proteoglycans* is relative.
- Second part of name suggests the predominant component.
- Proteoglycans are predominantly glycans (or polysaccharides), whereas glycoproteins contain more protein (by weight).
- Proteoglycans are glycosaminoglycans usually joined covalently to a membrane or secreted protein.
- They are found primarily in extracellular fluids where their high carbohydrate contents allow them to bind large amounts of water.

## Glycoconjugates

### • Glycoproteins

- They are conjugated proteins in which the prosthetic groups are carbohydrate molecules (proteins with covalently bonded carbohydrate).
- The oligosaccharide content is usually less than is found in proteoglycans.
- Glycoproteins are ubiquitous in nature and are found in almost all organisms. They occur in cells in both soluble and membrane-bound forms as well as in the intracellular matrix and extracellular fluids.
- The carbohydrate groups stabilize the molecule through hydrogen bonding, protecting the molecule from denaturation and shield the protein from hydrolysis.

## Glycoconjugates

### Glycoproteins

- The sugars may be neutral sugars, amino sugars, uronic acids or neuraminic acid (NANA).
- The carbohydrate portions of glycoproteins perform important biological functions. They,
  - carry determinants of human ABO blood grouping.
  - stabilize the protein conformation.
  - are involved in immunoprotection (cytokines, immunoglobulins).
  - are involved in cell-cell or cell-molecule recognition events (host-parasite interactions).
  - are involved in blood clotting.

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## Glycoconjugates

Glycoprotein	Molecular weight	Carbohydrate content (%)	Source
<i>Enzyme</i> (e.g. Bromelin)	33,000	36	Pineapple
<i>Lectin</i> (e.g. Soybean lectin)	120,000	6	Soybeans
<i>Membrane component</i> (e.g. Glycophorin)	31,000	60	Human erythrocytes
<i>Serum glycoprotein</i> (e.g. IgG immunoglobulin)	150,000	10	Human serum
<i>Structural glycoprotein</i> (e.g. Collagen)	300,000	0.4	Rat skin
<i>Cytokine</i> (e.g. Interferon)	26,000	20	Human leukocytes

Source: Adapted from Sharon, N and Lis, H. (1981) Glycoproteins, C&EN

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## Glycoconjugates

### Glycoproteins (ABO Blood Group Antigens)

- Blood group classification depends on differences in oligosaccharide structures bonded to a protein called **glycophorin**, and lipids (glycosphingolipids) of red blood cell membranes.
- It is the terminal sugar of the oligosaccharide that distinguishes the different blood group cells.
- These minor differences in sugars result in the differences in compatibility of the blood types.
- A-type** blood group determinant has a terminal N-acetylgalactosamine on the oligosaccharide branch.
- B-type** group determinant has  $\alpha$ -D galactose as the terminal residue.
- Some oligosaccharides on AB group blood cells have a terminal N-acetylgalactosamine, others have galactose.

## Glycoconjugates

- The oligosaccharides function as the antigenic determinants of the various blood groups.
- Agglutination (clumping) of blood cells occurs when incompatible blood types are mixed and is due to antigen-antibody interaction.
- A person with blood type A makes antibodies against type B blood (anti-B antibodies) in the blood serum and vice versa.
- Persons with blood types A or B do not make antibodies against blood type O. But blood O group persons make antibodies against blood A and B group types.

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## Glycoconjugates

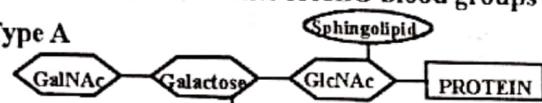
- Type O group persons are described as "universal donors". However because they produce antibodies against both type A and type B blood groups they are not "universal acceptors".
- People with type AB blood group are considered "universal recipients" since they have neither anti-A nor anti-B antibodies in their blood. If they had, they would be destroying their own cells.

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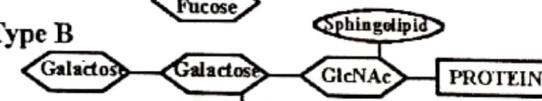
## Glycoconjugates

### Oligosaccharide determinants of ABO blood groups

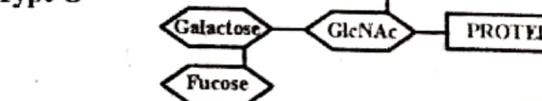
#### Blood Type A



#### Blood Type B

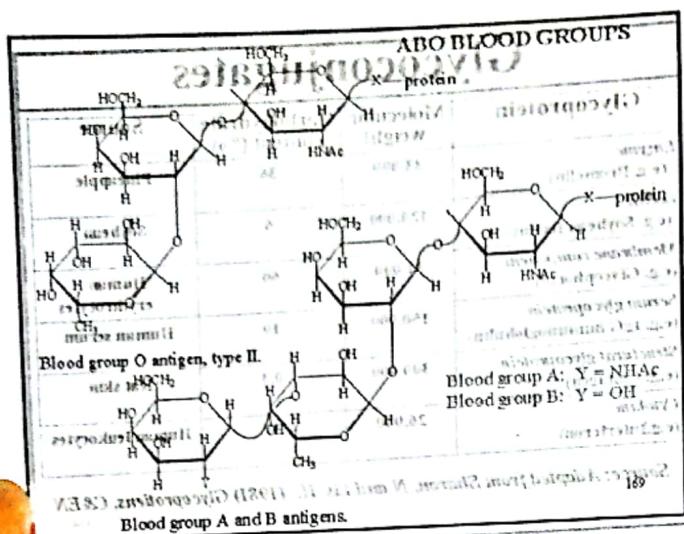


#### Blood Type O



GlcNAc = N-acetylgalactosamine; GlcNAc = N-acetylglucosamine

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## Glycoconjugates

### Lectins

- Are a group of sugar-binding and cell-agglutinating glycoproteins or proteins inherent to plant tissues.
- Have been isolated from a wide variety of natural sources (plants, fungi, bacteria, fish eggs, molluscs, mammalian cell membranes, etc.).
- They bind reversibly and specifically to certain carbohydrates. Among the best known lectins are:
  - **Concanavalin A** (specific for binding  $\alpha$ -mannose and  $\alpha$ -glucose)
  - **Wheat germ agglutinin** (specific for binding  $\beta$ -N-acetylglucosamine and  $\alpha$ -N-acetylneurameric acid)
  - **Soybean agglutinin** (specific for binding  $\beta$ -D-N-acetylgalactosamine and galactose)
  - **Peanut agglutinin** (specific for binding galactose)

## Glycoconjugates

### Lectins

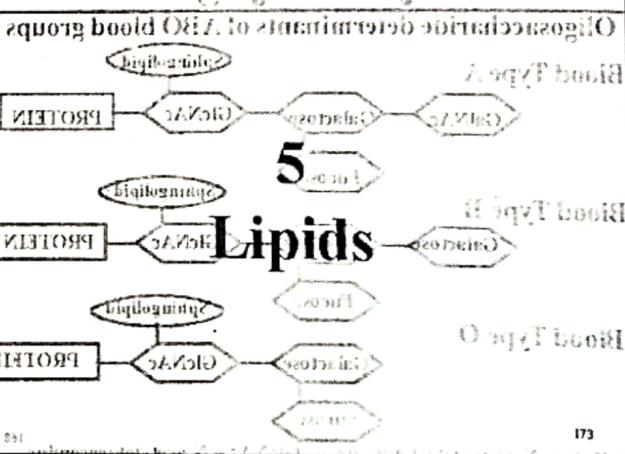
- Oligosaccharide-lectin interactions mediate many biological processes.
- They agglutinate cells and/or precipitate carbohydrates.
- Are used to evaluate carbohydrate composition of glycoproteins.
- Their exact physiological role is unknown. They are useful probes for investigating cell surfaces. They have wide *in vitro* applications including:
  - blood group determination studies,
  - lymphocyte subpopulation studies,
  - cell fractionation studies,
  - bacterial typing,
  - histological and ultrastructural studies of normal and pathological conditions.

## Glycoconjugates

### Glycolipids

- Usually membrane lipids with oligosaccharide head groups.
- Function as sites for recognition by carbohydrate-binding proteins.

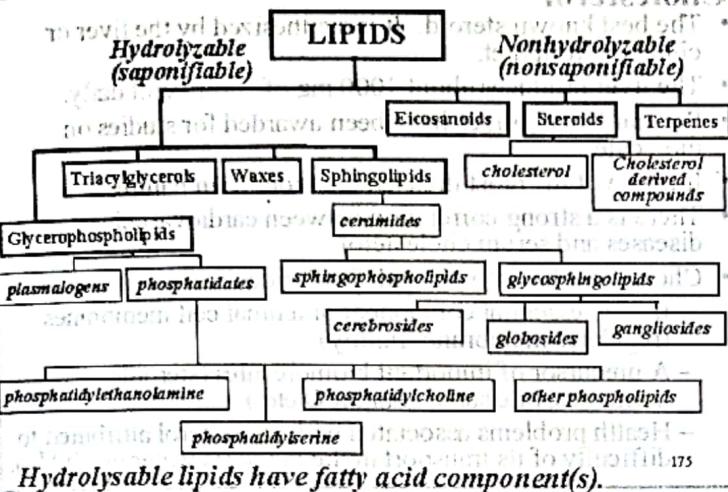
## Glycoconjugates



## Classification of Lipids

- The term "lipid" comes from the Greek word "lipos" meaning fat or lard.
- They include a great variety of chemical structures.
- Lipids are defined by their physical behavior rather than their chemical nature.
- Solubility behavior is the main distinguishing characteristic.
- They have a hydrophobic nature and are more soluble in non polar solvents than water.
- Fats and oils (triacylglycerols) are lipids best known for their role in energy production.
- A 70 kg human has 400,000 kJ total body fat fuel reserve compared to 2,700 kJ total glycogen energy reserve.

# Classification of Lipids



# Terpenes

- A class of natural products found in both plants and animals.
- Name comes from **turpentine** which is rich in terpenes.
- Important terpenes in plants and animals include:
  - Limonene** (provides odor in citrus fruits)
  - $\beta$ -carotene (source of colour in carrots)
  - Gibberellic acid (a plant growth hormone)
- Squalene** (an intermediate in steroid synthesis)
- Some **pheromones** (which are hormone-like sex attractants released by insects)
- Carotenoids** (light absorbing pigments in insect wings, skin of fish, egg yolk, lobsters and plant). **Lycopene**, the red pigment of tomato, is a powerful antioxidant carotenoid that helps prevent certain cancers and reduce the risk of cardiovascular diseases.

# Terpenes

- Most essential oils (odorous components of plants) are mixtures of terpenes.
- Vitamins A, D, E, K are terpenes or terpene-derived compounds.
- All terpenes are constructed by head-to-tail joining of isoprene units (the head is the end closer to the methyl branch).

Isoprene



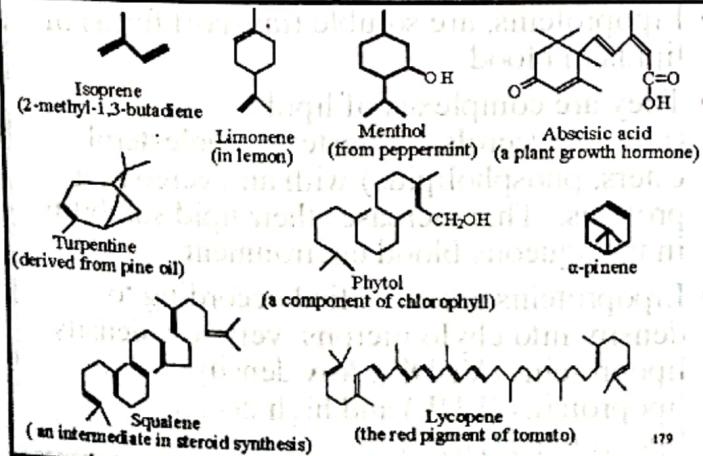
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# Terpenes

- Terpenes may contain 2, 3 or more isoprene units. May be open chain or cyclic! May contain double bonds, hydroxyl, carbonyl, or other groups.
- They are classified according to the number of isoprene units.
- A **terpenoid** is a terpene-like structure with other elements other than carbon and hydrogen.

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# Terpenes



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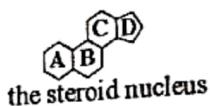
# Classification of Terpenes

Class	Isoprene units	Example	Source	Structure
Monoterpenes	2	citral	lemongrass	
Sesquiterpenes	3	farnesene	oil of citronella	
Diterpene	4	phytol	a plant alcohol	
Triterpene	6	squalene	olive oil, yeast	
Tetraterpene	8	$\beta$ -carotene	orange	
Polyterpene	3000-6000	rubber	natural rubber	

# Steroids

## Steroids

- Steroids are classified as polyprenyl compounds (i.e. built from isoprene units), and are therefore terpenoid lipids.
- The dominant feature of steroids is the cyclic ring system made of four fused rings, generally arranged in a 6-6-5 fashion (three are six carbon rings, and are designated A, B, C. The fourth, a five-membered-carbon ring, is designated D).
- The differences in steroids are found in the length of the side chain at carbon-17 and the placement of various functional groups on the rings.



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## Steroids

### Cholesterol

- Obtained from eating milk, eggs, meat, lobster etc.
- Synthesized by the liver from fats, carbohydrates and proteins.
- Cholesterol occurs in cell membranes, myelin sheath, brain and nerve cells.
- Total plasma cholesterol levels are considered high if they exceed 200-220 mg/dL.
- Cholesterol levels in the bile above saturating amounts may result in gallstone formation.
- Gallstones are composed largely of cholesterol with little amounts of fatty acids, phospholipids, and calcium salts.

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### Cholesterol

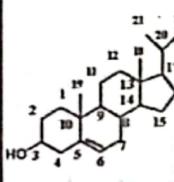
- The best known steroid. It is synthesized by the liver or obtained from diet.
- The liver produces about 1000 mg of cholesterol daily.
- Several Nobel prizes have been awarded for studies on molecule.
- It is one of the most dreaded compounds in nature.
- There is a strong correlation between cardiovascular diseases and serum cholesterol.
- Cholesterol has "good" and "bad" sides.
  - It is an essential component of animal cell membranes (regulates membrane fluidity).
  - A precursor of important biomolecules (steroids hormones, bile salts, vitamin D etc.).
  - Health problems associated with cholesterol attributed to difficulty of its transport in the blood (is water insoluble).

## Steroids

### Cholesterol

- Cholesterol is the precursor in the biosynthesis of all steroids.
- It is synthesized in most animals, not in plants or prokaryotes.
- Occurs up to 25% in cell membranes.
- Has a rigid ring structure that affects cell membrane fluidity.
- The higher the cholesterol level, the more rigid the cell membrane.
- Atherosclerosis, hardening of arteries, is accompanied by the build up of cholesterol which is deposited on the inner surface of the arteries.
- This can lead to high blood pressure which may cause heart attack, stroke or kidney dysfunction.

**Cholesterol**  
(a steroid alcohol)

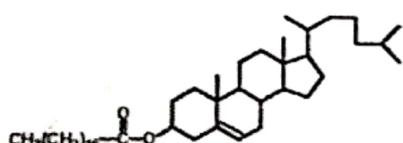


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## Steroids

### Cholesterol esters

- Cholesterol esters are the prevalent forms of cholesterol in the blood.
- They are formed from the esterification of the alcohol group on carbon 3 of cholesterol with a fatty acid.



**Cholesteryl ester**

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## Cholesterol and Lipoproteins

- Lipoproteins are soluble transport forms of lipids in blood.
- They are complexes of lipids (triacylglycerols, cholesterol, cholesterol esters, phospholipids) with an overcoat of proteins. This increases their lipid solubility in the aqueous blood environment.
- Lipoproteins are classified according to density into chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL).

## Lipoproteins

### Chylomicrons

- Consist of about 99% of lipid material.
- They are assembled in the intestine from dietary triacylglycerol (TAG) and cholesterol.
- They contain mainly TAG and are the least dense of lipoproteins.
- The TAG is hydrolyzed by the action of lipoprotein lipase lining the blood vessels near cells that use TAG.
- The removal of TAG results in cholesterol-rich chylomicron remnants that are taken up by liver and assembled into VLDL.

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## Lipoproteins

### Very Low Density Lipoprotein (VLDL)

- Assembled in the liver. Contains mainly TAG but lesser amounts than in chylomicrons.
- Delivers synthesized triacylglycerols to adipose and other peripheral tissues for storage or energy.

### Low Density Lipoprotein (LDL)

- Contains mainly cholesterol and is involved in the regulation of cholesterol metabolism.
- LDL is the major carrier of cholesterol in blood.
- Considered bad cholesterol because it moves cholesterol from the liver to extrahepatic tissues after circulation in the blood stream (i.e. puts cholesterol into circulation and increases plasma cholesterol).

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## Lipoproteins

- Production of LDL receptors can be stimulated to take up LDL from circulation into cells where enzymes break it down liberating cholesterol from cholesterol esters and hence reduce the level of plasma cholesterol.
- High LDL level and low HDL level in the bloodstream signifies faulty cholesterol transport, which can lead to atherosclerosis condition.
- High Density Lipoprotein (HDL)-**
- Contains mainly protein, phospholipid and cholesterol and little TAG.
- HDL picks up cholesterol from peripheral tissue, dying cells, membranes, and turns it over to the liver.
- **Good cholesterol** because it removes cholesterol from circulation (lowers plasma cholesterol).

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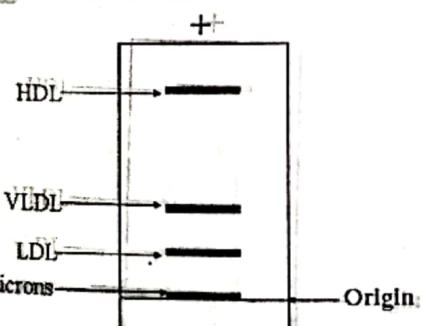
## Composition of Lipoproteins

Density (g/mL)	Composition (% by weight)			
	protein	cholesterol	phospholipids	TAG
Chylomicrons (<0.95)	2	4	9	85
VLDL (0.95-1.006)	10	20	18	50
LDL (1.006-1.063)	25	45	20	10
HDL (1.063-1.2)	55	17	24	4

Source: Adapted from Kritchevsky, D. (1986) Atherosclerosis and Nutrition. Nutr. Int. 2, 290-297

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## Electrophoresis of Lipoproteins



Lipoproteins can be identified by electrophoresis. Net charge is due to protein and phospholipid content.

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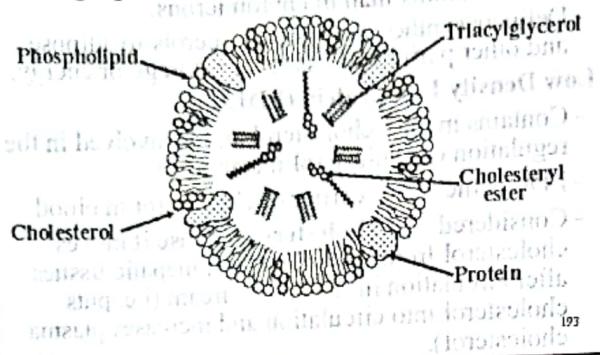
## Lipoprotein Structure

- Lipoproteins are large, spherically shaped molecules.
- They consist of a shell (outside layer) of proteins, phospholipids, and cholesterol surrounding an inner core of more hydrophobic (nonpolar) lipids such as triacylglycerols and cholesterol esters.

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## Lipoprotein Structure

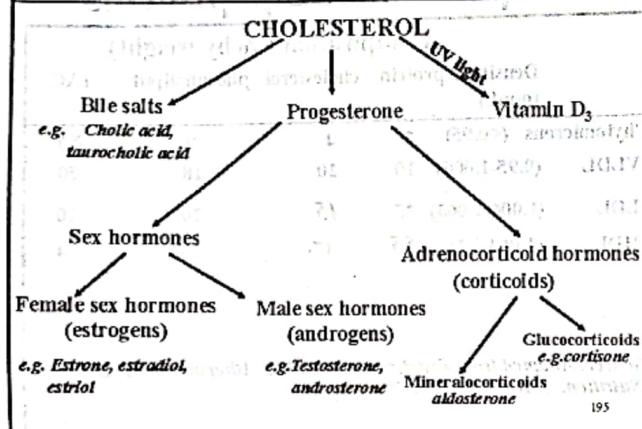
Cross sectional schematic representation of a lipoprotein



## Self-Test Question

- Identify the lipoproteins described below:
  - Which lipoprotein has the highest percentage of protein?
  - Which lipoprotein removes cholesterol from circulation?
  - Which lipoprotein carries TAG from the intestine to fat cells?
  - Which lipoprotein has the highest percentage of triacylglycerol?
  - Which lipoprotein carries TAG synthesized in the liver to the muscles?
  - Which lipoprotein has the highest density?

## Biomolecules Derived from Cholesterol

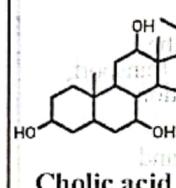


## Steroids

### Bile salts

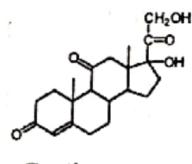
- Are synthesized from cholesterol in the liver and stored in the gallbladder.
- They are released into small intestines after ingestion of a fatty meal.
- Act as biological detergents which solubilize fats by converting dietary fats into mixed micelles of bile salts and triacylglycerols.
- They include, cholic acid, taurocholic acid, and glycocholic acid.

### Cholic acid



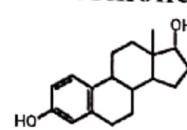
## Steroids

### Glucocorticoids

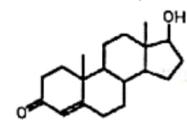


- Synthesized in the adrenal cortex.
- Affect protein and carbohydrate metabolism.
- Promote gluconeogenesis, glycogenesis, and fat breakdown.
- Depress protein synthesis in muscles and make amino acids available for gluconeogenesis.
- Suppress inflammation, immune and allergic responses.
- Cortisone (a glucocorticoid) inhibits inflammatory responses by inhibiting phospholipases which release arachidonic acid from membranes.
- Cortisone is used in the treatment of rheumatoid arthritis and bronchial asthma.

### Sex hormones



Estradiol



Testosterone  
(principal male hormone)

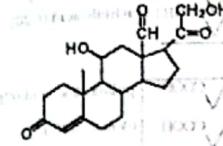
### Steroids

- They are made in the gonads.
- Control growth and development of reproductive organs.
- Estrone and estradiol help control the menstrual cycle.
- Estrogen causes growth of uterus lining and ripening of the ovum.
- Testosterone stimulates sperm production, promotes growth of male sex organs, facial hair and muscle.

## 2 Steroids

### Mineralocorticoids

- Produced in the adrenal gland.
- Regulates reabsorption of  $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{HCO}_3^-$  by the kidney.
- Increases blood pressure by increasing  $\text{Na}^+$  ion and water reabsorption in the kidneys.

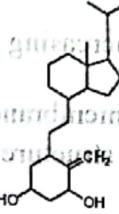


**Aldosterone**

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### Vitamin D<sub>3</sub>

- Made from cholesterol by a rearrangement process initiated by UV radiation.



**1,25-Dihydroxycholecalciferol**  
(biologically active form of vitamin D<sub>3</sub>)

- Involved in phosphorus and calcium metabolism.
- Deficiency causes rickets; a disease characterized by malformation of bone which leads to osteomalacia.

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## Anabolic Steroids

- They are involved in increasing muscle mass and decreasing body fat.
- Some athletes take advantage of this to develop their muscles.
- Their use is forbidden in many sporting activities because they provide unfair advantage.
- Testosterone is an anabolic steroid; so are many synthetic compounds.
- The disadvantages of testosterone as an anabolic steroid include:
  - Undesirable effects on the development of secondary sexual characteristics in women such as growth of facial hair, irregular menstruation, deeper voice and baldness. They can also cause hypertension, retention of fluid and acne.
  - Administration by injection since they are nonpolar and are ineffective when taken orally. Synthetic anabolic steroids such as methandienone and methenolone have been produced which are more polar and are taken orally.

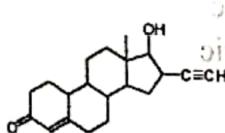
### Synthetic Anabolic Steroids

Compound name (IUPAC)	Chemical structure	Relative activity
Methandienone		0.21
Stanozolol		0.14
Nandrolone		0.01
Oxandrolone		0.005

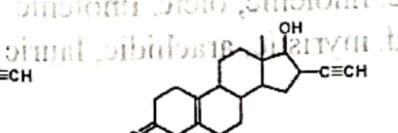
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## Contraceptive Steroids

- Progesterone prevents ovulation.
- Progesterone-like compounds are used for birth control.
- Synthetic derivatives of testosterone (e.g. norethynodrel, norethindrone) are often used in birth control pills formulation.



**Norethindrone**



**Norethynodrel**

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## Fatty Acids

- Are long-chain carboxylic acids.
- Carbon length ranges from 4 as in butyric acid to 36 carbons as in some brain fatty acids.
- Those common in nature usually consists of an even number of carbons (12 to 24).
- Most prevalent in nature are 16 and 18 carbon fatty acids.
- They are either saturated (no carbon-carbon double bonds) or unsaturated (one or more carbon-carbon double bonds).
- They are rarely found in their free forms in cells, but exist usually as esters of glycerol (mono-, di- and triacylglycerols).

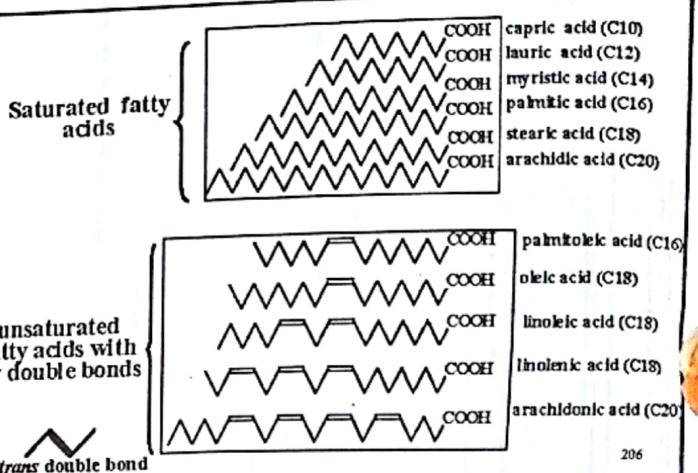
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## Fatty Acids

- For saturated fatty acids, the higher the number of carbons, the higher the melting point.
- For unsaturated fatty acids the higher the number of C=C, the lower the melting point.
- Solubility in water decreases with increasing chain length.
- Unsaturation leads to more flexible membranes.
- Cis* double bonds disrupt membrane structure than *trans* double bonds.

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## Fatty Acids



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## Fatty Acids

### Physical Properties of Saturated Fatty Acids

Number of carbons	Common name (source)	Systematic name	Melting point (°C)	Solubility in water (mg/mL)
12:0	Lauric acid ( <i>Laurus</i> , Latin, laurel plant)	n-Dodecanoic acid	44	0.063
14:0	Myristic acid ( <i>Myristica</i> , Latin, nutmeg genus)	n-Tetradecanoic acid	54	0.024
16:0	Palmitic acid ( <i>Palma</i> , Latin, palm fruit)	n-Hexadecanoic acid	63	0.008
18:0	Stearic acid ( <i>Stearyl</i> , Greek, "hard fat")	n-Octadecanoic acid	70	0.003
20:0	Arachidic acid ( <i>Arachis</i> , Latin, legume genus)	n-Eicosanoic acid	77	

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## Fatty Acids

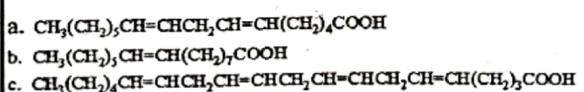
### Physical Properties of Unsaturated Fatty Acids

Number of carbons	Common name (source)	Systematic name	Melting point (°C)
16:1( $\Delta^9$ )	Palmitoleic acid (cod liver oil, butter fat)	cis-9-Hexadecenoic acid	1
18:1( $\Delta^9$ )	Oleic acid ( <i>Oleum</i> , Latin for olive oil)	cis-9-Octadecenoic acid	13
18:2( $\Delta^9,12$ )	Linoleic acid ( <i>Linum</i> , Greek for flax)	Cis,cis-9,12-Octadecadienoic acid	-5
18:3( $\Delta^9,12,15$ )	$\alpha$ -Linolenic acid (linseed oil)	Cis,cis,cis-9,12,15-Octadecatrienoic acid	-11
20:4( $\Delta^{5,8,11,14}$ )	Arachidonic acid (corn oil)	Cis,cis,cis,cis-5,8,11,14-Eicosatetraenoic acid	-50

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## Self-Test Questions

1. Work out the shorthand nomenclature for each of the following fatty acids.



2. Draw the chemical structures of the fatty acids denoted by the following shorthand nomenclature.

- a. 10:1 $\Delta^4$   
 b. 18:2 $\Delta^{9,12}$   
 c. 18:3 $\Delta^{9,12,15}$

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## Self-Test Questions

3. Assume you have triacylglycerol samples containing the sets of fatty acids listed below. Is each sample a liquid (oil) or solid (fat) at room temperature?

- a. palmitic, stearic, myristic  
 b. oleic, linoleic, arachidonic  
 c. linolenic, oleic, linolenic  
 d. myristic, arachidic, lauric

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## Essential Fatty Acids

- Both fish and vegetable oils have high amounts of polyunsaturated fatty acids (PUFA).
- Humans can synthesize all but two of the polyunsaturated fatty acids they need. The two are linoleic acid and linolenic acid.
- They are called essential fatty acids because they have to be obtained from the diet.
- They are usually obtained from plant and fish sources.

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## Essential Fatty Acids

- These 18-carbon fatty acids (linoleic and linolenic acids) are precursors for the synthesis of arachidonic acid (a 20 carbon fatty acid), which is a precursor for the synthesis of hormone-like substances called eicosanoids (*eicos* is Greek word for 20).
- Eicosanoids are important in the regulation of blood pressure, blood clotting, blood lipid levels, immune and inflammation responses.

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## Essential Fatty Acids

### Omega-3 Fatty Acids

- These are polyunsaturated fatty acids in which the last double bond is three carbon atoms away from the methyl end of the fatty acid. They are mostly found in fish oils and chloroplast of green leafy vegetables. The common omega-3 fatty acids are linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA).



Linolenic acid  
(18:3 Δ<sup>9,12,15</sup>)



Eicosapentaenoic acid (EPA)  
(20:5 Δ<sup>5,8,11,14,17</sup>)



Docosahexaenoic acid (DHA)  
(22:6 Δ<sup>4,7,10,13,16,19</sup>)

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## Essential Fatty Acids

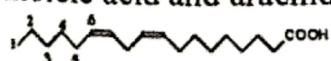
- Omega-3 fatty acids appear to lower blood platelet aggregation and hence reduce the tendency of blood to clot. High levels of these fatty acids could cause bleeding.

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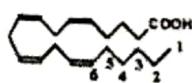
## Essential Fatty Acids

### Omega-6 Fatty Acids

- These are polyunsaturated fatty acids in which the last double bond is located six carbon atoms from the methyl end of the fatty acid chain.
- They are found mostly in seeds of most plants except coconut, cocoa and palm.
- The two common omega-6 fatty acids are linoleic acid and arachidonic acid.



Linoleic acid  
(18:2 Δ<sup>9,12</sup>)



Elcosatetraenoic (arachidonic) acid  
(20:4 Δ<sup>5,8,11,14</sup>)

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## Essential Fatty Acids

### Omega-9 Fatty Acid

- These are fatty acids in which the last double bond is located 9 carbon atoms from the methyl end of the fatty acid chain.
- Oleic acid is an omega-9 fatty acid



Oleic acid

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## Essential Fatty Acids

- Omega-6 and omega-3 fatty acids are essential because humans cannot make them and must obtain them from their diet.
- It has been suggested that human beings evolved on a diet with an omega-6 to omega-3 essential fatty acids ratio of approximately 1.
- Omega-6/omega-3 ratios range from 4 in certain rural populations to as high as 50 in some urban populations.

## Essential Fatty Acids

- High omega-6/omega-3 fatty acid ratio promote the pathogenesis of cancer, cardiovascular diseases, hypertension, diabetes, arthritis, osteoporosis, inflammatory and autoimmune diseases, etc.
- Mammalian cells cannot convert omega-6 to omega-3 fatty acids because they lack the converting enzyme, omega-3 desaturase.

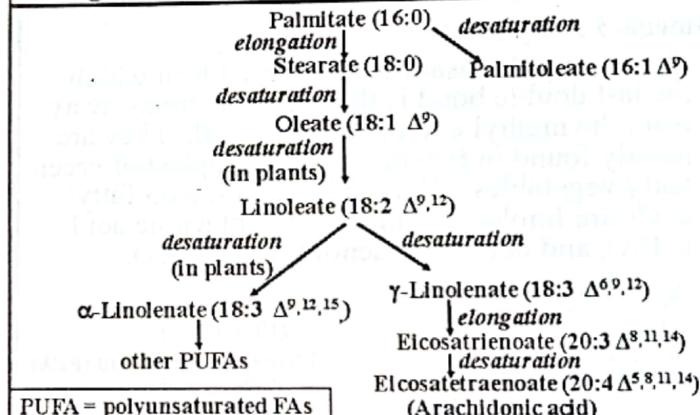
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## Elongation and Desaturation of Fatty Acids

- Fatty acids are synthesized in the cytosol.
- The longest length of fatty acid normally synthesized is the 16-carbon fatty acid, palmitic acid.
- Palmitic acid undergoes two post-synthetic modification processes:
  - **Elongation:** addition of carbons units to form long chain fatty acid, LCFA. This takes place in the mitochondrion and/or endoplasmic reticulum. It is catalyzed by *elongases*.
  - **Desaturation:** oxidation to produce one or more double bonds (takes place in the membrane of the smooth endoplasmic reticulum).

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## Elongation and Desaturation of Fatty Acids



Source: Redrawn from Nelson, D. L. and Cox, M. M (2000) Lehninger Principles of Biochemistry,

220

## Eicosanoids

- Eicosanoids are a diverse group of hormone-like molecules which affect a wide range of physiological functions in mammals.
- They are among the most potent of biological compounds.
- They are produced in mammalian tissues from polyunsaturated fatty acids with a 1,4-pentadienyl structure element such as linoleic, linolenic, dihomo-γ-linolenic, eicosatetraenoic (arachidonic), eicosapentaenoic, and decosahexaenoic acids.

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## Eicosanoids

- Are not synthesized and stored, but are produced in very small amounts ( $< 10^{-14} M$ ) as and when needed.
- Unlike hormones, eicosanoids are short-lived and do not travel in the blood to their site of action. They act in cells that produce them or in neighboring cells and are referred to as paracrine hormones or autocrine regulators or local hormones.
- They are unstable (half-life of only 30 seconds) and difficult to study.
- They regulate many cell functions and play crucial roles in a variety of physiological processes including regulation of smooth muscle contraction and various immune and inflammatory functions.

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## Eicosanoids

- The actions of eicosanoids are multiple and variable (stimulatory and inhibitory), depending on the tissue type and nature of receptors with which they interact.
- Eicosanoid receptors are coupled to either phospholipase or adenylate cyclase.
- Eicosanoids are very specific. For example, whereas prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) lowers blood pressure a closely related prostaglandin, PGF<sub>2α</sub> raises blood pressure.

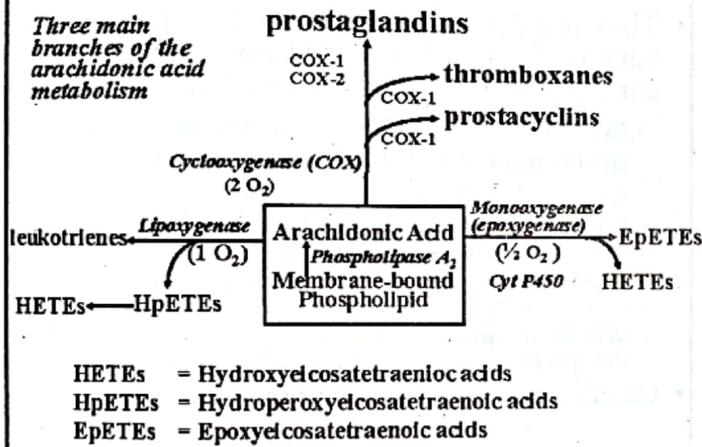
223

## Eicosanoids

- Arachidonic acid is the major substrate for eicosanoid synthesis in many species. It is stored as glycerol ester in membrane phospholipids from where it is released by the action of phospholipase A<sub>2</sub>.
- Arachidonic acid may be acted upon, after release, by *cyclooxygenase* to produce **prostaglandins** and **thromboxanes** or by *lipoxygenase* to produce **leukotrienes** or by *epoxygenase (monooxygenase)* to produce **epoxyeicosatetraenoic acids (EpETEs)** and **hydroperoxyeicosatetraenoic acids (HETEs)**.
- Arachidonic acid is usually needed for the production of pro-inflammatory eicosanoids, while eicosapentaenoic acid (EPA) is needed for the production of anti-inflammatory eicosanoids.

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## Pathways of Eicosanoids Synthesis



## Eicosanoids: Prostaglandins (PG)

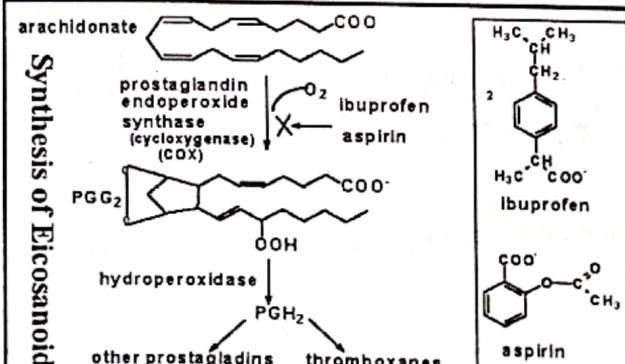
- They have a 5-membered ring in their structure.
- Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>)
- The subscript number in a name indicates the number of double bonds in the molecule.
  - Were first discovered in semen where they are stored after synthesis in the prostate gland (hence name). They are found throughout the body (lungs, liver, uterus etc.).
  - Are potent mediators of inflammation.
  - Regulate synthesis of cyclic AMP (cAMP).
  - Promote vasodilation of coronary arteries and antagonize platelet aggregation.
  - Regulate smooth muscle contraction during menstruation and labor, induce abortion, vomiting, and nausea. Control fertility and contraception. Promotes or mitigates inflammation and pain.

## Eicosanoids: Prostaglandins

- Eicosanoids are involved in inflammation, pain, and fever responses in tissues.
- Two types of anti-inflammatory drugs affect prostaglandin synthesis
  - Steroidal anti-inflammatory drugs (SAIDs):** e.g. hydrocortisone, betamethasone, and prednisolone.
  - These inhibit a specific phospholipase A<sub>2</sub> that is involved in arachidonic acid release from membrane phospholipids.
- Nonsteroidal anti-inflammatory drugs (NSAIDs):** e.g. aspirin, ibuprofen (Advil, Motrim), indomethacin, naproxen (Aleve and Naprosyn), and phenylbutazone, acetaminophen (Paracetamol).
  - These inhibit cyclooxygenase (COX).
  - There are two isoforms of COX in animals. COX-1 carries out normal physiological production of prostaglandins, while COX-2 is induced by cytokines, mitogens, and endotoxins in inflammatory cells.

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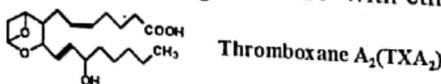
## Eicosanoids



Cyclooxygenase activity which results in the production of both pro- and anti-inflammatory eicosanoids is inhibited by aspirin, and ibuprofen. This is the basis for the analgesic (pain relieving) property of these drugs.

## Eicosanoids: Thromboxanes

- Have a 6-membered ring structure with ether.

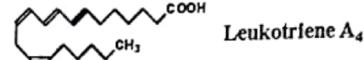


- First isolated from blood platelets (thrombocytes).
- Facilitate blood clot formation through platelet aggregation and vasoconstriction.
- Aspirin prevents strokes caused by blood clots in the brain by inhibiting the synthesis of thromboxanes.

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## Eicosanoids: Leukotrienes

- Leukotrienes are hydroxy-fatty acid derivatives of arachidonic acid.
- They contain no ring structure, have a triene (three conjugated double bonds) in their structure.



- First isolated from white blood cells (leukocytes).
- They play a major role in inflammatory responses.
- Cause contraction of smooth muscle in lungs.
- Asthmatic attack and allergic reactions may be caused by over production of leukotrienes.
- Leukotriene synthesis is a target of some anti-asthmatic drugs.

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## Waxes

- Are non polar esters of usually saturated long chain fatty acids and long chain monohydroxylic alcohols. There can be between 14 to 36 carbon atoms in the fatty acids or alcohols found in waxes and are unbranched.
- Waxes also contain hydrocarbons, alcohols, fatty acids, aldehydes, and sterols (steroid alcohol), etc.
- Waxes are extremely insoluble in water because of the long hydrophobic tails.
- They serve as protective coating for leaves, stems and fruits, water repellents for feathers and furs, lubrication for the skin, etc. They also serve as a storage form of energy.

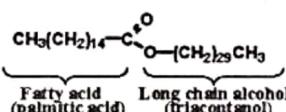
231

## Waxes

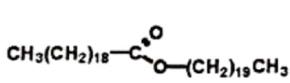
- They are derived from several sources and vary in chemical composition. They include ear wax, leaf wax, wool wax, beeswax, etc.
  - Beeswax:** an ester composed of palmitic acid and the alcohol triacontanol plus 20% hydrocarbons. Used in candles, shoe polish and as paper wax.
  - Carnauba wax:** obtained from a Brazilian palm plant leaf. Used in furniture, floor and car waxes.
  - Jojoba wax:** obtained from Jojoba plant seed. Used in cosmetics, candles etc.
  - Whale oil (spermaceti wax):** obtained from the head of the sperm whale. It is a mixture of waxes.
- Canning "wax" not a wax but paraffin (an alkane).

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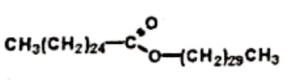
## Waxes



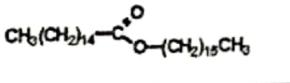
Beeswax  
(myricyl palmitate)  
(component of honeycomb)



Carnauba wax  
(melissyl cerotate)  
(in Brazilian palm leaves)



Jojoba wax  
(found in Jojoba seed)



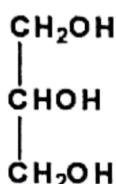
Spermaceti wax  
(cetyl palmitate)  
(from head of sperm whale)

## Self-Test Question

- When water birds have had their feathers fouled with crude oil after an oil spill, they are cleaned to remove the spilled oil on their feathers. Why are they not released immediately after they are cleaned?

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## Glycerol Lipids



Structure of Glycerol

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## Glycerol Lipids

### Acylglycerols

- The three hydroxyl groups of glycerol can be esterified with one, two or three fatty acids to give mono-, di-, and triacylglycerols respectively. Saturated fatty acids usually bind to the  $\alpha$ -carbon, whereas unsaturated fatty acids almost always bind to the  $\beta$ -carbon.

— **Triacylglycerols** (Fats and Oils): Are called neutral fats and are destined for storage and provide energy. They provide more energy on a mole for mole basis than carbohydrates since they are more reduced and are stored without water.

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## Glycerol Lipids

- Phosphatidic acid:** Is formed when two of the hydroxyl groups of glycerol are esterified with fatty acids and the third hydroxyl group is esterified with phosphoric acid.
- **Glycerophospholipids** (derivatives of phosphatidic acid).
  - Phosphatidylcholine** (lecithin): has the phosphate group of phosphatidic acid esterified with the aminoalcohol, choline.
  - Phosphatidylethanolamine** (cephalin): has the phosphate group of phosphatidic acid esterified with ethanolamine.
  - Phosphatidylserine** (cephalin): the phosphate group of phosphatidic acid is esterified with serine.
  - Lecithins and cephalins are abundant in the brain and nerve tissues, egg yolk, yeast, and wheat germ.

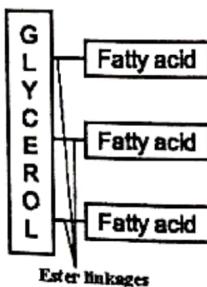
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## Glycerol Lipids

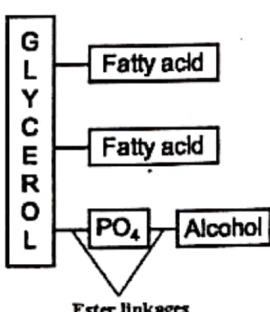
- **Phosphatidylinositol (inositides):** the phosphate of phosphatidic acid is esterified with inositol (a hexahydroxyalcohol).
- **Phosphatidylglycerols and diphosphatidylglycerols:** contain an additional molecule of glycerol bound to the phosphate residue of phosphatidic acid.
- **Plasmalogens** (phospholipids with ether-linked fatty acids): ether lipids in which one of the two acyl chains of glycerol is in an ether rather than an ester linkage. About half of heart phospholipids are plasmalogens.

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## Triacylglycerols and Glycerophospholipids



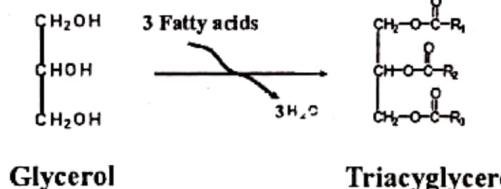
Triacylglycerols  
Neutral fat (Fats/Oils)



Glycerophospholipids

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## Triacylglycerols



Glycerol

Triacylglycerol

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## Triacylglycerols

- Fats and oils are triacylglycerols (triglycerides).
- Fats are triacylglycerols with a high percentage of saturated fatty acids. They are solids or semisolids at room temperature. They are generally obtained from animals.
- Melting point of fat is increased with increasing saturated fatty acid composition and decreases with increasing unsaturated fatty acid content. The melting point is decreased by decreasing number of carbons in the fatty acid components of the fat.

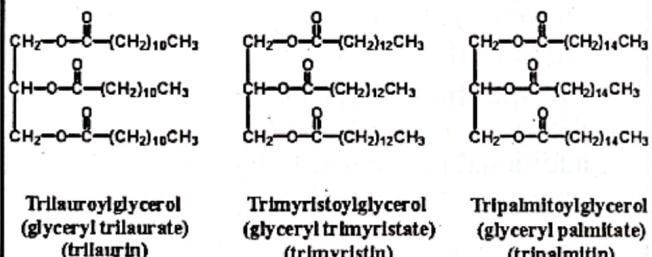
241

## Triacylglycerols

- Oils are triacylglycerols with a high percentage of unsaturated fatty acids. They are generally obtained from plants and are liquids at room temperature.
- Oils become rancid during storage under aerobic conditions due to autoxidation (peroxidation) of the double bonds in unsaturated fatty acids.

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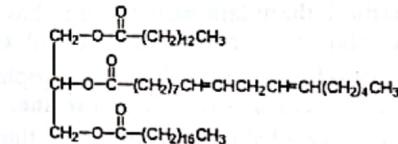
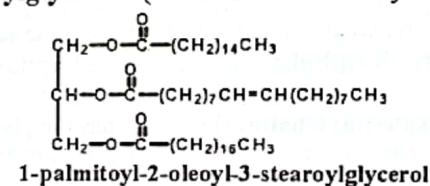
## Triacylglycerols



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## Triacylglycerols

Mixed triacylglycerols (contain different fatty acids)



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## Naturally Occurring Fats and Oils

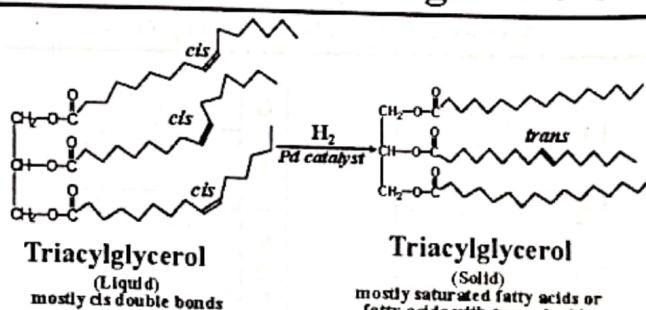
Most naturally occurring fats and oils are mixed triacylglycerols (the three fatty acid portions are usually not the same)

Percentage composition of main fatty acids in common fats and oils

Source	Palmitic acid	Stearic acid	Oleic acid	Linolenic acid
Soybean oil	10	-	25	55
Corn oil	10	5	45	38
Butter	25	10	35	-
Human fat	25	8	46	10
Palm oil	39	4	40	5

Source: Taken from Ouellette, J. R. (1997) *Introduction to General, Organic and Biological Chemistry*. 4th Edition. Prentice Hall, New Jersey.

## Hydrogenation of Vegetable Oils



Hydrogenation converts oil into solid fat.  
Margarine is a partially hydrogenated vegetable oil.

Source: Redrawn from Boyer, R. (1999) *Concepts in Biochemistry*.

## **Hydrogenation of Vegetable Oils**

- Molecules of saturated fatty acids pack tightly together resulting in stronger secondary attractive forces.
  - Molecules of *cis* unsaturated fatty acids do not pack tightly and have weaker secondary attractive forces because the *cis* double bonds cause the chains to kink. *Cis* double bonds lower melting point by preventing firm contact between fatty acid chains.
  - For practical reasons oils are not completely hydrogenated during the commercial hydrogenation process. A complete hydrogenation product would be hard and not useful as margarine.

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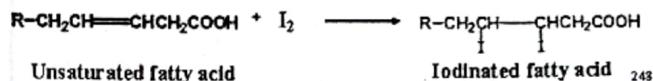
## Saponification of Triacylglycerol

- Soaps are the  $\text{Na}^+$  or  $\text{K}^+$  salts of fatty acids.
  - Saponification of triacylglycerol is the historical origin of soap making.
  - Soaps are formed when a triacylglycerol is treated with heat and alkali in a process called **saponification** (the reverse of esterification).
  - During saponification, there is hydrolysis of TAG to produce glycerol and the alkali salts of the three fatty acids which are hydrolyzed from triacylglycerol.
  - The glycerol product of saponification, commercially called glycerin, is of major importance in the cosmetic industry.
  - Glycerin is used as a lubricating component of skin creams, hand lotions and soaps.

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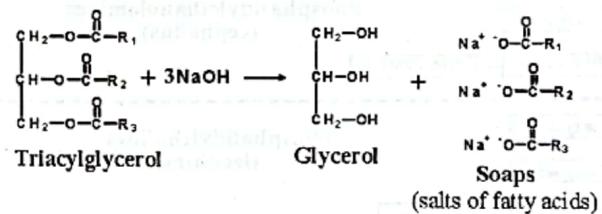
## Iodination of Unsaturated Fatty Acids

- The halogen elements (chlorine, bromine and iodine) react with unsaturated fatty acids to form products in which the halogen is added across the double bonds.
  - The amount of halogen added is a measure of the degree of unsaturation.
  - The amount of iodine that reacts with the fatty acid can be used to determine the *iodine number*.



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## Saponification of Triacylglycerol



Saponification can be used quantitatively to determine the average molecular weight of a fatty acid in a triacylglycerol. The saponification value is an indication of the nature of the fatty acids in the fat/oil. The longer the carbon chain of a fatty acid the less acid released per gram fat/oil hydrolyzed.

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### **Acid Value of Triacylglycerol**

- Microorganisms hydrolyze the ester bonds in fats/oils to release free fatty acids.
  - The amounts of free fatty acids in a fat/oil is used to assess the age and quality of the fat/oil.
  - The amount of free fatty acids is determined by titration of the fat/oil with KOH.
  - The acid value is the number of milligrams of KOH required to neutralize the free fatty acids in 1 gram of fat/oil.

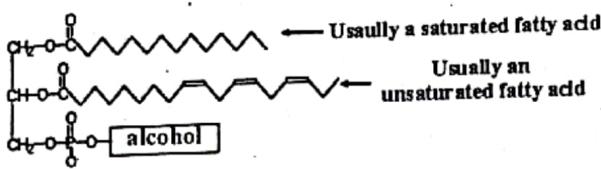
25

### **Self-Test Question**

1. The hydrolysis of an optically active triacylglycerol gives 1 mole each of glycerol and oleic acid and two moles of stearic acid. Write the structure for the triacylglycerol. Indicate using asterisks the chiral center(s). How many stereoisomers of the triacylglycerol are possible?
  2. Because peanut oil floats on the top of peanut butter, many brands of peanut butter are hydrogenated. A solid product then forms that is mixed into peanut butter and does not separate. If the triacylglycerol in peanut oil contains one palmitic acid, one oleic acid, and one linolenic acid is completely hydrogenated, what is the structure of the product formed?

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## Glycerophospholipids



General structure of a glycerophospholipid

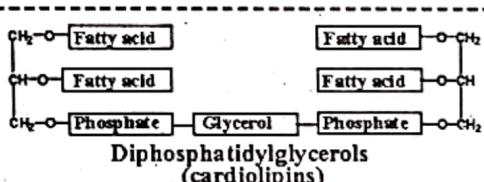
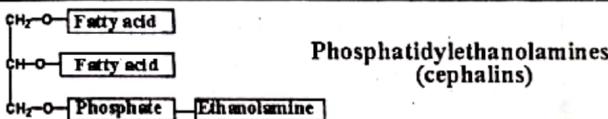
alcohol = ethanolamine, choline, serine, inositol, etc

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H <sub>3</sub> C—CH—CH <sub>2</sub> O O (GLYCEROL)			FATS AND OILS (TAGs)
FA	FA	FA	
FA	FA	(P)	PHOSPHATIDIC ACID
I	I	(P) O—CH <sub>2</sub> —CH <sub>2</sub> —N—CH <sub>3</sub> (Choline)	PHOSPHATIDYLCHOLINES (lecithins)
J	I	(P) O—CH <sub>2</sub> —CH <sub>2</sub> —N—H (Ethanolamine)	PHOSPHATIDYLETHANOLAMINES (Cephalins)
FA	FA	(P) O—CH <sub>2</sub> —CH <sub>2</sub> —NH <sub>2</sub> (Serine)	PHOSPHATIDYLSERINES (Cephalins)
I	I	(P) OH OH CH <sub>2</sub> —CH—CH <sub>2</sub> (Inositol)	PHOSPHAINOSITIDES (Inositides)
FA	FA	(P) OH OH OH (Glycerol) CH <sub>2</sub> —CH—CH <sub>2</sub>	PHOSPHATIDYLGlycerols
FA	FA	(P) OH CH <sub>2</sub> —CH—CH <sub>2</sub> FA FA O CH <sub>2</sub> —CH—CH <sub>2</sub>	DIPHOSPHATIDYLGlycerols (Cardiolipins)
O CH CH R	FA	(P) O—CH <sub>2</sub> —CH <sub>2</sub> —N—CH <sub>3</sub> O—CH <sub>2</sub> —CH <sub>2</sub> —NH <sub>2</sub> (Ethanolamine)	PLASMALOGENS

Source: Adapted from J. Murch, O. Novakova and K. Karel, *Biochemistry in Schmidle Perspective*, Akademie, 1977.

## Glycerophospholipids



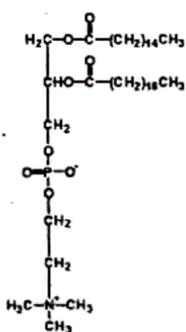
255

## Glycerophospholipids

- **Phosphatidylcholines** (lecithins) are found in micelles of protoplasm of body cells and function as emulsifying agents to transport fat molecules.
- **Phosphatidylethanolamines** (cephalins) found in the heart, liver, and brain tissues are essential in blood clotting.
- **Phosphatidylserines** (cephalins). Found in soybean phospholipid, are brain enhancers. They improve concentration, renovate memory and facilitate focused activity. Reduce the risk of cognitive dysfunction or dementia in the elderly.

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## Glycerophospholipid



1-Palmitoyl-2-stearoyl-phosphatidylcholine

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## Self-Test Question

1. In the preparation of sauces that involve mixing water and melted butter, egg yolks are added to prevent separation. How do egg yolks prevent separation? Hint: Egg yolks are rich in phosphatidyl choline (lecithin).

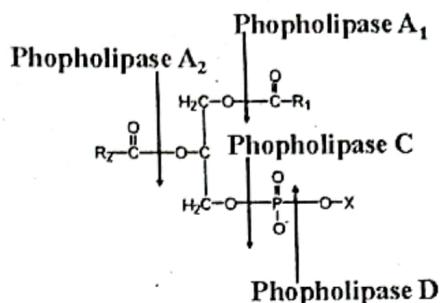
258

## Phospholipases

- Phospholipases A<sub>1</sub> and A<sub>2</sub> hydrolyze the ester bonds of glycerophospholipids at C-1 and C-2 of glycerol respectively.
- Phospholipases C and D hydrolyze the phosphodiester bonds in the head group.

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## Phospholipases



### A Phosphoacylglycerol

Site of action of phospholipases designated A<sub>1</sub>, A<sub>2</sub>, C and D

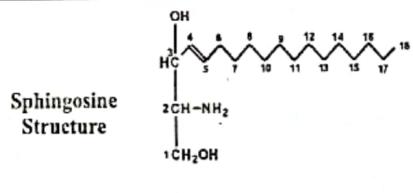
260

## Sphingolipids

- Contain sphingosine, an 18-carbon aminoalcohol backbone instead of the glycerol in glycerol lipids.
- A fatty acid is bound to the nitrogen of sphingosine through an amide linkage.
- Fatty acid components with 24 carbon atoms are the most common among sphingolipids.
- The terminal hydroxyl group is either free (as in ceramides) or esterified with phosphocholine to give a sphingophospholipid, sphingomyelin.
- Some sphingolipids have carbohydrate units in their structure (the glycosphingolipids).

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## Sphingolipids



Sphingosine

(an 18-carbon aminoalcohol)

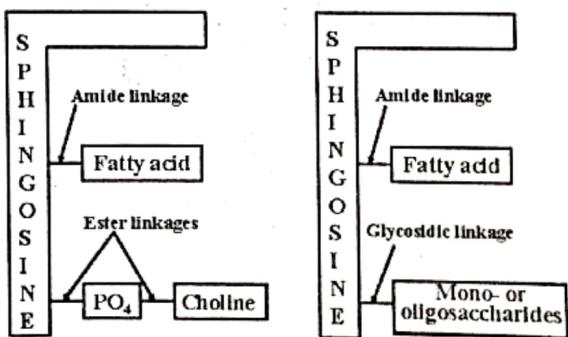
262

## Sphingolipids

### Glycosphingolipids (glycolipids)

- Found usually in membranes and function as sites for recognition by carbohydrate-binding proteins.
  - A sugar (such as glucose or galactose) bound to terminal hydroxy group produces a **cerebroside** (e.g. **glucocerebroside**). Sulfonation of the sugars in cerebrosides gives rise to **sulfolipids**.
  - **Globosides** are glycosphingolipids with a disaccharide or trisaccharide bound to the terminal hydroxyl group of sphingosine.
  - **Gangliosides** are formed when an oligosaccharide is glycosidically linked to the terminal hydroxyl group. N-Acetylneurameric acid is usually the terminal sugar in gangliosides.

## Sphingolipids



Sphingophospholipids  
(Sphingomyelins)  
are phospholipids

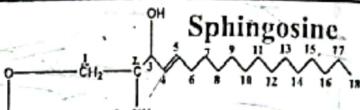
Glycosphingolipids

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## Sphingolipids

- Sphingolipids are more stable to hydrolysis because of the presence of an amide linkage rather than the ester linkages found in glycerophospholipids.
- Sphingomyelins are found in myelin sheath of nerve fibers.
- Loss of the sheath leads to slowing and eventual cessation of nerve impulse.
- In multiple sclerosis, the myelin sheath is destroyed progressively by sclerotic plaques which affect the brain and spinal cord. It is a crippling and fatal condition.
- Cerebrosides and gangliosides are found in the nerve and brain cell membranes.

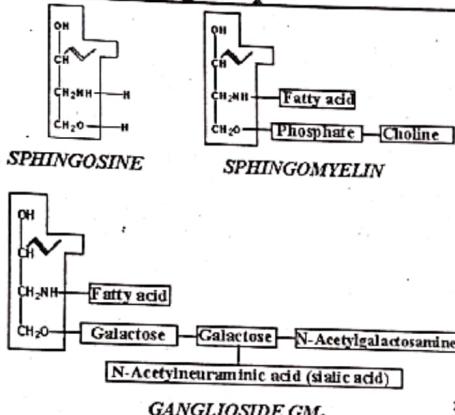
## Sphingolipids

		
	H	Fatty acid
SPHINGOLIPIDS	Phosphocholine	CERAMIDES
	Gal or Glc.	SPHINGOMYELINS
	Oligosaccharide	CEREBROSIDES
	Sulfate saccharides: Glc-SO <sub>3</sub> H, Gal-SO <sub>3</sub> H	GANGLIOSIDES
		SULFOLIPIDS
		GLYCOSPHINGOLIPIDS

Source: Adapted from Musil, J., Novakova, O., and Kunz, K. (1977) Biochemistry in Schematic Perspective, Avicenum,

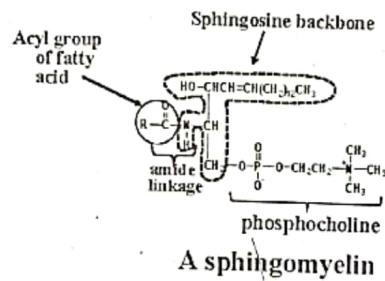
265

## Sphingolipids



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## Sphingolipids



Overall structure of sphingomyelin drawn to look like a glycerophospholipid

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## Self-Test Questions

1. Assign and describe the polar head and non polar tails to the following lipids:
  - (a) Cerebrosides
  - (b) Cholesterol
  - (c) Fatty acid
  - (d) Phosphatidylserine
  - (e) Sphingomyelin
2. Describe how the structural features of a ceramide differ from that of a cerebroside.
3. How are glycosphingolipids similar to sphingophospholipids? In what ways do the two types of compounds differ?

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## Self-Test Questions

4. Which of the molecular components in the following list are present in the structures of both a triacylglycerol and a phosphatidyl choline?
  - (a) Only one fatty acid
  - (b) Sphingosine
  - (c) Phosphate
  - (d) Three fatty acids
  - (e) Alcohol
  - (f) Glycerol
  - (g) Sugar
  - (h) Two fatty acid
5. Which components from the list above do galactocerebroside and sphingomyelin have in common?
6. Which component(s) from the list above does phosphatidyl ethanolamine and sphingomyelin have in common?

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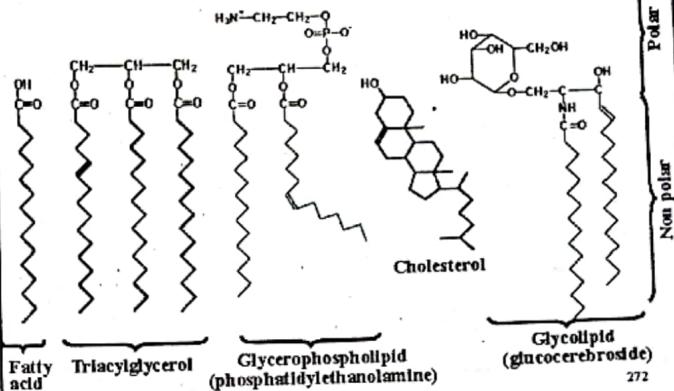
## Self-Test Questions

7. For each class of lipids in the left column in the Table below, indicate with an "X" the components in the top row that are found in these lipids. Indicate with "some" if not all of the lipids in a class contain a component. Indicate in the last column whether the lipid is found in a membrane.

	Fatty acid	Glycerol	Sphingosine	Phosphate	Carbohydrate	Membrane Lipid?
Triacylglycerol	X					
Phospholipid				X		
Sphingolipid			X			
Ceramide			X			
Ganglioside			X			
Cholesterol						

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## Amphiphatic Nature of Lipids



## Cell Membranes (% Lipid)

Lipid Type	Human Red Blood Cells	Bacteria
Glycerophospholipid		
with choline	19	0
with ethanolamine	18	65
with serine	8	0
Triacylglycerol	0	18
Sphingomyelin	18	0
Glycosphingolipids	10	0
Cholesterol	25	0
Others	2	17

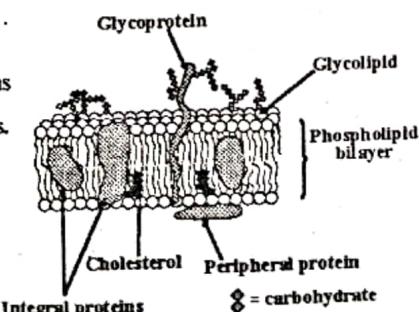
Source: Taken from Mathews, C. K., van Holde, Ahen, K. G. (1996), Biochemistry; Addison/Wesley/Longman/Benjamin Cummings.

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## Cell Membranes

The membrane is not a rigid but a fluid-like structure. A *fluid mosaic model* of membranes envisages the membrane as a "sea" of lipids interspersed with proteins.

Cholesterol makes up 20-25% of the lipid bilayer in animals. Cholesterol adds to the rigidity and strength of membranes.



Phospholipids = Glycerophospholipids and sphingophospholipids

Properties of membranes are due mainly to the lipid, protein and carbohydrate composition. NB. The lipid bilayer does not include triacylglycerols as they are completely hydrophobic with no hydrophilic part.

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## Cell Membranes

- Principal functions of membranes are to:
  - separate cells and cell components,
  - maintain concentration and electrochemical gradients,
  - ensure transport of nutrients and products,
  - act as support for surface antigens,
  - give rise to and propagate nerve impulse.

## Cell Membranes

### Composition

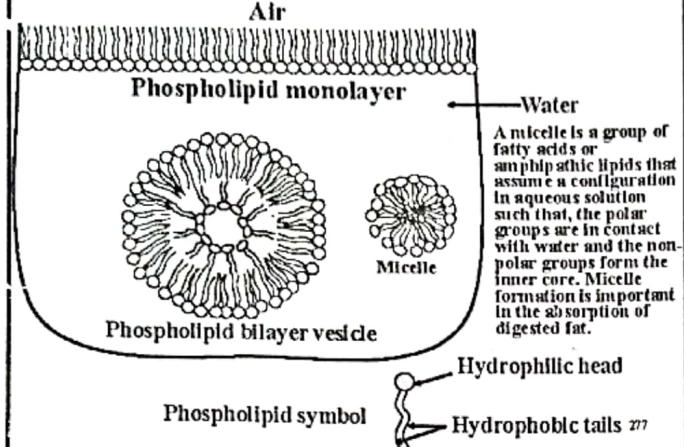
#### Percent by weight

Membrane	Protein	Lipid	Carbohydrate
Myelin	18	79	3
Human erythrocyte plasma membrane	49	43	8
Amoeba plasma membrane	54	42	4
Mycoplasma cell membrane	58	37	1.5
Halobacterium purple membrane	75	25	0

Adapted from Guidotti, G. (1972) Membranes Proteins, Ann. Rev. Biochem. 41:731.

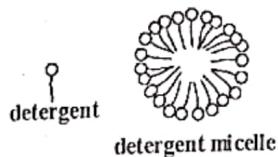
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## Phospholipids in Water



## Soaps and Detergents

- Soaps and detergents help to remove water-insoluble dirt.
- They do this by forming micelles in solution.
- The interior of micelles is a hydrophobic environment into which nonpolar molecules (greasy dirt) may dissolve



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## Cleaning Action of Detergent



Soap molecules dislodge greasy molecules by attaching themselves to the grease through their non polar tails. The polar portion of the soap keeps the grease in aqueous solution so it can be washed away.

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## 6 Proteins

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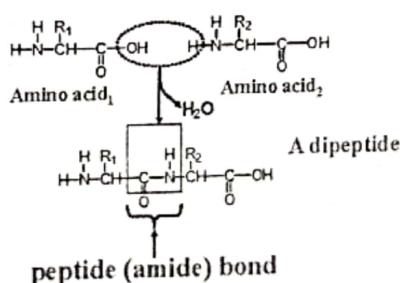
## Proteins

- The term protein comes from the Greek word *proteios*, meaning first rank.
- They are biopolymers constructed from amino acids that display a wide range of functions and structures.
- The human body has over 100,000 different kinds of proteins.
- Proteins demonstrate individuality in many ways. The dissimilarity among proteins is due to differences in amino acid sequence, isoelectric point (pI), structure, and the presence of non amino acid components (prosthetic groups).

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## Amino Acids

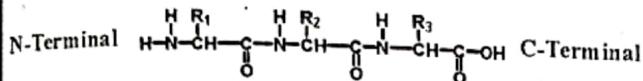
- Amino acids are the main building blocks of proteins.
- The amino and carboxyl groups of amino acids can react in a head-to-tail fashion with the elimination of water to form amide (peptide) linkages.



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## Amino Acids

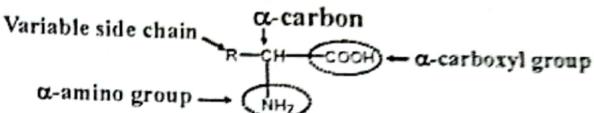
- The amino acid residues (or amino acid units) are joined together by peptide bonds in a peptide or protein.
- Peptides and proteins are named from the amino ( $\text{NH}_2$ ) to the carboxyl ( $\text{COOH}$ ) terminus.



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## Amino Acids

- Contain an amino group and an carboxylic acid group both attached to the  $\alpha$ -carbon and are thus called  $\alpha$ -amino acids.



- Approximately 250 different amino acids are found in nature, but only about 20 are commonly found in plant and animal proteins.
- The 20 amino acids can be combined in a variety of ways to form muscles, tendons, skin, fingernails, hair, enzymes, silk, hormones, antibodies, feathers, etc.

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## Amino Acids

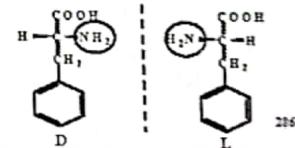
Name	One-Letter abbreviation	Three-Letter abbreviation	Name	One-Letter abbreviation	Three-Letter abbreviation
Glycine	G	Gly	Cysteine	C	Cys
Alanine	A	Ala	Asparagine	N	Asn
Valine	V	Val	Glutamine	Q	Gln
Leucine	L	Leu	Tyrosine	Y	Tyr
Isoleucine	I	Ile	Tryptophan	W	Trp
Methionine	M	Met	Aspartate	D	Asp
Phenylalanine	F	Phe	Glutamate	E	Glu
Proline	P	Pro	Histidine	H	His
Serine	S	Ser	Lysine	K	Lys
Threonine	T	Thr	Arginine	R	Arg

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## Chirality of Amino Acids

The  $\alpha$ -carbons of amino acids, apart from glycine, have four different atoms or group of atoms attached and hence are chiral. Amino acids are therefore optically active and exist as enantiomeric (mirror image) D- and L- forms. The L-enantiomers are the most common among proteins. Proteins with the D-isomeric forms of amino acids are biologically less active. The D-forms have been found in cells of certain microorganisms. The gradual conversion of amino acids such as aspartic acid from the L- to the D- isomer has been observed in certain tissues with age. The conversion of amino acids from L- to D- form has been implicated in the aging process.

Enantiomers of phenylalanine



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## Classification of Amino Acids

- Amino acids are classified into two major groups: **essential** amino acids (those required in the diet) and **non essential** amino acids (those made by the body).
- They are also classified based on the nature (polar or nonpolar) of the side chain ( $\text{R}$ -group).

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## Classification of Amino Acids

Non essential (dispensable)	Essential (nondispensable)
Alanine	Histidine*
Arginine*	Isoleucine
Asparagine	Leucine
Aspartic acid	Lysine
Cysteine#	Methionine
Glutamic acid	Phenylalanine
Glutamine	Threonine
Glycine	Tryptophan
Proline	Tyrosine#
Serine	Valine

\* Essential for infants not adults # Essential for premature infants  
Arginine is classified as a semi-essential or conditionally essential amino acid. This implies under normal conditions, the body is able to synthesize enough arginine to meet physiological needs

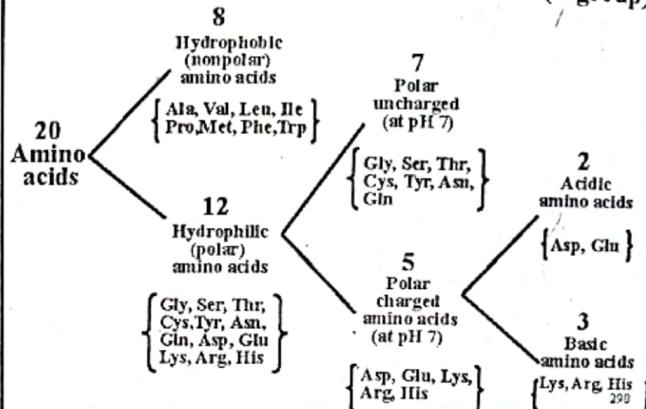
## Classification of Amino Acids

- Amino acids differ in their R-group (side chain) which determines differences in the physical and chemical properties of amino acids and the proteins they form.
- They are classified according to the nature of the R-group as,
  - nonpolar,
  - polar uncharged, or polar neutral, (those with non ionizable R-groups),
  - polar charged, or polar ionizable, (those with acidic or basic R-groups).

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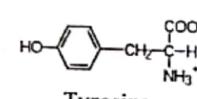
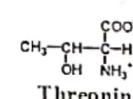
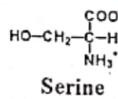
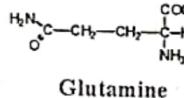
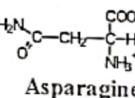
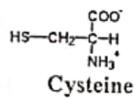
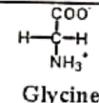
## Classification of Amino Acids

Classification based on nature of side chain (R group)



## Classification of Amino Acids

Polar, Uncharged Amino Acids

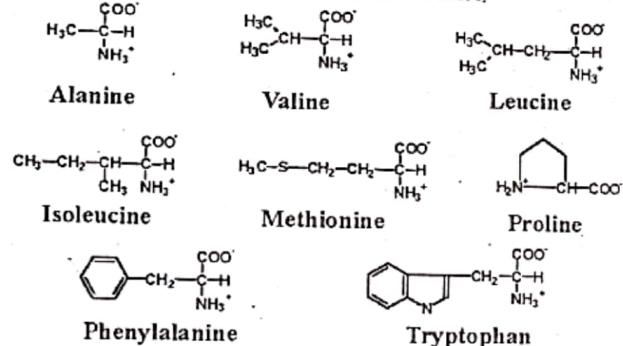


Structures are drawn as they exist at pH 7.  
NB. Glycine is sometimes classified as hydrophobic.

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## Classification of Amino Acids

Hydrophobic Amino Acids



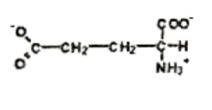
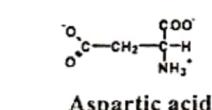
Structures are drawn as they exist at pH 7

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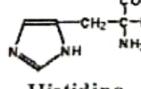
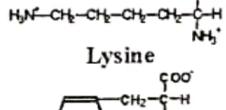
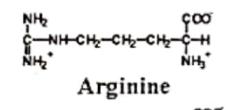
## Classification of Amino Acids

Polar, Charged Amino Acids

Acidic Amino Acids



Basic Amino Acids



Structures are drawn as they exist at pH 7

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## Self-Test Question

1. Name amino acid(s) in which the R group contains the following:

a hydroxyl group \_\_\_\_\_

a second chiral carbon atom \_\_\_\_\_

an amide group \_\_\_\_\_

an aromatic ring \_\_\_\_\_

a sulfur atom \_\_\_\_\_

an amino group \_\_\_\_\_

an acid group \_\_\_\_\_

a branched side chain \_\_\_\_\_

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## Dipolar Nature of Amino Acids

- Amino acids contain a basic amino group and an acidic carboxyl group in the same molecule.
- Though it is convenient to write amino acid structures with the -COOH and -NH<sub>2</sub> groups, the two groups usually exist in the ionic state (-COO<sup>-</sup> or -NH<sub>3</sub><sup>+</sup>) depending on the pH environment in which an amino acid finds itself.
- Amino acids undergo an **internal acid-base reaction** to yield a **dipolar ion**, also called a **zwitterion** (from German *zwitter*, "hybrid"), with a net charge of zero at physiological pH.

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## Dipolar Nature of Amino Acids

- Because of the resultant ionic charges, the properties of an amino acid resemble those of a salt rather than those of an uncharged molecule.
- The melting points of solid amino acids are high because of the salt properties of the zwitterions (e.g. glycine = 260°C).
- The dipolar structures of the amino acids account for the following properties of amino acids:
  - Crystalline in their pure form
  - High melting point
  - Mostly water soluble
  - Not very soluble in most organic solvents
  - Large dipole moments

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## Dipolar Nature of Amino Acids

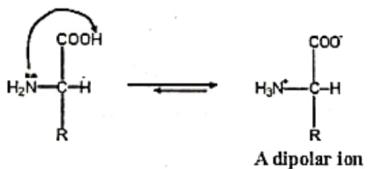
Consider the following compounds:

Ethylamine	Acetic acid	Glycine
CH <sub>3</sub> CH <sub>2</sub> NH <sub>2</sub>	CH <sub>3</sub> COOH	NH <sub>2</sub> CH <sub>2</sub> COOH
mp = -84°C	mp = 16°C	mp = 232°C

Ethylamine is a gas and acetic acid is liquid at room temperature, whereas glycine is a solid.

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## Dipolar Nature of Amino Acids



The dipolar ion is the more common form in neutral solution

In aqueous solution the amine group has a greater attraction for the carboxyl proton. Aqueous solutions of neutral amino acids are slightly acidic because the -NH<sub>3</sub><sup>+</sup> group is a stronger acid than the -COO<sup>-</sup> group is a base.

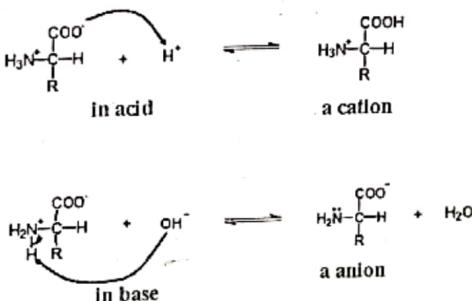
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## Acid-base Properties of Amino Acids

- A compound that is both an acid and a base is described as **amphotropic**.
- Amino acids are amphotropic compounds and their aqueous solutions behave as buffers.
- Amino acids contain ionizable groups which act as weak acids or bases.
- These groups take on or give off proton(s) when the pH is changed.

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## Acid-base Properties of Amino Acids



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## Acid-base Properties of Amino Acids

### Acidic amino acids



The side chain carboxyl group of glutamic acid is more acidic than water and so it can donate a proton to water to produce a hydronium ion, making the solution acidic.

### Basic amino acids



The  $\epsilon$  NH<sub>2</sub> group of lysine is more basic than water. It accepts a proton from water resulting in the release of an OH<sup>-</sup> group making the solution basic.

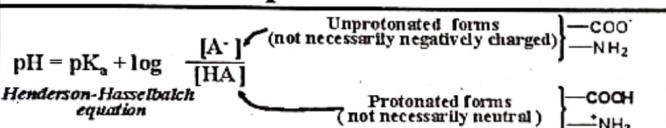
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## Acid-base Properties of Amino Acids

- Each ionization group of an amino acid can exist in one of two states (charged or neutral)
- The charge on a functional group is determined by relationship between its pK<sub>a</sub> and the pH of the solution.
- This relationship is described by the Henderson-Hasselbalch equation.

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## Acid-base Properties of Amino Acids



From equation, if pH>pK, unprotonated form (-COO<sup>-</sup>, NH<sub>2</sub>) predominates. If pH<pK, protonated form (-COOH, -NH<sub>3</sub><sup>+</sup>) predominates

If pH=pK, the protonated and unprotonated forms are of equal concentration.

The ratio of [A<sup>-</sup>] to [HA], and not the individual components determines the pH of a solution.

When concentration of the unprotonated [A<sup>-</sup>] and protonated [HA] forms of a given ionizable group are equal, then log<sub>10</sub> 1 = 0, and pH = pK<sub>a</sub>. Thus the pK<sub>a</sub> is defined as the pH at which the concentration of the ionizable group under consideration is half unprotonated and half protonated or are equal (i.e 1:1 or 50:50). That is, half the number of the amino acid species in solution have the ionizable group under consideration protonated, whereas the other half have the group in the unprotonated form.

## Acid-Base Properties of Amino Acids

- pK<sub>a</sub> is an intrinsic property of an ionizable group.
- It is the negative log<sub>10</sub> of the acid dissociation constant of the ionizable group.
- The higher the pK<sub>a</sub>, the more basic the ionizable group, that is the more readily the group accepts a proton. The lower the pK<sub>a</sub>, the more acidic the ionizable group, that is the more readily the group donates a proton.
- pH is a property of a solution. The higher the pH, the more basic the solution. The lower the pH, the more acidic the solution.
- At a pK<sub>a</sub> equal to the pH, the ionizable group is at its best buffering capacity, i.e pH at which solution resists changes in pH most effectively. The best buffering region is in the range of pH = pK<sub>a</sub>  $\pm$  1 pH unit.

## Acid-Base Properties of Amino Acids

### pK<sub>a</sub> Values of Amino Acids

Name	pK <sub>1</sub>	pK <sub>2</sub>	pK <sub>R</sub>	Name	pK <sub>1</sub>	pK <sub>2</sub>	pK <sub>R</sub>
Glycine	2.4	9.8		Asparagine	2.0	9.8	
Alanine	2.3	9.9		Glutamine	2.2	9.1	
Valine	2.3	9.6		Tryptophan	2.4	9.4	
Leucine	2.4	9.6		Cysteine	1.8	8.8	8.3
Isoleucine	2.4	9.7		Tyrosine	2.2	9.1	10.9
Methionine	2.3	9.2		Aspartate	2.0	10.0	3.9
Phenylalanine	1.8	9.1		Glutamate	2.2	9.7	4.3
Proline	2.0	10.6		Histidine	1.8	9.2	6.0
Serine	2.1	9.2		Lysine	2.2	9.2	10.8
Threonine	2.6	10.4		Arginine	1.8	9.0	12.5

NB: The amino side chain of histidine has a lower pK than  $\alpha$ -amino group

## Acid-Base Properties of Amino Acids

- pK<sub>1</sub> values are assigned to the  $\alpha$ -carboxyl group, pK<sub>2</sub> values to the  $\alpha$ -amino group, and pK<sub>R</sub> to ionizable groups in the side chain (R group).
- The  $\alpha$ -carboxyl group of amino acids is characterized by pK<sub>a</sub> values in the range 2 to 3
- The  $\alpha$ -amino group is characterized by a pK<sub>a</sub> around 10.
- At pH values between about 4 and 9, amino acids exist in a dipolar ion (zwitterion) form.

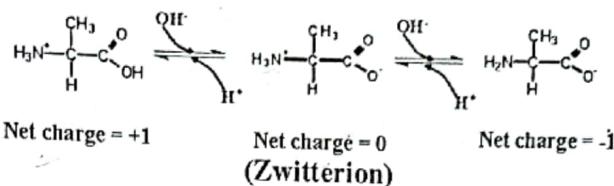
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## Titration Curve of Amino Acid

- The pH has a profound effect on the charge of an amino acid or protein.
- Addition of an acid ( $H^+$ ) makes a protein more positive as the proton suppresses the  $COO^-$  charge of the amino acid (or  $COO^-$  of the aspartic and glutamic acid side in a protein), and promotes the ionization of the nitrogen in the imidazole group of histidine and other amino groups.
- The addition of a base ( $OH^-$ ) makes a protein more negative since the base removes the positive charge on the imidazole nitrogen and other charged amino groups and promotes a negative charge on hydroxyl group of tyrosine and the sulphydryl group of

## Acid-Base Properties of Amino Acids

### Ionization States of Alanine



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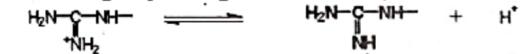
## Acid-Base Properties of Amino Acids

### Ionizable R Groups of Amino Acids

#### Sulphydryl group of cysteine



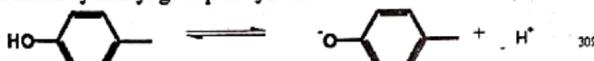
#### Guanidinium group of arginine



#### Imidazole group of histidine



#### Phenolic hydroxyl group of tyrosine



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## Self-Test Questions

- Which of the following amino acids has a net charge of +2 at  $pH < 2$ ? Which has a net charge of -2 at  $pH > 10$ ? aspartic acid, alanine, arginine, glutamic acid, leucine, lysine.
- Suggest a reason why amino acids are usually soluble at pH extremes than they are at neutral pH.

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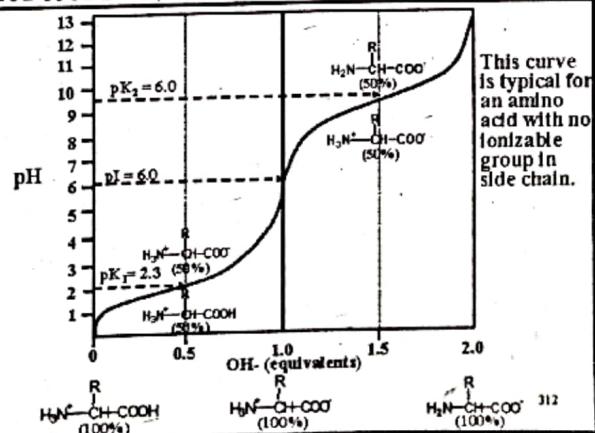
## Drawing Titration Curves

### Rules

- Identify the ionizable groups and their  $pK_a$  values for an amino acid (or peptide).
- Label the x-axis from 0 to the number of ionizable groups in the amino acid. This is the number of equivalents of  $OH^-$  ions required for titration.
- Label the y-axis from 0 to 14 (the pH axis).
- Write the  $pK_a$  values, from the lowest  $pK_a$  to highest  $pK_a$  at each 0.5 equivalents (i.e. 0.5, 1.0, 1.5 etc.), as you go from left to right on the x-axis.
- Draw a smooth curve to connect the  $pK_a$  values with inflection at the equivalent points.
- Determine the predominant net charge at the different points on the curve.

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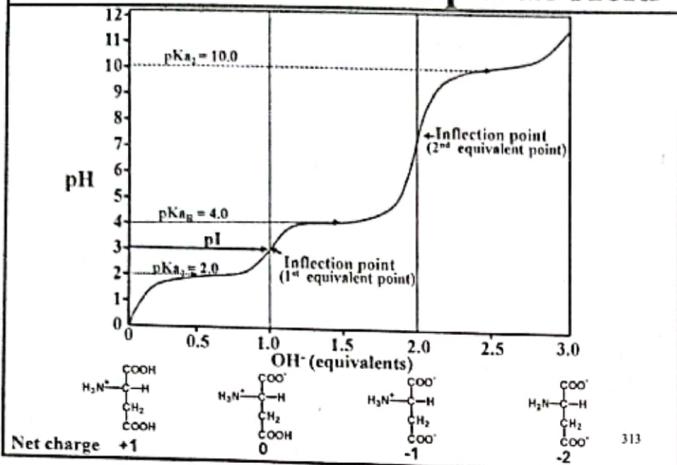
## Titration Curve of Amino Acid



This curve is typical for an amino acid with no ionizable group in side chain.

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## Titration Curve of Aspartic Acid



## Isoelectric Point

- Isoelectric point (pI), is the pH at which the net charge on an amino acid, peptide or protein is zero.
- The isoelectric point is a physical constant diagnostic of amino acids and proteins.
- A mixture of amino acids can be separated by their isoelectric points using electrophoresis (a process of measuring the migration of ions in an electric field).
- For amino acids having no additional acidic or basic groups in their side chain, the pI is nearly the midpoint of the two pK<sub>a</sub> values;

$$pI = \frac{pK_1 + pK_2}{2}$$

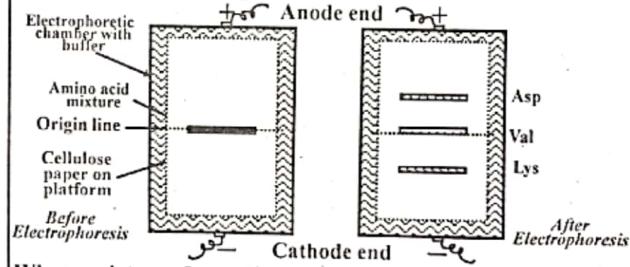
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## Isoelectric Point

- When a mixture of amino acids is applied to a piece of filter paper connected to electrodes and the voltage is turned on, the positively charged amino acids move to the negative electrode (cathode) and the negatively charged amino acids move to the positive electrode (anode). At the isoelectric point the amino acid no longer migrates since the overall (net) charge is zero.
- Proteins and amino acids are least soluble at their isoelectric points as their dipolar nature results in interaction among protein (amino acid) molecules which leads to their aggregation and precipitation out of solution.

## Isoelectric Point

### Electrophoresis of Amino Acids



When a mixture of aspartic acid ( $pI = 2.8$ ), valine ( $pI = 6.0$ ), and lysine ( $pI = 9.7$ ) in a buffer of  $pH 6$  is applied to filter paper placed in an electric field, the aspartic acid would be negatively charged since the  $pH > pI$ , and would move to the positive electrode (anode). The valine would be neutral ( $pH = pI$ ) and would stay at the origin. The lysine would be positively charged ( $pH < pI$ ) and would move toward the negative electrode (cathode).

## Isoelectric Point

### Rules for determining pI from a titration curve.

- Draw the titration curve for the amino acid or peptide.
- Identify the point on the curve where the net charge on the amino acid or peptide is zero.
- Identify the two pK<sub>a</sub> points just below and above the zero net charge point.
- The isoelectric point lies midway between the two pK<sub>a</sub> values that indicate the protonation and deprotonation of the isoionic (electrically neutral) form.
- The average of the two pK<sub>a</sub> values is the pI:

$$pI = \frac{pK_1 + pK_2}{2}$$

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## Isoelectric Point

Protein	Isoelectric (isoionic) point (pI)
Pepsin	1.1
Casein (milk protein)	4.6
Egg albumin	4.7
Serum albumin	4.9
Urease	5.0
Hemoglobin	6.8
Myoglobin	7.0
Cytochrome c	10.7
Lysozyme	11.0

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## Isoelectric Point

- The side chains of amino acids such as aspartic acid and glutamic acid have appreciable acidic properties.
- Those of amino acids such as lysine, histidine, arginine, cysteine, and tyrosine have appreciable basic properties.
- The isoelectric structures of the amino acids vary according to the basicity or acidity of the side-chain group.
- Peptides or amino acids with pI values 2 or more units below 7 are called acidic. Those with pI values 2 or more units above 7 are called basic. Those with pI  $\pm$  1 of 7 are neutral.

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## Self-Test Questions

3. A mixture of proteins with the isoelectric points in bracket, is applied to a gel in a buffer of pH 6.8 for electrophoresis. Albumin (4.9), hemoglobin (6.8), and lysozyme (11.0).
- Which protein will migrate towards the positive electrode?
  - Which protein will migrate towards the negative electrode?
  - Which protein will remain at the same place it was originally applied?

321

## Self-Test Questions

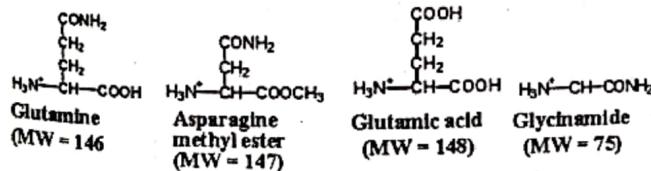
1. Match each amino acid with its isoelectric point. Choose among the following values: 10.76, 6.30, 5.07, 2.77
- |                     |
|---------------------|
| Cysteine _____      |
| Aspartic acid _____ |
| Proline _____       |
| Arginine _____      |

2. Predict the approximate isoelectric point of each of the following amino acids:
- |                     |
|---------------------|
| serine _____        |
| histidine _____     |
| glutamic acid _____ |
| glutamine _____     |
| lysine _____        |

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## Self-Test Questions

6. What will the paper electrophoretic separation pattern look like when a mixture of the following compounds is separated in a buffer of pH 6.0.



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## Self-Test Questions

4. The isoelectric points of pepsin and chymotrypsin are 1.1 and 9.6 respectively. What do these values indicate about the amino acid composition of pepsin and chymotrypsin?
5. If a mixture of the following three pentapeptides is electrophoresed at pH 7.5, what would be their pattern of migration?
- Thr-Phe-His-Asp-Met.
  - Ile-Glu-Asp-Cys-Asp.
  - Glu-Tyr-Asp-Glu-Lys.

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## Self-Test Question

- 7.
- 
- Titration Curve for Histidine
- a. At what point is the amino acid electrically neutral?  
b. What is the structure of the amino acid at point V?  
c. What is the net charge on the amino acid at point I?  
d. At what point is the net charge on the amino acid +1?  
e. Calculate the isoelectric pH (pI) of histidine.  
f. What is the net charge on the amino acid at point VII?

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## Self-Test Questions

8. Calculate the pH at which the  $\epsilon$ -amino group of lysine is 20% dissociated.
9. Calculate the pH at which the  $\gamma$ -carboxyl group of glutamic acid is two-thirds dissociated.

325

## Amino Acid-Derived Bioactive Compounds

### Decarboxylation

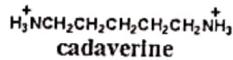
- Most amino acids transfer amino groups via transamination or are oxidatively deaminated.
- Some are decarboxylated, mostly after death in higher organisms.
- Many important neurotransmitters are produced through decarboxylation reactions of certain amino acids.

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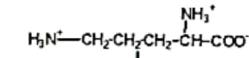
## Amino Acid-Derived Bioactive Compounds

### Decarboxylation

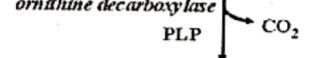
- Polyamines (biogenic amines) such as spermidine and spermine, which are used in DNA packaging, are derived from the decarboxylation of ornithine.
- The biogenic amines, cadaverine and putrescine originate from the decarboxylation of lysine and ornithine respectively.
- Arg, Lys, His, and Tyr are also decarboxylated by bacterial decarboxylases in the intestines.
- Amines with low molecular weight have sharp penetrating odors similar to that of ammonia. Amines with high molecular weight smell like decaying fish. Putrescine and cadaverine are responsible for the odor of decaying animal tissue.



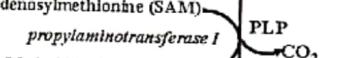
327



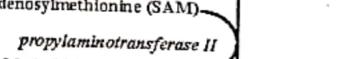
Ornithine



Putrescine



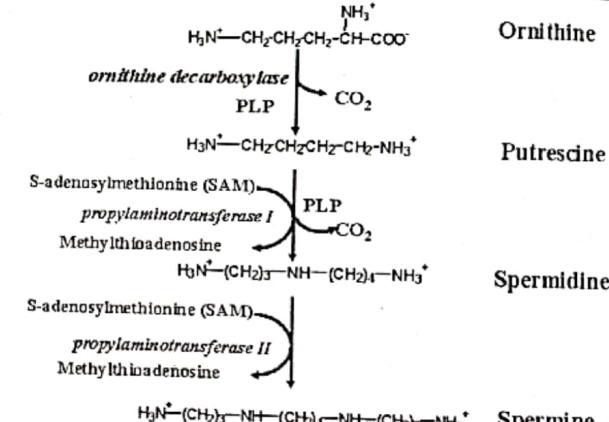
Spermidine



Spermine

328

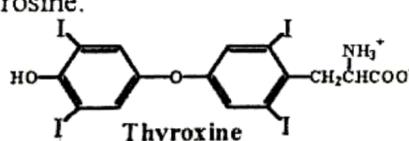
## Amino Acid-Derived Bioactive Compounds



## Amino Acid-Derived Bioactive Compounds

### Bioactive Compounds Derived from Tyrosine

- Dopamine, norepinephrine and epinephrine are neurotransmitters produced from tyrosine.
- High levels of dopamine have been implicated in **schizophrenia** (a mental disorder) whereas low levels have been implicated in **Parkinson's disease** (a nervous disorder that causes the limbs to shake).
- Tyrosine is also the precursor for the synthesis of the skin pigment melanin.
- Thyroxine, a thyroid hormone, is synthesized from two molecules of tyrosine.

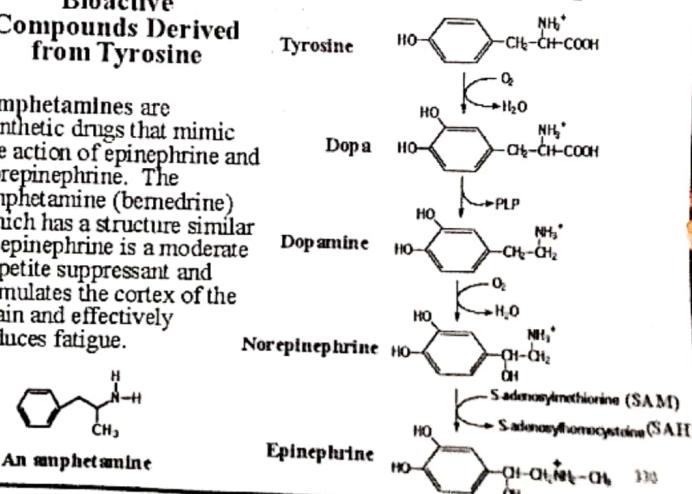


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## Amino Acid-Derived Bioactive Compounds

### Bioactive Compounds Derived from Tyrosine

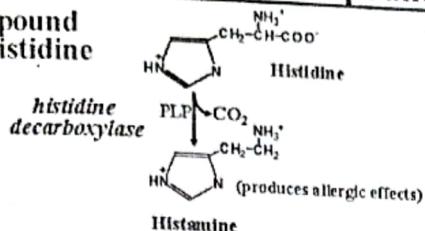
Amphetamines are synthetic drugs that mimic the action of epinephrine and norepinephrine. The amphetamine (bernedrine) which has a structure similar to epinephrine is a moderate appetite suppressant and stimulates the cortex of the brain and effectively reduces fatigue.



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## Amino Acid-Derived Bioactive Compounds

### Bioactive Compound Derived from Histidine

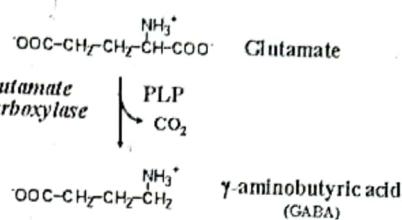


Histamine, a decarboxylated product of histidine, is a powerful vasodilator. It stimulates acid secretion in the stomach. Histamine is released as part of the allergic response. Anti-histamines are drugs which occupy histamine receptor sites in nerves and prevent the allergic effects of histamines.

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## Amino Acid-Derived Bioactive Compounds

### Bioactive Compound Derived from Glutamate

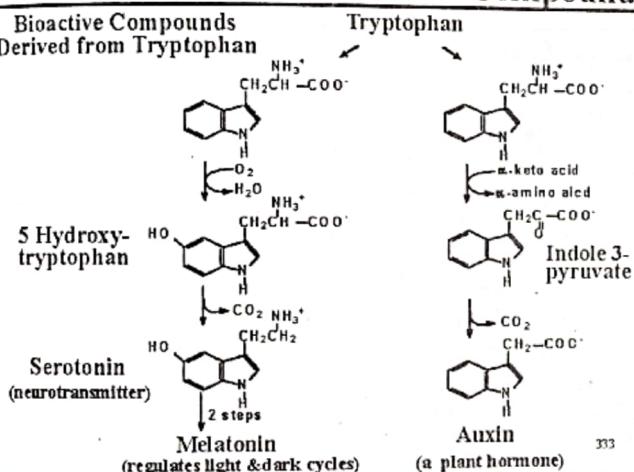


GABA is a neurotransmitter. Low levels have been associated with epileptic seizures.

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## Amino Acid-Derived Bioactive Compounds

### Bioactive Compounds Derived from Tryptophan



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## Amino Acid-Derived Bioactive Compounds

Compound	Function	Precursor
GABA	neurotransmitter	Glu
Auxin	plant growth hormone	Trp
Catecholamines	neurotransmitter	Tyr, Phe
Glutathione	reducing agent	Gly, Glu, Cys
Histamine	allergic response	His
Melanin	skin pigments	Tyr, Phe
Nitric oxide	cell messenger	Arg
Phosphocreatine	muscle energy	Gly, Arg, Met
Porphyrin	heme and chlorophyll	Gly
Purine bases	RNA, DNA, cofactors	Asp, Gly, Gln
Pyrimidine bases	RNA, DNA, cofactors	Asp
Serotonin	neurotransmitter	Trp
Spermine, spermidine	DNA packaging	Met, Orn
Thyroxine	hormone	Tyr
Alkaloids	bases in plants	Orn, Asp, Lys, Tyr Trp, Phe, His <sup>334</sup>

Source: Adapted from Boyer, R. (1999) Concepts in Biochemistry.

## Amino Acid-Derived Compounds

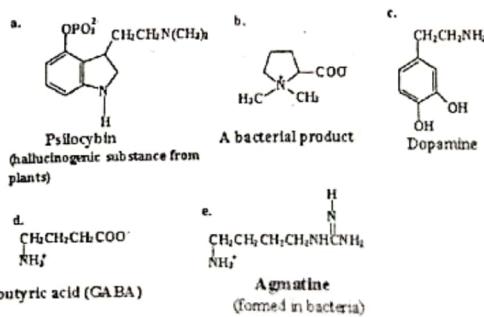
### Monosodium Glutamate

- The monosodium salt of glutamic acid (monosodium glutamate, MSG) is used as a food flavor (taste) enhancer.
- It is added to many canned and frozen foods.
- It is sold under many brand names.

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## Self-Test Question

1. The natural products drawn below are synthesized from amino acids. For each product, name the amino acid precursor.



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## Peptides

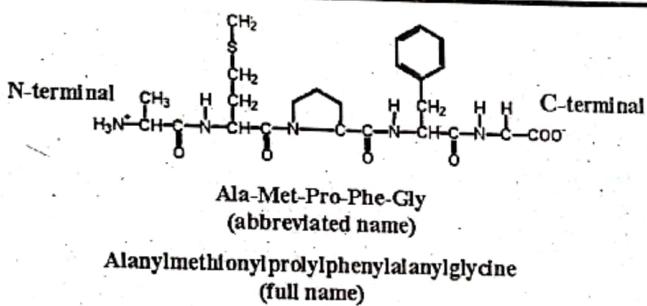
- The amide linkages between amino acids are called peptide bonds.
  - A peptide is a molecule with one or more peptide bonds.
  - A dipeptide has 2 amino acid residues, a tripeptide has 3, and a tetrapeptide has 4 etc.
  - Oligopeptides have quite a few amino acid residues, polypeptides have many.
  - Peptides are named from amino- or N-terminus to carboxyl- or C-terminus.

## Peptides

## **Stems for Naming Peptides**

- Glycine (**glycyl**)
  - Alanine (**alanyl**)
  - Valine (**valyl**)
  - Leucine (**leucyl**)
  - Isoleucine (**isoleucyl**)
  - Serine (**seryl**)
  - Threonine (**threonyl**)
  - Cysteine (**cysteinyl**)
  - Methionine (**methionyl**)
  - Proline (**prolyl**)
  - Aspartic acid (**aspartyl**)
  - Glutamic acid (**glutamyl**)
  - Asparagine (**asparaginyl**)
  - Glutamine (**glutaminyl**)
  - Phenylalanine (**phenylalanyl**)
  - Tyrosine (**tyrosyl**)
  - Tryptophan (**tryptophyl**)
  - Arginine (**arginyl**)
  - Histidine (**histidyl**)
  - Lysine (**lysyl**)

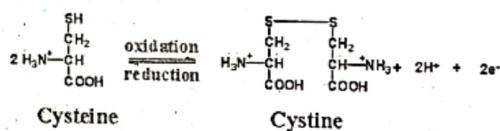
Peptides



A peptide is named from the N- to the C-terminal

Peptides

## Oxidation-Reduction of Cysteine



In proteins, the side chain sulphydryl groups of two cysteine residues can be oxidized to form a disulfide bridge that connects (cross-links) widely spaced parts of the polypeptide. There can be either oxidized or reduced cysteines in proteins. They are generally all reduced or all oxidized. The dimer of cysteine is called cystine.

Peptides

## pK<sub>a</sub> Values of Amino Acids in Peptides

- The  $pK_a$  of the terminal carboxyl of a peptide increases whereas  $pK_a$  of the terminal amino group decreases with each addition of an amino acid.
  - Electrostatic interactions between  $\text{COO}^-$  and  $\text{NH}_3^+$  of the zwitterion affects the ionization of the carboxyl group. This interaction decreases as the length of peptide increases, hence the increase in  $pK_a$  of the carboxyl group (i.e. its ability to donate the carboxyl proton decreases).
  - Ionization of the protonated amino group reduces the electrostatic interaction. As the distance between the charged groups increases, removal of the proton from the amino group is easier, hence the decrease in its  $pK_a$  (i.e. its ability to donate the  $\text{NH}_3^+$  proton increases).<sup>341</sup>

Peptides

## **pKa Values of Amino Acids in Peptides**

Amino acid or peptide	pKa <sub>1</sub>	pKa <sub>2</sub>
Ala	2.34	9.69
Ala-Ala	3.12	8.30
Ala-Ala-Ala	3.39	8.03

*Source: Adapted from Nelson, D. L. and Cox, M. M. (2000) Lehninger, Principles of Biochemistry.*

## pK<sub>a</sub> Values of Groups Found in Proteins

Amino Side Chain	pK <sub>a</sub> (25°C)
α-Carboxyl (terminal)	3.0-3.2
β-Carboxyl (aspartic)	3.0-4.7
γ-Carboxyl (glutamic)	4.4
Imidazolium (histidine)	5.6-7.0
α-Amino (terminal)	7.6-8.4
Sulfhydryl (cysteine)	8-9
ε-Amino (lysine)	9.4-10.6
Phenolic hydroxyl (tyrosine)	9.8-10.4
Guanidinium (arginine)	11.6-12.6

Source: Taken from Edsall, J. T. In *Proteins, Amino Acids and Peptides*, Reinhold, New York

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## Determining Net Charge on a Peptide at a Given pH

- Draw the given peptide structure.
- Identify the side chain and terminal ionizable groups.
- Write the pK<sub>a</sub> values for each of these groups. If the exact value is not given, use the approximate values.
- Note that the pK<sub>a</sub> value of a group in a peptide may differ from the pK<sub>a</sub> of the same group in a free amino acid.
- Determine the state of protonation, and hence charge, on each group at the given pH.
- Determine the net charge on the peptide, by summing up the charges.

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## Self-Test Questions

- What is the value of the ratio [aspartate]/[aspartic acid] in a solution of aspartic acid at a pH of 5.0?
- Draw the molecular structure for the tripeptide Ala-Pro-Asp in its completely protonated form.
- Consider the following peptides: Phe-Glu-Ser-Met and Val-Trp-Cys-Leu. Do these peptides have different net charges at pH 1? At pH 7? Indicate the net charges at both pH values.
- Write the structures of the following peptides.
  - glycylglycine
  - alanylleucylmethionine

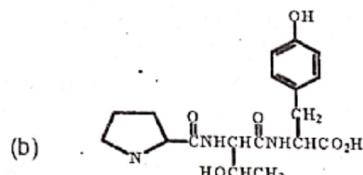
## Self-Test Questions

- A solution contains a mixture of three tripeptides; Tyr-Arg-Ser, Glu-Met-Phe, and Asp-Pro-Lys. Assume that the α-COOH groups have a pK<sub>a</sub> of 2.0 and the α-NH<sub>2</sub> groups have a pK<sub>a</sub> of 10. At which pH value (2.0, 6.0, or 13.0) would electrophoresis provide the best resolution of the three tripeptides in the mixture?

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## Self-Test Questions

- Write the full and abbreviated name for each of the following peptides.



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## Self-Test Questions

- Write the structure of the principal ionic species of the following peptides in the indicated solution. What is the net charge on each peptide?
  - glycyllysine in dilute aqueous HCl
  - glycylglutamic acid in dilute aqueous NaOH
  - glycyltyrosine in dilute aqueous NaOH

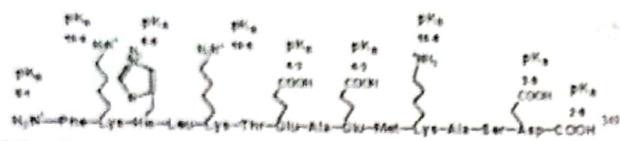
348

## Self-Test Questions

8. Partial hydrolysis of bradykinin results in the following tripeptides:
- pro-pro-gly
  - arg-pro-pro
  - phe-ser-pro
  - pro-phe-arg
  - ser-pro-phe
  - pro-gly-phe
  - gly-phe-ser

What is the amino acid sequence in bradykinin?

9. Calculate the net charge on the peptide below at pH = 6.0.



## Biologically Active Peptides

Peptide hormone	Amino acid residues	Function
Angiotensin II	8	Increases blood pressure
Bradykinin	9	Produces pain
Calcitonin	32	Reduces blood Ca levels
Leucine enkephalin (leu-enkephalin)	5	Moderates pain
Glucagon (liver)	29	Promotes glucose release from glycogen
Oxytocin (pituitary)	9	Stimulates milk production and affects uterine contraction
Insulin (pancreas)	51	Regulates blood glucose level

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## Biologically Active Peptides

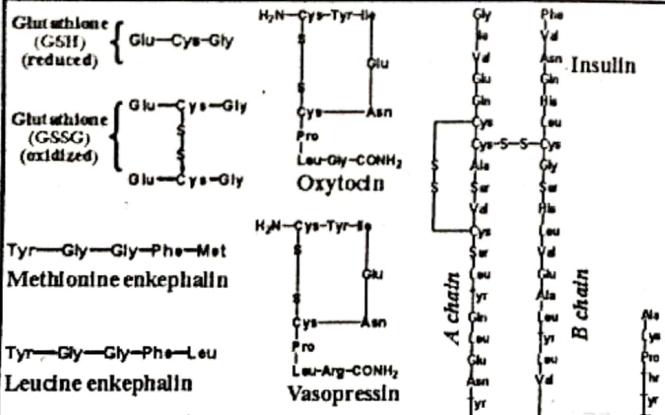
- Enkephalins:** Are natural pain killer peptides produced by the brain. They bind to specific receptor sites on the surface of brain cells to reduce pain. They are thought to be responsible for the temporary loss of pain during severe injury and the analgesic effects of acupuncture. They have short-lived effects. The receptor sites that bind enkephalins also have high affinity for opiates such as morphine, heroin etc., and are also referred to as opiate receptors.
- Angiotensin II:** Has a composition of Asp-Arg-Val-Tyr-Ile-His-Phe. It increases blood pressure by constricting blood vessels.
- Bradykinin:** A pain-causing peptide released into the blood plasma in response to toxins in wasp stings or tissue damage.

## Biologically Active Peptides

- Glutathione:** It is involved in oxidation-reduction reactions.
- Vasopressin:** Regulates water balance and blood pressure by adjusting the amount of water reabsorbed by the kidneys (it is an antidiuretic hormone).
- Endorphins:** Are natural pain killer peptides. Four groups have been identified ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ). Amino acid composition of endorphins range from 16-31 depending on the group.

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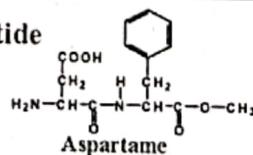
## Biologically Active Peptides



Source: Adapted from Boyer, R. (1999) *Concise Biochemistry*.

## Aspartame

A Sweet Peptide



Aspartame, a synthetic methyl ester derivative of the dipeptide L-Aspartyl-L-phenylalanine, is sweet and of important commercial value. It is about 200 times as sweet as sucrose (table sugar). It is used in the soft drink industry as a sugar substitute. Its use is controversial as there has been concerns about its safety. Because of its phenylalanine content, phenylketonurics are advised to stay away from it. It is marketed under the trade name "NutraSweet".

## Protein Structure

### Importance of amino acid side chains

- Amino acid side chains are not involved in forming the peptide bonds of a protein.
- The nature of the side chains helps to determine the overall structure and reactivity of the proteins in which they are found.
- For example, water-soluble proteins contain many amino acids with polar side chains, while an insoluble muscle protein contains a greater proportion of amino acids with nonpolar side chains.

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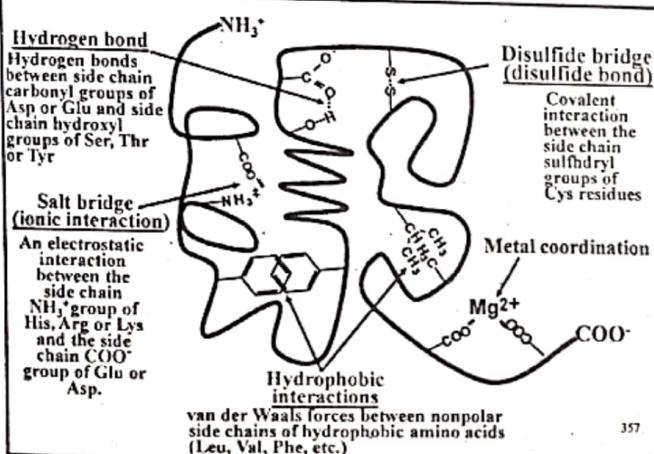
## Protein Structure

### Importance of amino acid side chains

- The side chain groups may form H-bonds, electrostatic and hydrophobic interactions and disulfide bonds.
- Some side chains may undergo covalent modification (e.g. phosphorylation, methylation, adenylation) which alter the physical and biological characteristics of proteins.
- Side chains may also function as proton donors or acceptors in a reaction mechanism (when the protein is an enzyme) or influence the conformational structural element and thereby alter the nature of its contribution to the structure of the molecule.

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## Protein Structure



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## Protein Structure

### Levels of protein structure

- The structures of proteins determine their functions.
- Four different levels of protein structure have been defined. These are:
  - primary structure:** the sequence of amino acid residues in the polypeptide chain held together by peptide bonds.
  - secondary structure:** the folding of the polypeptide chain into a coil ( $\alpha$ -helix) or a sheet ( $\beta$ -sheet) stabilized by hydrogen bonding.
  - tertiary structure:** the intrachain overall folding of a protein into a three-dimensional compact structure stabilized by interactions between the side chains of amino acids within the protein.

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## Protein Structure

- quaternary structure:** the interaction between two or more subunits (polypeptide chains) in a protein to form a larger biologically active protein.
  - Multi-subunit proteins are important in the regulation of metabolic function of proteins.
  - Such proteins are functional only if all subunits are bound to one another.
- All proteins have primary, secondary and tertiary structures, but only some (multi-subunit proteins) have quaternary structure.

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## Protein Structure

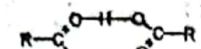
### Stabilizing Bonds and Forces in Proteins

Level of Structure	Bond or Interaction
Primary	Peptide bond (covalent)
Secondary	Hydrogen bond (noncovalent)
Tertiary	Hydrogen bond Ionic interaction (noncovalent) Hydrophobic interaction (noncovalent) Disulfide bond (covalent)
Quaternary	Hydrogen bond Hydrophobic interaction Ionic interaction

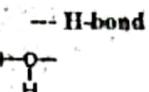
360

## Protein Structure

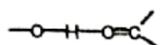
### Hydrogen bonding possibilities



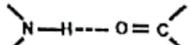
Hydrogen-bonded dimer of carboxylic acids



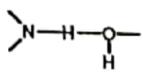
Hydroxyl-hydroxyl H-Bonds



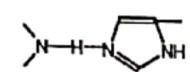
Hydroxyl-carbonyl H-Bonds



Amide-carbonyl H-Bonds



Amide-hydroxyl H-Bonds

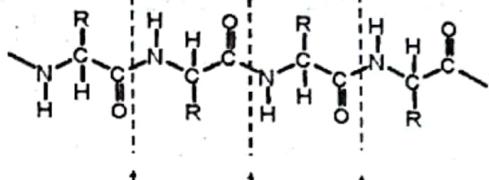


Amide-imidazole H-Bonds

H—O bonds are stronger than H—N bonds because nitrogen is less electronegative than oxygen

## Protein Structure

### Primary Structure

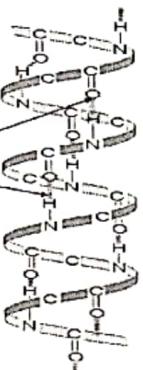


Peptide bonds joining amino acid residues

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## Protein Structure

### Secondary Structure ( $\alpha$ -helix)



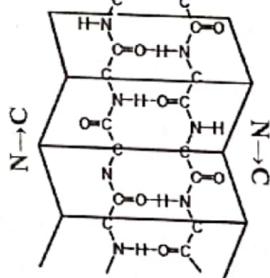
Polypeptide backbone in ribbon form

Intramolecular hydrogen bonding shown between N-H and C=O groups of backbone

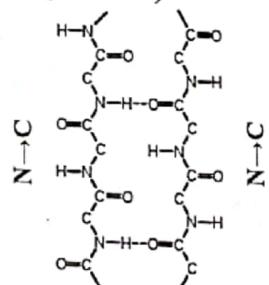
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## Protein Structure

### Secondary Structure ( $\beta$ -Sheet)



Anti-parallel  $\beta$ -pleated sheet  
(peptide chains run in the opposite direction)

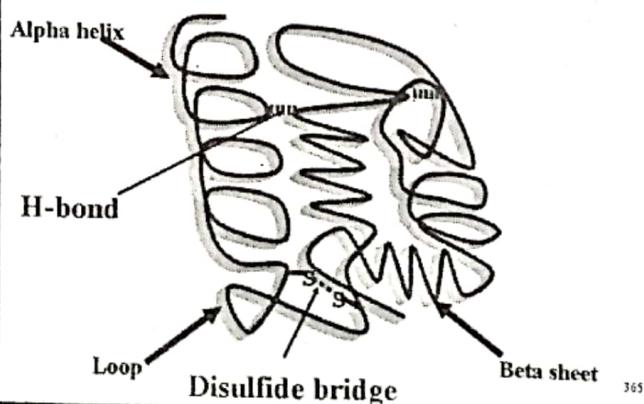


Parallel  $\beta$ -pleated sheet  
(peptide chains run in the same direction)

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## Protein Structure

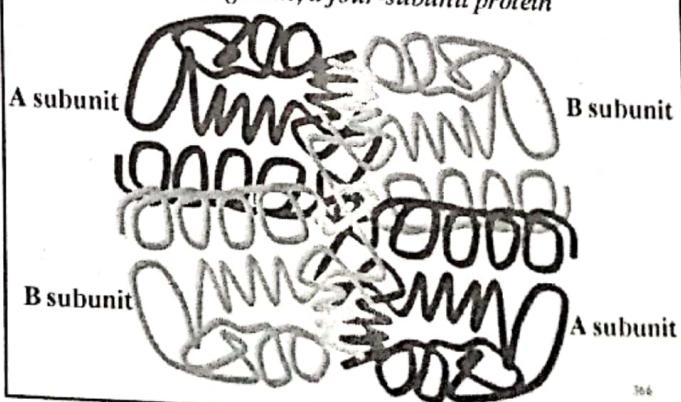
### Tertiary Structure



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## Protein Structure

### Quaternary Structure Hemoglobin, a four-subunit protein



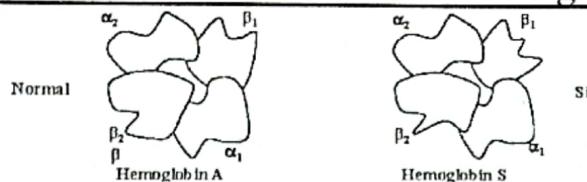
366

## Protein Structure

- Hemoglobin is a protein with a quaternary structure.
- It is a tetramer with  $2\alpha$  and  $2\beta$  subunits ( $\alpha_2 \beta_2$ ).
- Each subunit has a heme group with  $\text{Fe}^{2+}$  which binds  $\text{O}_2$ .
- There are two stable forms of the protein, a "relax" form (R) which binds  $\text{O}_2$  and a "taut" form (T) which cannot bind  $\text{O}_2$  but binds  $\text{HCO}_3^-$ .

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## Normal and Sickle-cell Hemoglobin



*Source: Redrawn from Lehninger, Principles of Biochemistry, Nelson and Cox, 2000*  
Conformational differences between hemoglobin A and hemoglobin S arise from a single base-pair substitution in the hemoglobin gene which results in a single amino acid change from a polar charged glutamic acid to valine, a hydrophobic amino acid. This change introduces a hydrophobic area on the surface of the hemoglobin molecule which leads to the formation of insoluble hemoglobin strands that result in the sickle shape of the red blood cell.

## Proteins Structure

Protein	Mol. Weight	Amino acid residues	Number of subunits
Insulin	5703	51	2
Lysozyme	14,300	130	1
Myoglobin	16890	153	1
Hemoglobin	65,500	574	4
Ferritin	450,000	4100	24
Glutamate dehydrogenase	1,000,000	8300	40

Average molecular weight of an amino acid = 100

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## Protein Denaturation

- Proteins in their natural state are called native proteins.
- The overall shape of a protein is maintained by many weak interactions.
- These interactions can be broken by physical (e.g. heat) and chemical (e.g. changes in pH) forces etc.
- Denaturation is the destruction of the native conformation of a protein and results in the loss of many biological properties of the protein.
- Denaturation refers to changes in the secondary, tertiary and quaternary structures of proteins but not the primary structure.

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## Protein Denaturation

### Mechanisms of protein denaturation.

- Heat:** alters the interactions involved in stabilizing secondary and tertiary structures due to the molecular vibrations it produces within the protein molecule. Results in the cleavage of noncovalent interactions (e.g. H-bonds) which lead to the unfolding of the protein making it non functional.
  - Results in coagulation due to intermolecular protein-protein interaction e.g. cooking an egg.
- Radiation** (laser beam, ionizing radiation etc.) : alters secondary and tertiary structures
- Change in pH:** disrupt salt bridges (and other ionic attractions e.g. metal coordination). e.g. curdling of milk.

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## Protein Denaturation

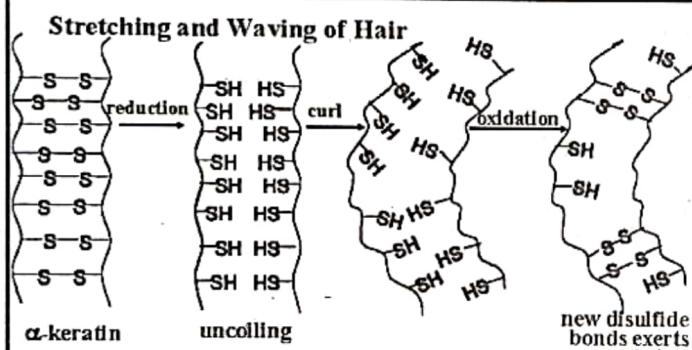
- Reduction** of disulfide linkages: Breaks S-S bonds to form SH groups or **oxidation** of sulfhydryl groups to give S-S linkages) e.g. stretching of hair.
- Chaotropic agents:** (ethanol, urea, guanidine)
  - Form competing hydrogen bonds with amino acid side chain residues.
  - Denaturation is partially or completely reversible when agent is removed.
  - At 70% concentration ethanol penetrates bacteria cells and kills them by disrupting the H-bonds of the native protein. 95% alcohol denatures mainly surface proteins.
- Agitation:** Stretches polypeptide chain until stabilizing interactions are disrupted. e.g. whipping of cream and beating of egg.

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## Protein Denaturation

- Heavy-metal salts of  $\text{Ag}^+$ ,  $\text{Hg}^+$ ,  $\text{Pd}^{2+}$ ,  $\text{Cd}^{2+}$ , etc. interact with sulphydryl groups and carboxylate ions and result in the precipitation of proteins as insoluble salts of heavy metals. Advantage is taken of this in using raw egg white or milk as an antidote for heavy metal poisoning. The egg and milk proteins are acidic and their deprotonated side chain carboxyl groups interact with and are denatured by the metal ions to form metal ion-protein complexes which precipitate in the stomach. They can then be removed by inducing vomiting, otherwise the protein will be digested and metal ion released and absorbed into the blood system.
- Detergents: (e.g. sodium dodecyl sulfate, SDS) bind strongly to proteins through hydrophobic interactions.

## Protein Denaturation



$\alpha$ -Keratin (an  $\alpha$ -helix cross-linked by disulfide bridges)

Source: Adapted from Nelson, D. L. and Cox, M. M. (2000), Lehninger Principles of Biochemistry.

## Protein Denaturation

### Stretching and waving of hair

- the breaking and making of disulfide linkages in the hair protein, keratin, and the ability of the helical protein to stretch, form the basis of "permanent" waving of hair. The biochemical processes involved in the waving of hair include:
  - The application of a solution of reducing agent to the hair to reduce and cleave the disulfide bonds leading to the uncoiling of the helical structure of the hair protein.
  - The reducing solution is removed by washing the hair. This is followed by the application of an oxidizing agent which results in the creation of new disulfide bonds. The new disulfide bonds formed are different from the original ones and this provides the desired curl.

## Protein Hydrolysis

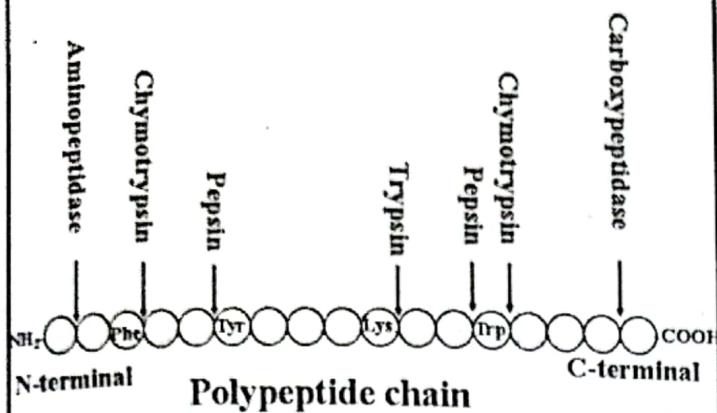
- Complete hydrolysis of proteins (breaking of peptide bonds) occurs when the protein is treated with 6 M HCl at 100°C for 24 hours under laboratory conditions.
- The concentration of acid in the stomach is only 0.1 M HCl.
- Enzymes (proteases) are required to be able to hydrolyze peptide bonds in proteins. The enzymes include:
  - Exopeptidases such as,
    - Carboxypeptidases: hydrolyze peptide bonds from the C-terminus of the protein
    - Aminopeptidases: hydrolyze peptide bonds from the N-terminus.

## Protein Hydrolysis

### Endopeptidases such as;

- Pepsin : hydrolyzes peptide bonds on the amino side of the aromatic amino acids ( $X^1\text{Tyr}$ ,  $X^1\text{Phe}$ ,  $X^1\text{Trp}$ )
- Trypsin: hydrolyzes peptide bonds on the carbonyl side of basic amino acids ( $\text{Lys}^1X$ ,  $\text{Arg}^1X$ )
- Chymotrypsin: hydrolyzes peptide bonds on the carbonyl side of aromatic amino acids. ( $\text{Tyr}^1X$ ,  $\text{Phe}^1X$ ,  $\text{Trp}^1X$ )
- Elastase: cleaves peptide bonds on the carbonyl side of glycine and alanine ( $\text{Gly}^1X$ ,  $\text{Ala}^1X$ )

## Protein Hydrolysis



## Protein Hydrolysis

Trypsin Action	Chymotrypsin Action
H <sub>2</sub> N—Val—Lys—Ser—Arg—Asn—COOH	H <sub>2</sub> N—Leu—Tyr—Met—Phe—Gln—Trp—Thr—COOH
<i>Trypsin digestion</i>	<i>Chymotrypsin digestion</i>
H <sub>2</sub> N—Val—Lys—COOH	H <sub>2</sub> N—Leu—Tyr—COOH
H <sub>2</sub> N—Ser—Arg—COOH	H <sub>2</sub> N—Met—Phe—COOH
H <sub>2</sub> N—Asn—COOH	H <sub>2</sub> N—Gln—Trp—COOH
	H <sub>2</sub> N—Thr—COOH

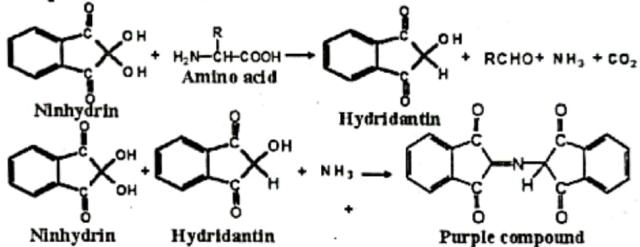
380

## Detection and Estimation of Amino Acids

- Methods for amino acid detection and estimation include those that require fluorescent or other UV-absorbing tags.
  - The colorimetric methods using either ninhydrin or Sanger's reagent can detect microgram quantities of amino acids. Ninhydrin is a versatile reagent and is used to detect proteins on paper, thin layer chromatography as well as in effluents from liquid chromatographic columns. Ninhydrin reacts with free amino groups and is directed towards the N-terminal residue of a protein.
  - The fluorescent methods that use reagents such as dansyl chloride and fluorescamine are able to detect nanogram quantities.
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## Detection and Estimation of Amino Acids

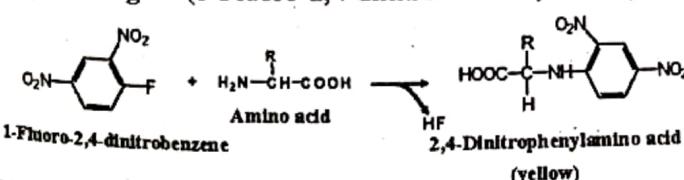
### Ninhydrin reaction



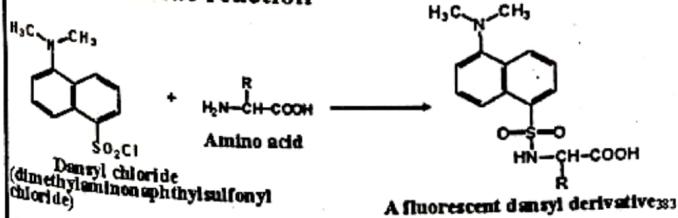
Ninhydrin is a powerful oxidizing agent that causes oxidative deamination of the  $\alpha$ -amino group of amino acids to release ammonia, carbon dioxide, and the corresponding aldehyde. The ninhydrin is reduced to hydridantin. The ammonia reacts with another molecule of ninhydrin and hydridantin to give a purple compound that absorbs at 570 nm. Because the color is proportional to the amino groups present, the ninhydrin reaction provides a convenient quantitative colorimetric assay for amines. The reaction can also be followed by measuring manometrically the evolution of  $\text{CO}_2$ . For imino acids like proline, a product is formed that gives a yellow color<sup>382</sup> which absorbs at 440 nm.

## Detection and Estimation of Amino Acids

### Sanger's reagent (1-Fluoro-2,4-dinitrobenzene, FDNB)



### Dansyl chloride reaction



## Protein Sequencing

Protein sequencing involves the following steps:

### 1. Unfolding of protein

- By denaturation using heat or other denaturing agents (e.g. urea, guanidine HCl etc.).

### 2. Breaking of disulfide bridges

- By reduction with mercaptoethanol, to produce SH groups, and the protection of SH groups from oxidation by reacting the polypeptide with iodoacetic acid, an alkylating agent which reacts with and stabilizes the SH groups.
- A disulfide bond may not be broken by hydrolysis, but can be oxidized to break by reagents such as performic acid.<sup>384</sup>

## Protein Sequencing

### 3. Determination of polypeptide subunits

This can be done by determining the number of N- and C-terminal residues present in the linearized protein. It is accomplished by reacting the protein with dansyl chloride, a fluorescent reagent, which forms a covalent linkage with the terminal amino group of the protein. The dansyl polypeptide complex is hydrolyzed under acidic conditions and the amino acids separated by high performance (pressure) liquid chromatography (HPLC). The number of fluorescent peaks, which is an indication of the number of dansyl-amino acid complexes, is suggestive of the number of free amino groups originally present in the polypeptide and hence the number of polypeptide subunits.

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## Protein Sequencing

### 5. Sequence determination by sequential Edman degradation

- Although methods previously described that use 1-Fluoro-2,4-dinitrobenzene (FDNB) and dansyl chloride for the determination of the N-terminal residue are powerful detection methods, they cannot be used repetitively on the same peptide as the peptide is completely degraded in the HCl-hydrolysis step.
- Edman devised a selective degradation method for labeling the amino terminal residues and sequentially removing them one residue at a time without disrupting other peptide bonds in the polypeptide.

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## Protein Sequencing

- The first step in the three-step method involves the formation of a phenylthiocarbamyl-peptide (PTC-peptide) by reacting the N-terminal amino group with Edman's reagent (phenylisothiocyanate, PITC). The PTC tag provides a UV absorbing group for easy detection following chromatographic separation.
- The second step involves the acid (trifluoroacetic acid) hydrolysis of the PTC-peptide which cleaves only the N-terminal peptide bond resulting in the cyclization of the N-terminal amino acid residue into a thiazolinone derivative. The rest of the polypeptide remains intact.

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## Protein Sequencing

### 4. Trypsin digestion (selective fragmentation)

Polypeptides with longer than 50 amino acids cannot be directly sequenced and have to be cleaved into smaller fragments by using chemical reagents or enzymes (proteases such as trypsin and chymotrypsin) that specifically cleave peptide bonds at known locations. Trypsin hydrolyzes peptide bonds after Lys and Arg provided the preceding residue is not a proline. Cyanogen bromide (CNBr) treatment specifically breaks peptide bonds after methionine residues and converts methionine into homoserine lactone.

The smaller fragments are separated by HPLC.

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## Protein Sequencing

- Amino acid sequence of polypeptides with less than 50 amino acid residues can be determined by Edman degradation.
- About a tenth of a microgram quantity of protein can be sequenced by Edman degradation. The method is a nondestructive and subtractive method of protein sequencing.
- Edman degradation involves the stepwise cleavage of a polypeptide from the N-terminal to form a 3 phenyl-2-thiohydantoin (PTH) derivative of the N-terminal amino acid after each cycle of degradation.

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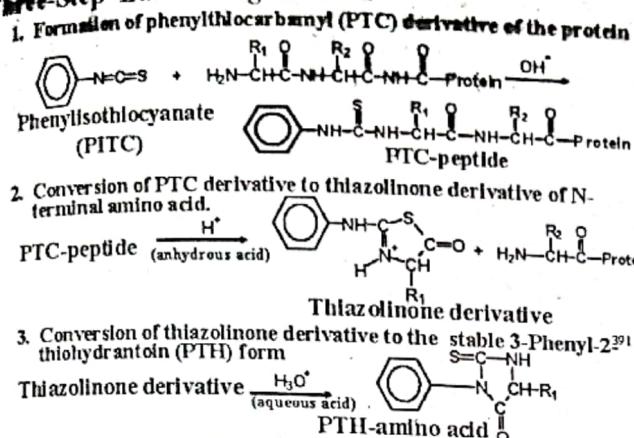
## Protein Sequencing

- The last step involves the conversion of the thiazolinone derivative to the more stable 3-phenyl-2-thiohydantoin (PTH) form of the amino acid.
- The remaining polypeptide is subjected to further cycles of Edman degradation.
- The combination of Edman degradation and liquid chromatography allows the identification of amino acids for protein sequencing.
- An automatic amino acid analyzer (sequenator) is now available that can be programmed to monitor the addition of reagents, the separation of products and identification of PTH derivatives of amino acids.

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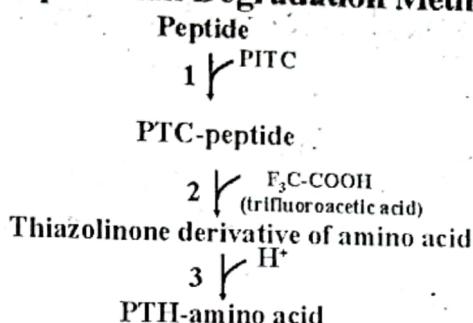
## Protein Sequencing

### Three-Step Edman Degradation



## Protein Sequencing

### Three-Step Edman Degradation Method



PTH-amino acids and PTC-peptides can be separated, quantitated and purified using liquid chromatography and UV detection.

## Protein Sequencing

### Sequence Reconstruction

- The amino acid sequence of a polypeptide is reconstructed after degradation by:
  - identifying the C-terminal fragments of enzyme digests. Trypsin digestion generates fragments with C-terminal lysine or arginine.
  - identifying cyanogen bromide cleaved fragments. Cyanogen bromide cleaves peptide bonds after methionine residues.
- Positions in a polypeptide with disulfide bridges can be identified by initially fragmenting the polypeptide with trypsin and cyanogen bromide before the reduction of disulfide bonds and subsequent alkylation of sulphydryl groups.

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## Self-Test Questions

1. A solution of a peptide of unknown sequence was divided into two samples. One sample was treated with trypsin and the other with chymotrypsin. The smaller peptides obtained by trypsin treatment had the following sequences:

Leu-Ser-Tyr-Ala-Ile-Arg  
Asp-Gly-Met-Phe-Val-Lys

The peptides obtained by chymotrypsin treatment had the following sequences:

Val-Lys-Leu-Ser-Tyr  
Ala-Ile-Arg  
Asp-Gly-Met-Phe

Deduce the sequence of the original peptide.

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## Self-Test Questions

2. The following reagents are useful for characterizing proteins. Describe how each can be used and what information can be obtained. Write all appropriate reactions.
- (a) Trypsin (b) Dansyl chloride (c) SDS  
(d) Ninhydrin (e) Cyanogen bromide (f) 6 M HCl  
(g) Sanger's reagent (h) Phenylisothiocyanate
3. A peptide has the following amino acid composition: Ala, Arg, Asp<sub>2</sub>, Glu<sub>2</sub>, Gly<sub>3</sub>, Leu, Val<sub>3</sub>. The following peptides were isolated after partial hydrolysis and their sequence determined.
- (i) Asp-Glu-Val-Gly-Gly-Glu-Ala (ii) Val-Asp-Val-Asp-Glu  
(iii) Val-Asp-Val (iv) Glu-Ala-Leu-Gly-Arg  
(v) Leu-Gly-Arg (vi) Val-Gly-Gly-Glu-Ala-Leu-Gly-Arg
- What was the amino acid sequence in the original peptide?

## Self-Test Questions

4. Amino acid composition as determined by acid hydrolysis of a peptide with 6M HCl at 110 °C for 24 hours in an evacuated sealed tube, followed by chromatographic analysis of the mixture revealed the presence of the following amino acids: Ala, Leu, Arg, Met, Phe, Thr, 2Val. Given the following information, what is the probable sequence of the peptide? Treatment of the peptide with,
- (a) Sanger's reagent yielded DNP-Ala  
(b) Trypsin: gave two fragments (Ala, Arg, Thr) and (Leu, Met, Phe, 2Val) which when treated with Sanger's reagent yielded DNP-Ala and -Val respectively.  
(c) cyanogen bromide: gave two fragments (Ala, Arg, Homoserine lactone, Thr, 2Val) and (Leu, Phe), DNP-Ala and -Leu, respectively.

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## Protein Classification

### By solubility

- **Globular proteins**
  - are tightly folded polypeptide chains
  - soluble in water
  - perform informational role
  - e.g. enzymes, transport proteins, immunoglobulins, hormones etc.
- **Fibrous proteins**
  - are extended parallel layers or sheets
  - are insoluble in water
  - perform a structural role
  - e.g. coordinated motion proteins (myosin), mechanical support proteins (collagen, elastin, keratin). Blood clot protein (fibrin).

## Protein Classification

### By composition

- **Conjugated proteins:** contain groups other than amino acids called **prosthetic groups** as opposed to **simple proteins** which contain only amino acids. They include:
  - **Glycoproteins:** contain < 4% carbohydrate, e.g. heparin, which inhibits blood clotting), ABO blood group proteins.
  - **Mucoproteins:** contain > 4% carbohydrate
  - **Nucleoproteins:** e.g. ribosomes, viruses
  - **Phosphoproteins:** e.g. casein (milk protein)
  - **Hemoproteins** e.g. hemoglobin, myoglobin, cytochromes
  - **Lipoproteins:** e.g. fibrin (in blood)
  - **Flavoprotein:** e.g. succinate dehydrogenase
  - **Metalloproteins:** e.g. Iron (in ferritin), Molybdenum (in dinitrogenase), Copper (in plastocyanin), Calcium (in calmodulin).

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## Protein Classification

### By function

- **Enzyme proteins**
  - Trypsin, chymotrypsin, catalyze peptide bond hydrolysis
  - Lysozyme, catalyzes glycosidic linkage hydrolysis
- **Transport proteins** (in blood)
  - Myoglobin and hemoglobin transport oxygen
  - Serum albumin transports free fatty acids
- **Storage proteins**
  - Ferritin, stores iron in spleen and liver
  - Casein, stores amino acids in milk

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## Protein Classification

- **Defense proteins** (protection against disease)
  - e.g. Immunoglobulins (antibodies), cytokines
- **Hormonal proteins** (Communication and regulation)
  - e.g. insulin, promotes glucose uptake by cells.
  - e.g. glucagon, stimulates release of glucose by liver.
- **Contractile proteins** (Coordination of motion)
  - e.g. actin and myosin for muscle contraction.
- **Receptor proteins** (respond to stimuli)
  - e.g. neurotransmitter receptors, hormone receptors.
- **Toxic proteins** (serve a defensive role for plants and animals).
  - e.g. snake venom

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## Functions of Membrane Proteins

- **Transport**
  - Mediate the transport of molecules across membranes.
- **Catalytic**
  - Catalyze reactions that take place on membrane proteins.
- **Structural**
  - Form part of cell and organelle membrane structures.

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## Functions of Membrane Proteins

- **Receptors**
  - Proteins embedded in membranes of cells and have portions exposed on the cell surface.
  - They function as signal receptors, enzymes and ion channels.
  - Receptors are named according to the ligand they bind.
    - (e.g. acetylcholine receptor, (nor)epinephrine receptor, histamine receptor, GABA receptor, glutamate receptor, vasopressin receptor, insulin receptor, prostacyclin receptor, angiotensin receptor, opiate receptor, light (rhodopsin) receptor etc.)
  - Some membrane receptors function as enzymes (e.g. Tyrosine kinase)

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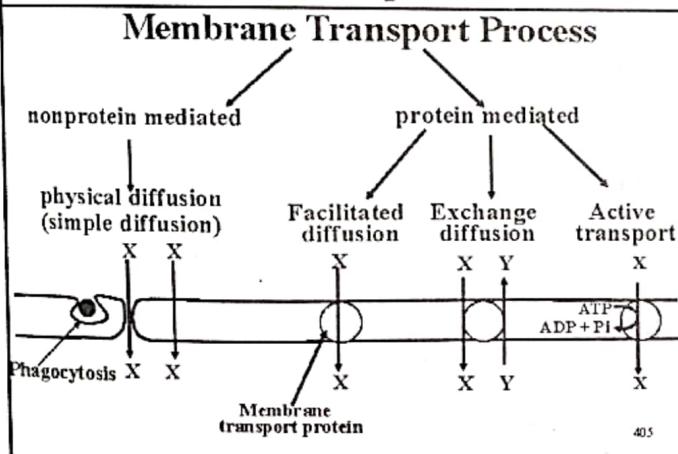
## Membrane Transport Proteins

- Transport of substrates across membranes depends both on the properties of the substrate being transported and the characteristics of the membrane.
- Depending on energy requirements, transport processes can be divided into:
  - Passive processes:** are spontaneous, proceed mainly by
    - Simple diffusion* through pores or lipid domains of the membrane.
    - Facilitated diffusion* mediated by membrane carrier proteins.
  - Active processes:** are also mediated by membrane proteins but are nonspontaneous processes (driven by energy). Enables nutrients and other molecules to be moved against a concentration gradient. This allows the cell to concentrate compounds.

## Membrane Transport Proteins

- Passive transport (Facilitated diffusion)**
  - does not require energy
  - occurs in the direction of higher to lower concentration
  - mediated by carrier (channel) proteins
- Active transport**
  - requires ATP energy or some other metabolic energy source.
  - occurs in the direction of lower to higher concentration
  - mediated by active transport proteins
    - e.g. sodium pump (pumps  $K^+$  in and  $Na^+$  out of cell)

## Membrane Transport Proteins

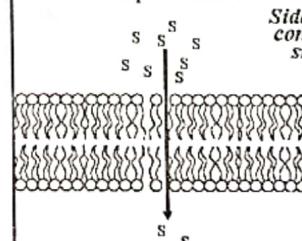


## Membrane Transport Proteins

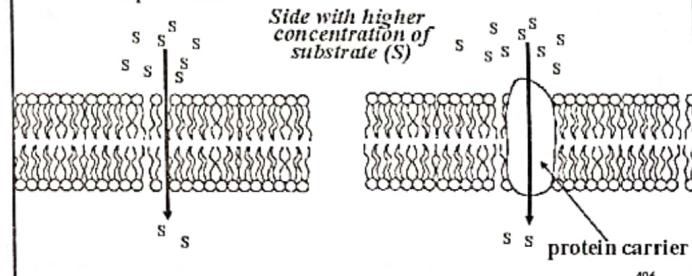
### Passive transport processes

#### Non-carrier Mediated

##### Simple diffusion

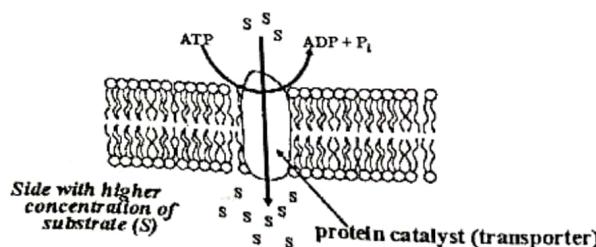


##### Carrier Mediated



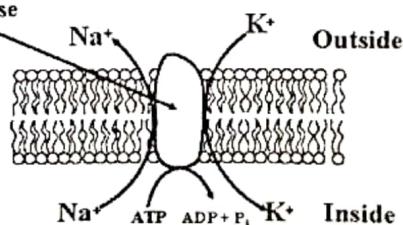
## Membrane Transport Proteins

### An Active Transport Process



## Membrane Transport Proteins

### $Na^+ - K^+$ ATPase

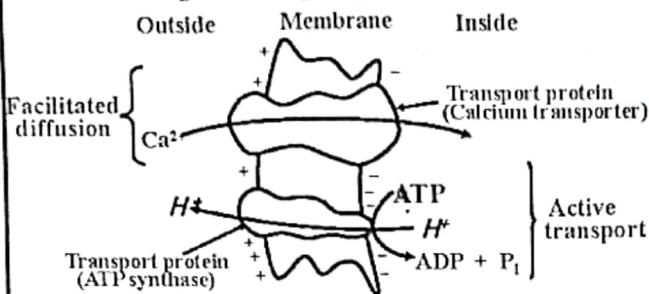


An active transport process which maintains a high  $Na^+$  concentration outside the cell and a high  $K^+$  concentration inside the cell against their gradients. The energy for maintaining this gradient is supplied by ATP hydrolysis. It is estimated that about a third of ATP produced by cells is used in maintaining  $Na^+$  and  $K^+$  concentrations across the cell membrane.



## Membrane Transport Proteins

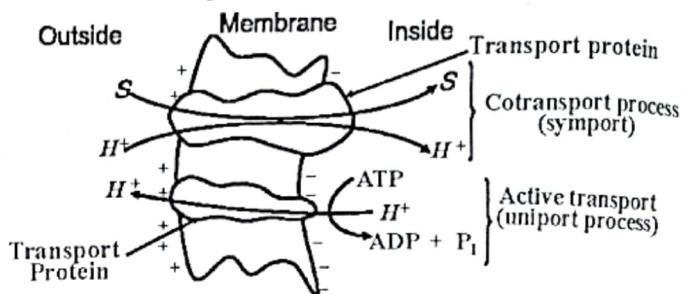
### Active Transport Coupled to Facilitated Diffusion



ATP hydrolysis is coupled to electrogenic (movement to create an electrical potential) movement of  $H^+$  from inside to outside. This results in a membrane potential positive on the outside. Calcium which is positively charged is electrophoresed (movement in response to an existing potential) by the existing membrane potential. This is how  $Ca^{2+}$  is accumulated by the mitochondrion.<sup>409</sup>

## Membrane Transport Proteins

### Active Transport Coupled to Facilitated Diffusion



Active transport of  $H^+$  from the inside to the outside of the cell is driven by ATP hydrolysis. This creates a membrane potential (positive on the outside and negative inside), also called *proton electrochemical gradient*. The  $H^+$  is electrophoresed back into the cell.  $H^+$  movement out is coupled to the movement of substrate ( $S$ ) into the cell. This is how lactose is accumulated by *E. coli*.<sup>410</sup>

## Membrane Transport Proteins

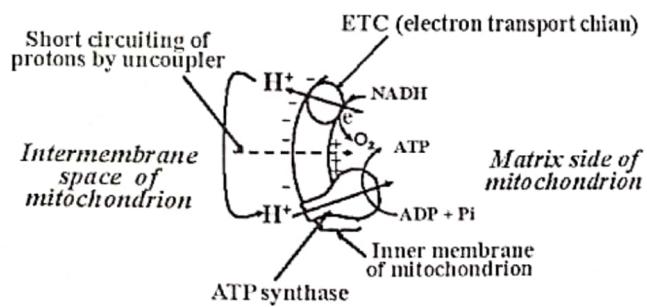
### Active Transport Coupled to Facilitated Diffusion in ATP Synthesis

- The energy derived from the downhill movement of electrons in the electron transport chain from substrates such as NADH and  $FADH_2$  to oxygen during their oxidation, drives protons from the matrix of the mitochondrion to the intermembrane space.
- The proton electrochemical gradient so created is used in driving the uphill synthesis of ATP from ADP and Pi by the ATP synthase.

<sup>411</sup>

## Membrane Transport Proteins

### Active Transport Coupled to Facilitated Diffusion in ATP Synthesis



The electron transport chain of the mitochondrion is coupled to ATP synthesis by a proton electrochemical gradient.<sup>412</sup>

## Membrane Transport Proteins

### Uncoupling Agents

- Compounds which facilitate the movement of protons across membranes instead of coupling the proton gradient to ATP production are known as uncouplers. They uncouple oxidation of substrate (NADH,  $FADH_2$ ) from phosphorylation of ADP to ATP in the mitochondrion.
- Uncouplers dissipate the proton gradient across the membrane.
- Dinitrophenol** is an uncoupler which acts by allowing protons to diffuse through the inner membrane.<sup>413</sup>

## Enzymes Proteins

- Cells produce remarkably efficient biocatalysts called **enzymes**, that are effective at significantly increasing rates of cellular reactions.
- There is universal participation of enzymes in reactions of living organisms.
- Enzymology is the study of:
  - the reactions catalyzed by enzymes,
  - the mechanism of an enzyme reaction,
  - the nature of enzymes themselves.

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## Enzyme Proteins

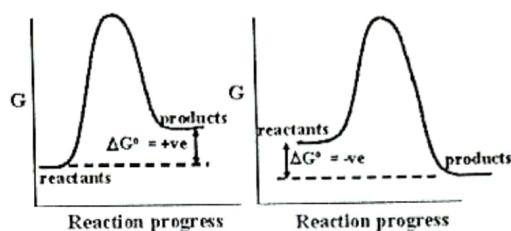
- Unlike non biological catalysts, enzymes are specific, can be regulated, and produce huge acceleration rates. An enzyme can increase a reaction rate by  $10^6$  to  $10^{14}$  fold.
- Enzymes are either **simple proteins**, in which the catalytic activity resides only in the polypeptide structure, or **conjugated proteins** which require a non-amino acid component called **cofactor**, such as a **metal ion** or a small organic molecule (**coenzyme**) to function.

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## Enzyme Proteins

### Spontaneous and nonspontaneous Reactions

Nonspontaneous (endergonic) reaction Spontaneous (exergonic) reaction



Although many cellular reactions proceed spontaneously (are exergonic) other reactions essential to life are endergonic ( $\Delta G$  is positive) and require an input of energy. By coupling a non-spontaneous reaction to a spontaneous energy-releasing reaction, the non-spontaneous one can be made to proceed.

## Enzyme Proteins

### Acceleration of Reaction Rates

- In an **uncatalyzed** reaction the reactant must climb over a higher hill of free energy, and hence a slower and longer pathway, before a downhill descent. In a **catalyzed** reaction the reactant goes over a lower barrier of free energy and hence a faster and shorter pathway downhill.

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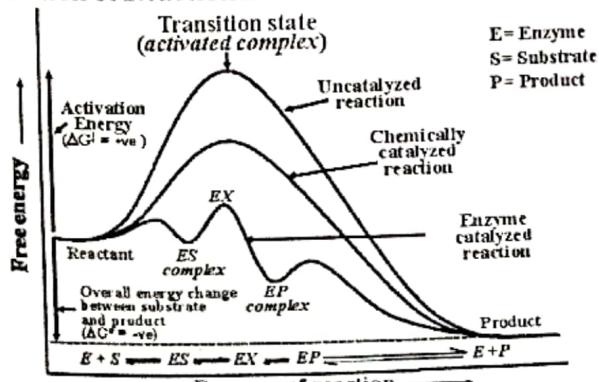
## Enzyme Proteins

- Enzymes accelerate reaction rates by lowering the activation energy which is accomplished either by;
  - increasing the stability of the transition state relative to the reactants or
  - decreasing the stability of the reactants;
    - (i) by weakening the bonds to be broken or disrupted to form the transition state or,
    - (ii) by increasing the reactivity of an attacking molecule.
- An enzyme catalyzed reaction also proceeds faster because it produces more transition steps each of which has a lower free energy of activation than the single step in an uncatalyzed reaction.



## Enzyme Proteins

### Acceleration of Reaction Rates



Source: Adapted from Pitskiewich, D. (1977). *Kinetics of Chemical and Enzyme-catalyzed Reactions*.

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## Enzyme Proteins

### Effect of Enzymes on $\Delta G^\circ$ and $K_{eq}$

- Enzymes cannot make a nonspontaneous reaction proceed.
- They only speed up spontaneous reactions.
- Catalysts have no effect on the *overall free energy* ( $\Delta G^\circ$ ) change of the reaction or the *equilibrium constant* ( $K_{eq}$ ) of the reaction. They only accelerate the rates at which the equilibria may be reached.

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## Enzyme Proteins

### Effect of catalyst on equilibrium constant ( $K_{eq}$ )

For example, in the reaction



$k_1$  might be  $10^{-2} \text{ min}^{-1}$  while  $k_2$  might be  $10^{-4} \text{ min}^{-1}$ . At equilibrium, the rates of the forward and reverse reactions are equal and hence  $k_1[S] = k_2[P]$

$$K_{eq} = [P]/[S] = k_1/k_2 = 10^{-2}/10^{-4} = 10^2$$

In the presence of a catalyst both  $k_1$  and  $k_2$  are enhanced to the same degree, thus if  $k_1$  increases  $10^5$  fold,  $k_2$  must also increase  $10^5$  fold. The  $K_{eq}$  remains unchanged.

$$K_{eq} = k_1/k_2 = 10^3/10 = 10^2$$

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## Enzyme Proteins

### The catalytic groups include:

- side chains of certain amino acids such as the hydroxyl group of serine, imidazole group of histidine and phenolic hydroxyl group of tyrosine, all of which act as nucleophiles.
- cofactors (nonprotein components of enzymes with mostly electrophilic properties).
  - Cofactors include metal ions (metal-ion cofactors) and small organic molecule cofactors called coenzymes.

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## Enzyme Proteins

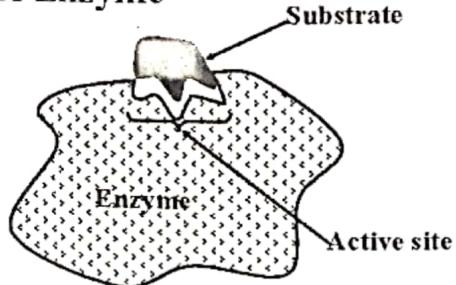
### Active Site of Enzymes

- Enzymes have a globular structure with a geometrically discrete region on their surface, where catalytic activity takes place, called the **active site** or **active centre**.
- Three essential features of the active site include:
  - a **shape** complementary to the substrate
  - **binding groups** that form bonds with the substrate (S) leading to the formation of an enzyme-substrate complex (ES), and
  - well oriented **catalytic groups**.

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## Enzyme Proteins

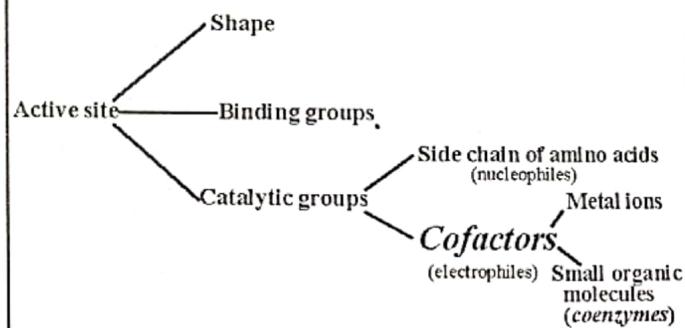
### Active Site of Enzyme



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## Enzyme Proteins

### Features of the active site



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## Enzyme Proteins

### Cofactors

- Amino acid side chains of enzymes alone (which usually function as nucleophiles) cannot catalyze all biological reactions in the cell since they are not good electron acceptors (electrophiles).
- Non amino acid components of enzymes (**cofactors**) which function as electrophiles are also required for catalysis.
- Some cofactors (**cosubstrates**) bind transiently to the enzyme, whereas others (**prosthetic groups**) are permanently bound to an amino acid moiety at the enzyme active site.

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# Enzyme Proteins

## Coenzymes

- Coenzymes are obligate cofactors for certain enzymes and function as catalytic groups at the active site of enzymes.
- They often function in the transfer of electrons or functional groups (hydrogen atom, acetyl, methyl, amino groups etc.)
- The coenzymes are generally the biologically active form of certain vitamins.

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# Enzyme Proteins

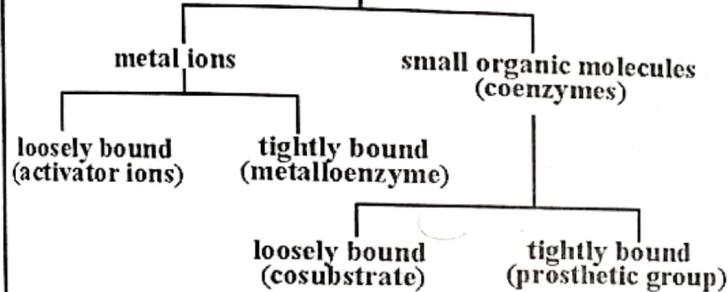
## Metal-ion cofactors

Cofactor	Enzyme
Fe <sup>2+</sup> , Fe <sup>3+</sup>	Cytochrome oxidase, catalase, peroxidase
Cu <sup>2+</sup>	Cytochrome oxidase, superoxide dismutase
Zn <sup>2+</sup>	Carbonic acid anhydrase, alcohol dehydrogenase
Mg <sup>2+</sup>	Pyruvate kinase, hexokinase, glucose 6-phosphatase
Mn <sup>2+</sup>	Ribonucleotide reductase, arginase
Ni <sup>2+</sup>	Urease
Mo <sup>6+</sup>	Dinitrogenase
K <sup>+</sup>	Pyruvate kinase
Se <sup>2+</sup>	Glutathione peroxidase

Enzymes that have metal ion cofactors tightly bound within the enzyme protein are called **metalloenzymes**, whereas those that have metal ions loosely bound are referred to as **metal-activated enzymes**.

# Enzyme Proteins

## Cofactors



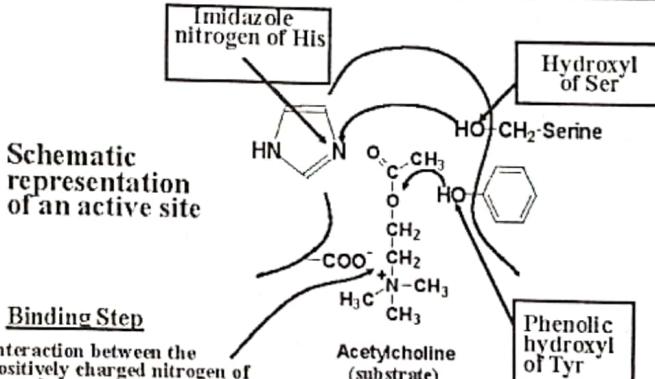
**Apoenzyme** = enzyme without cofactor (apo = without)

**Holoenzyme** = enzyme cofactor complex (holo = whole)

Source: Adapted from L.A. Moran and K. G. Scrimgeour, (1994) A Biochemistry Resource Book.

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# Enzyme Proteins



Source: Redrawn from Musil, J., Novakova, O. and Kunz, K. (1977) Biochemistry in Schematic Perspective, Avicenum.

# Enzyme Proteins

## Catalytic Function of Side Chains

Amino Acid	Reactive Side Chain	Catalytic Function
Serine	Hydroxyl (-OH)	Binds acyl groups
Tyrosine	Hydroxyl (-OH)	Form hydrogen bonds with ligands
Cysteine	Sulfhydryl (-SH)	Binds acyl groups
Lysine	$\alpha$ -amino (-NH <sub>3</sub> <sup>+</sup> )	Binds anions or transfer protons
Aspartic acid	$\beta$ -Carboxyl (-COOH)	Binds cations or transfer protons
Glutamic acid	$\gamma$ -Carboxyl (-COOH)	Binds cations or transfer protons
Arginine	Guanidinium $\text{H}_2\text{N}-\text{C}(=\text{NH})-$	Binds anions or transfer protons
Histidine	Imidazole 	Transfer protons

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# Enzyme Proteins

## Coenzymes

Precursor Vitamin	Coenzyme	Metabolic Function	Catalytic Classification
Vitamin A	cis-Retinal	Vision	Prosthetic group
Vitamin B <sub>1</sub> (Thiamine)	Thiamine pyrophosphate (TPP)	Transfer of aldehyde groups	Prosthetic group
Vitamin B <sub>2</sub> (Riboflavin)	FMN/FMH <sub>2</sub> (Flavin mononucleotide)	Oxidation-reduction reactions	Prosthetic group
	FAD/FADH <sub>2</sub> (Flavin adenine dinucleotide)	Oxidation-reduction reactions	Prosthetic group

Source: Adapted from Moran, L.A. and Scrimgeour, K.G. (1994). Biochemistry Resource Book.

## Enzyme Proteins

### Coenzymes

Precursor Vitamin	Coenzyme	Metabolic Function	Catalytic classification
Vitamin B <sub>5</sub> (Pantothenic acid)	Coenzyme A (CoA)	Transfer of acyl groups	Cosubstrate
Vitamin B <sub>6</sub> (Pyridoxine)	Pyridoxal phosphate (PLP)	Transfer of groups to and from amino acids	Prosthetic group
Vitamin B <sub>12</sub> (Cobalamin)	Adenosyl-cobalamin	Intramolecular rearrangement	Prosthetic group
Vitamin K	Vitamin K	Carboxylation of glutamate residues	Prosthetic group

Source: Adapted from Moran, L. A and Scrimgeour, K. G. (1994). Biochemistry Resource Book.

## Enzyme Proteins

### Coenzymes

Precursor Vitamin	Coenzyme	Metabolic Function	Catalytic Classification
Biotin	Biocytin	Carboxylation or carboxyl group transfer	Prosthetic group
Folic acid	Tetrahydrofolate	Transfer of one carbon units (methyl, formyl, etc)	Cosubstrate
Niacin	NAD <sup>+</sup> /NADH (Nicotinamide adenine dinucleotide)	Oxidation-reduction reactions	Cosubstrate
	(NADP <sup>+</sup> /NADPH Nicotinamide adenine dinucleotide phosphate)	Oxidation-reduction reactions	Cosubstrate

Source: Adapted from Moran, L. A and Scrimgeour, K. G. (1994). Biochemistry Resource Book.

## Enzyme Proteins

### Coenzymes

Precursor vitamin	Coenzyme	Metabolic function	Catalytic classification
None	Adenosine triphosphate (ATP)	Transfer of phosphate groups	cosubstrate
None	S-Adenosylmethionine (SAM)	Transfer of methyl groups	cosubstrate
None	Phosphoadenosine phosphosulfate (PAPS)	Transfer of sulfhydryl groups	cosubstrate
None	Nucleotide sugars	Transfer of carbohydrate groups	cosubstrate
None	Cytidine diphosphate (CDP)	Transfer of alcohols in lipid synthesis	cosubstrate

Source: Adapted from Moran, L. A and Scrimgeour, K. G. (1994). Biochemistry Resource Book.

## Structures of Vitamins

Vitamin	Structure	Deficiency Disease
<i>Water-soluble Vitamins</i>		
Niacin (nicotinamide)		Pellagra
Riboflavin (vitamin B <sub>2</sub> )		Growth Retardation
Thiamine (vitamin B <sub>1</sub> )		Beriberi

## Structures of Vitamins

Vitamin	Structure	Deficiency Disease
<i>Water-soluble Vitamins</i>		
Pantothenic acid		Dermatitis (chickens)
<i>Water-soluble Vitamins</i>		
Biotin		Dermatitis (human)
Pyridoxine (vitamin B <sub>6</sub> )		Dermatitis (rats)

## Structures of Vitamins

Vitamin	Structure	Deficiency Disease
<i>Water-soluble Vitamins</i>		
Folic acid		Anemia
<i>Water-soluble Vitamins</i>		
Lipoic acid		Growth deficiencies
L-Ascorbic acid		Scurvy

## Structures of Vitamins

Vitamin	Structure	Deficiency Disease
<u>Water-soluble Vitamin</u>		
Cobalamin vitamin B <sub>12</sub>		Pernicious anemia

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## Structures of Vitamins

Vitamin	Structure	Deficiency Disease
<u>Fat-Soluble Vitamins</u>		
Trans-Retinol (vitamin A)		Night blindness
Cholecalciferol (vitamin D)		Rickets

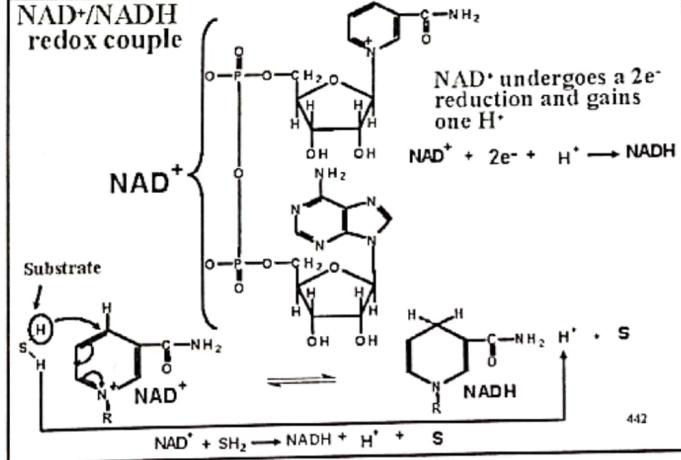
440

## Structures of Vitamins

Vitamin	Structure	Deficiency Disease
<u>Fat-Soluble Vitamins</u>		
Tocopherol (vitamin E)		Reproductive problems in rats
Phylloquinone (vitamin K <sub>1</sub> )		Problems in blood clotting

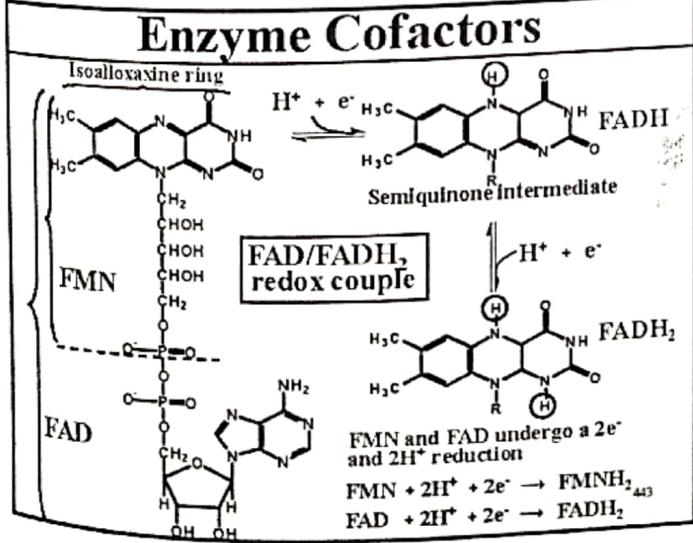
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## Enzyme Cofactors



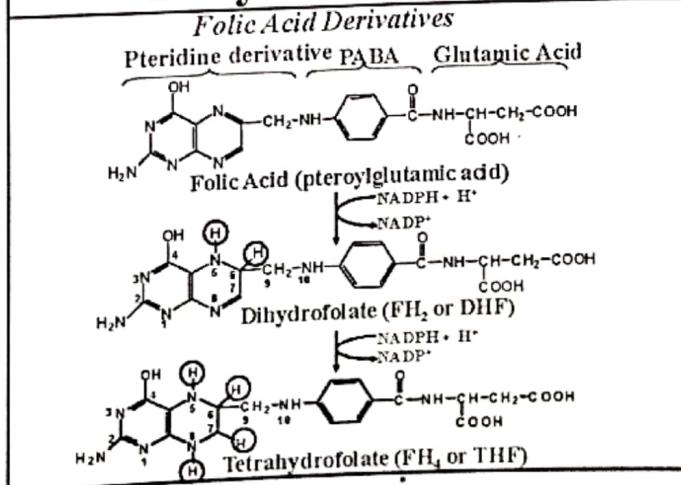
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## Enzyme Cofactors



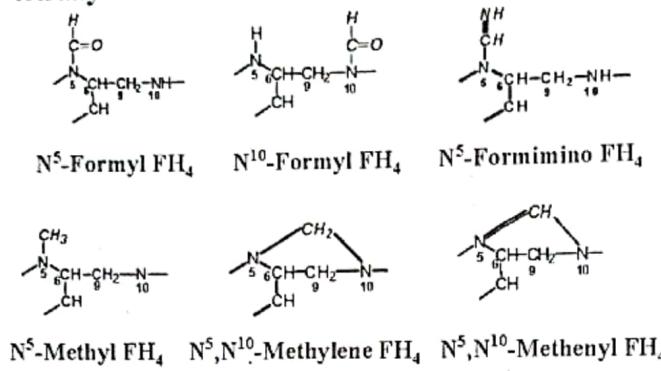
443

## Enzyme Cofactors



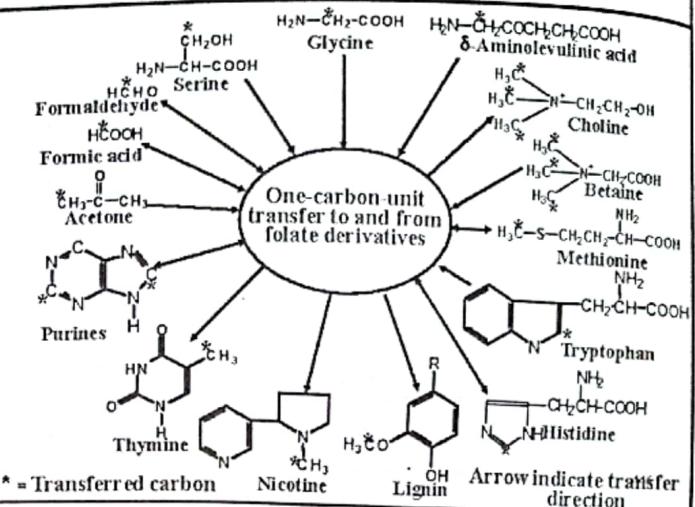
## Enzyme Cofactors

Partial structures of one-carbon derivatives of tetrahydrofolate



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## Enzyme Cofactors



## Enzyme Cofactors

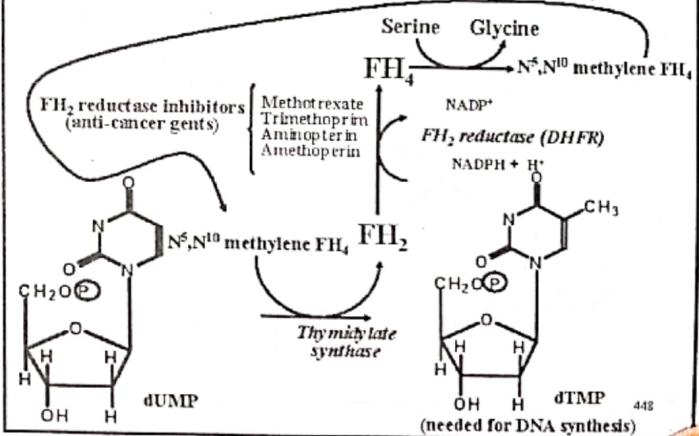
### Tetrahydrofolate Derivatives

- In the synthesis of thymine, needed for DNA synthesis, uracil is methylated at carbon 5 of the pyrimidine ring.
- This reaction is catalyzed by the enzyme thymidylate synthase which transfers the methyl group from methylene tetrahydrofolate.
- Cancerous cells have an unusually high level of DNA synthesis.
- Inhibition of thymidylate synthase by fluorouracil reduces the rate of tumor cell growth since thymine production is inhibited.
- Inhibition of FH<sub>2</sub> reductase (DHFR) by chemotherapeutic agents also slows down tumor cell growth as this prevents the conversion of FH<sub>2</sub> to FH<sub>4</sub>, which is required to form the N<sup>5</sup>,N<sup>10</sup>-methylene-FH<sub>4</sub>, needed to produce dTMP for DNA synthesis from dUMP.

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## Enzyme Cofactors

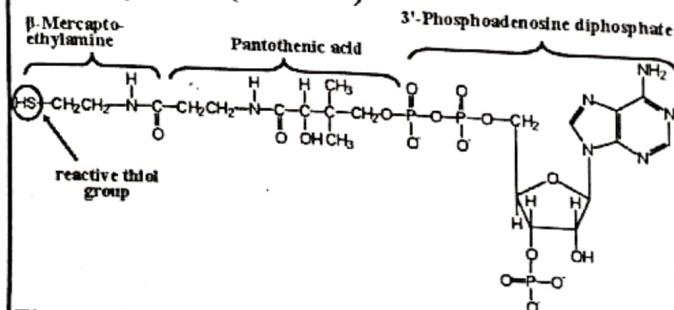
### Tetrahydrofolate derivatives in nucleotide biosynthesis



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## Enzyme Cofactors

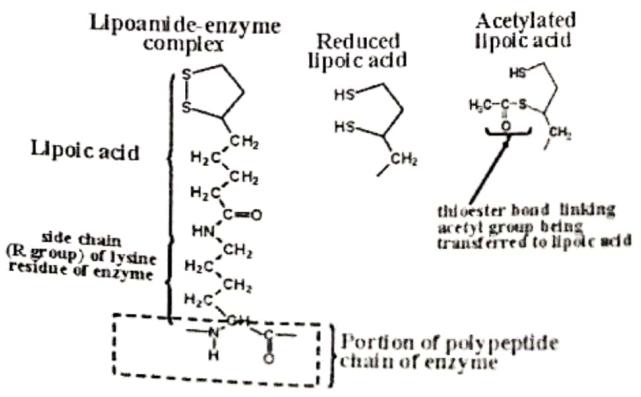
### Coenzyme A (CoASH)



The reactive thiol group (-SH) of the β-mercaptopropyl moiety involved in the transfer of acyl groups is shown in the abbreviated name of coenzyme A (CoASH)

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## Enzyme Cofactors



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## Enzyme Specificity

- Specificity is the ability to recognize only one or a small number of closely related substrates.
- Enzymes exhibit different degrees of specificity.
- Four degrees of specificity have been recognized.
  1. **Linkage specificity** is when an enzyme catalyzes the creation or cleavage of only certain bond types e.g.
  - Lipases hydrolyze ester linkages in triacylglycerols.
  - Proteases hydrolyze peptide linkages in proteins.

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## Enzyme Specificity

2. **Group (broad) specificity** is when an enzyme acts on similar substrates containing same functional groups.
  - Trypsin and chymotrypsin act on specific type of amino acids in a polypeptide
  - Hexokinase catalyzes the addition of phosphoryl group to hexose sugars.

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## Enzyme Specificity

3. **Absolute specificity** is when only one substrate is acceptable by an enzyme.
4. **Stereospecificity**
  - Enzymes are able to distinguish between optical and geometrical isomers.
  - An enzyme will use only one form of an enantiomeric pair, unless its function is to convert one enantiomer to the other.
  - Some enzymes can also distinguish between paired chemically alike substituents e.g. two hydrogen atoms in a molecule.

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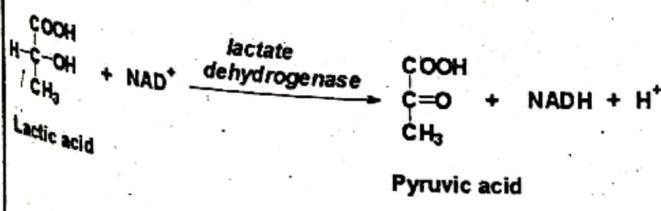
## Classification of Enzymes

Enzyme Class	Reaction Type	Reaction Description
1. Oxidoreductases	Oxidation-Reduction	Proton-electron transfer
2. Transferases	Group transfer	Transfer of functional group from one compound to another
3. Hydrolases	Hydrolytic cleavage	Bond cleavage by water
4. Lyases	Nonhydrolytic cleavage	Addition to double bond or elimination to form a double bond.
5. Isomerases	Isomerization	Intramolecular rearrangement
6. Ligases	Bond formation	Formation of carbon-carbon and other bonds using ATP

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## Enzyme Class and Reaction Type

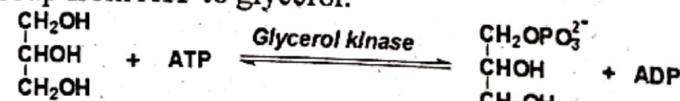
**Oxidoreductases:** Catalyze oxidation-reduction reactions. They include oxidases, peroxidases, oxygenases, dehydrogenases and reductases.



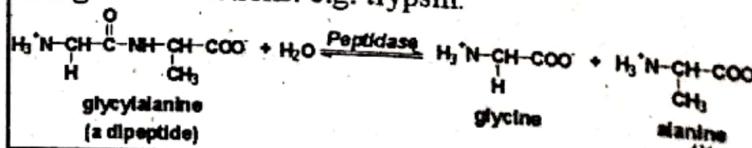
455

## Enzyme Class and Reaction Type

**Transferases:** Catalyze the transfer of groups of atoms from one compound to another. An example is glycerol kinase which transfers a phosphate group from ATP to glycerol.



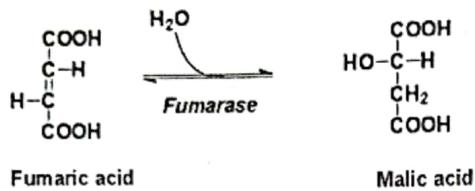
**Hydrolases:** Catalyze hydrolytic cleavage of esters, glycosidic and peptide bonds. They are important in digestive reactions. e.g. trypsin.



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## Enzyme Class and Reaction Type

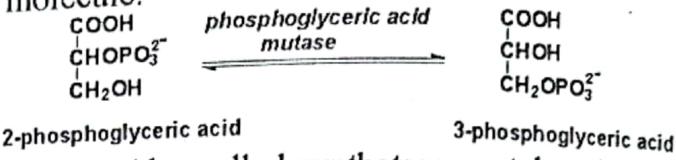
**Lyases:** Catalyze non hydrolytic cleavage reactions which involve the addition of small molecules such as water, ammonia or  $\text{CO}_2$  to double bonds such as  $\text{C}=\text{C}$  and  $\text{C}=\text{N}$ , or the reverse elimination reaction to create double bonds.



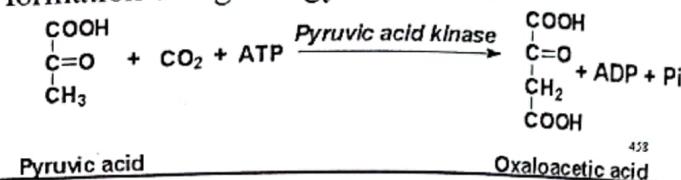
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## Enzyme Class and Reaction Type

**Isomerases:** Catalyze the isomerization or rearrangement of functional groups within a molecule.



**Ligases:** Also called synthetases, catalyze bond formation using energy from ATP.



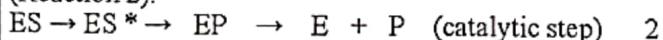
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## Mechanism of Enzyme Action

A model for the kinetic analysis of enzymes is based on a proposal by Michaelis and Menten. In this model, a non covalent complex is formed between the enzyme (E) and the substrate (S) to produce an enzyme-substrate complex (ES), in a fast and reversible step (Reaction 1).



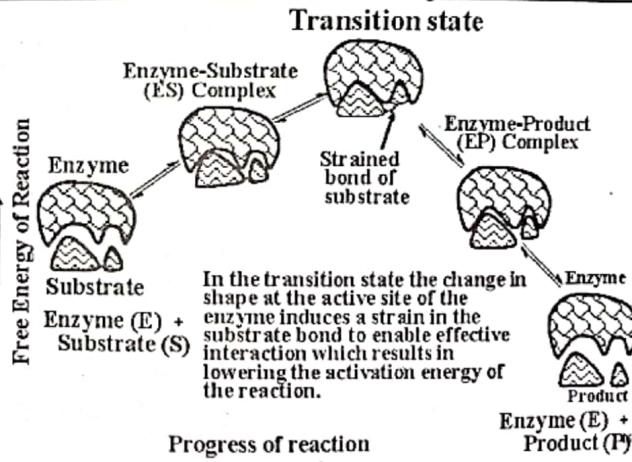
The ES complex undergoes the chemical reaction proper during which the enzyme-substrate complex, in a slow step, is converted to an enzyme-product (EP) complex which breaks down to regenerate the enzyme and product (Reaction 2).



$\text{ES}^*$  = transition state (energetically unstable)

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## Mechanism of Enzyme Action



## Mechanism of Enzyme Action

- **Lock-and-key model** (proposed by Emil Fischer in 1894)
  - In this model, the active site of an enzyme is envisaged as a rigid opening on the enzyme protein surface into which the substrate fitted like a key in a lock, and only substrates with the right shape and size fit into the cleft on the enzyme surface.
- **Induced-fit model** (proposed by Daniel Koshland in 1958)
  - This model is based on the fact that proteins are flexible molecules with flexible active sites that are close in shape to the substrate.
  - The substrate does not fit exactly but the active site of the enzyme changes conformation to accommodate the substrate in a close fit enzyme-substrate interaction.

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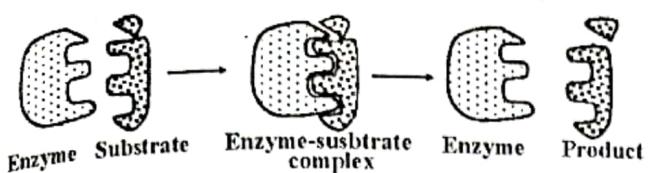
## Mechanism of Enzyme Action

- It should be noted that it is very simplistic to visualize an enzyme active site as either a rigid structure (as proposed by the lock-and-key model) or a flexible template that can be modified to fit the substrate (as proposed by the induced-fit model).

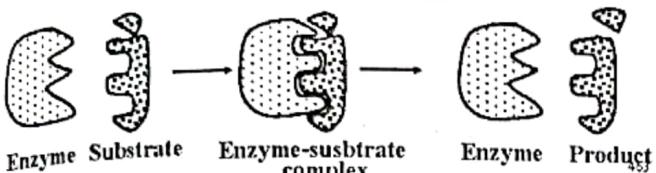
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## Mechanism of Enzyme Action

### LOCK-AND-KEY MODEL.

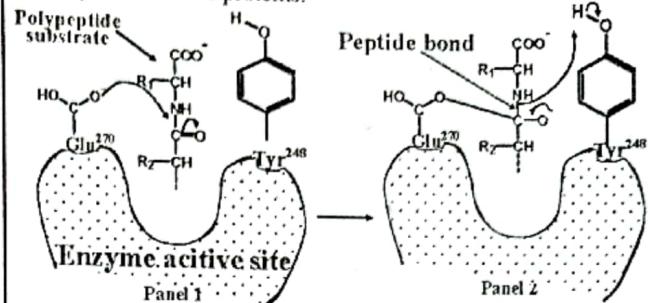


### INDUCED-FIT MODEL



## Mechanism of Enzyme Action

**Mechanism of action of carboxypeptidase:** Carboxypeptidase is a stomach protease that hydrolyzes peptide bonds from the carboxyl terminal of proteins.

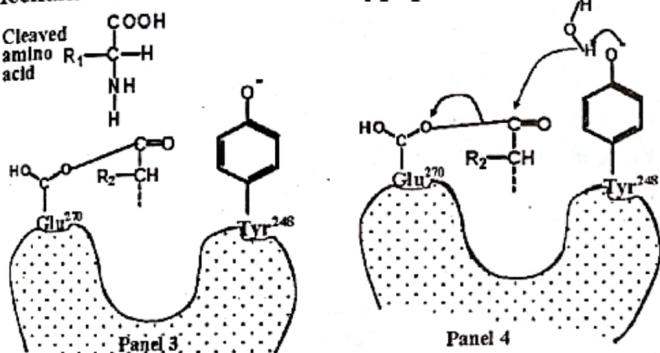


The ionized carboxyl group of Glu<sup>270</sup> acts as a nucleophile when it attacks the carbonyl of the polypeptide.

Hydroxyl group of Tyr<sup>248</sup> donates a proton to the peptide bond of the polypeptide. Panel 2 represents the transition state.

## Mechanism of Enzyme Action

### Mechanism of action of carboxypeptidase



Tyr<sup>248</sup> accepts a proton in order to enhance the nucleophilicity of the attacking molecule (water). The original enzyme is then regenerated.

## Enzyme Kinetics

### What is enzyme kinetics ?

- Enzyme kinetics is the study of the dependence of the rates of enzyme-catalyzed reactions on the following parameters:
  - substrate concentration,
  - pH,
  - temperature,
  - ionic strength,
  - other variables etc.

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## Enzyme Kinetics

### Why enzyme kinetics?

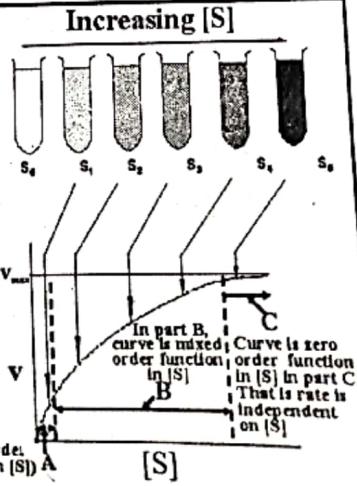
- Enzyme kinetics,
  - enables the biochemist to observe the work of an enzyme as a biological catalyst.
  - provides information about the mechanism of an enzyme catalyzed reaction.
  - allows the estimation of K<sub>m</sub> and V<sub>max</sub>. These give an indication of conditions of substrate and enzyme concentrations that may prevail in the cell.
  - enables the determination of the effect of pH, ionic strength, temperature etc. on enzyme activity and provides a fair insight into the properties of an enzyme *in vivo*.

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## Enzyme Kinetics

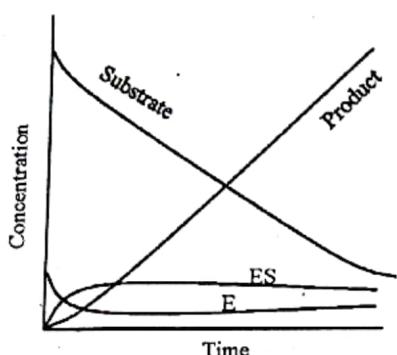
### Experimental Procedure

- Prepare each with a different [S]
- The same amount of enzyme (E) is added to each tube.
- The rate of [P] formation before [S] decreases significantly is measured, i.e., measure initial change in [P]
- $V = \Delta[P]/\Delta t$  is the initial velocity of reaction
- V is plotted as a function of initial [S].



## Enzyme Kinetics

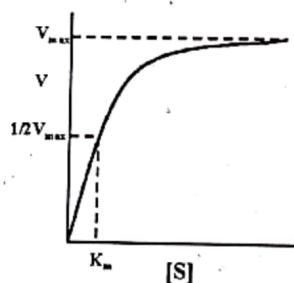
Time course for the concentrations of the components in a typical enzyme reaction



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## Enzyme Kinetics

### Michaelis-Menten Plot



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## Enzyme Kinetics

### Michaelis-Menten Equation

- The substrate ( $S$ ) dependence of the rate ( $v$ ) of an enzyme-catalyzed reaction is given by the Michaelis-Menten equation below:

$$v = \frac{V_{max} \times [S]}{K_m + [S]}$$

- $v$  is the rate of the reaction,  $K_m$  is the Michaelis-Menten constant, and  $V_{max}$  is the maximum rate attainable when the enzyme is saturated with substrate.
- The Michaelis-Menten equation is graphically represented by a rectangular hyperbola.

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## Enzyme Kinetics

### Turnover Number of Enzymes

Enzyme	Turnover number (min <sup>-1</sup> )
Carbonic anhydrase	$3.6 \times 10^7$
Ketosteroid isomerase	$1.7 \times 10^7$
Fumarase	$1.2 \times 10^6$
$\beta$ -amylase	$1.1 \times 10^6$
$\beta$ -galactosidase	12,500
Phosphoglucomutase	1,240
Succinate dehydrogenase	1,150

Source: Taken from Ouellette, R. J. (1997). Introduction to General, Organic and Biological Chemistry

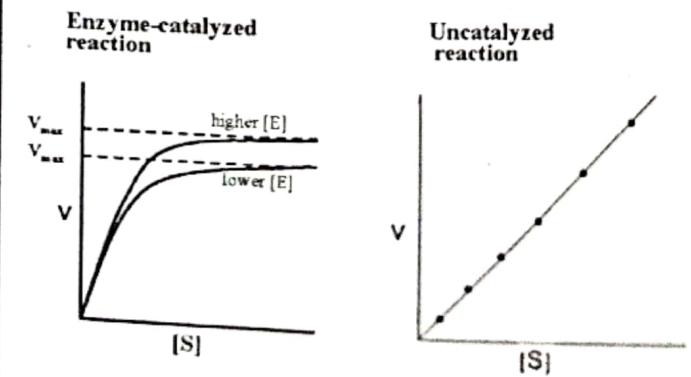
## Enzyme Kinetics

### Michaelis-Menten Plot

- The curve flattens due to saturation of the enzyme with substrate. No matter how much more substrate you add, the enzyme can only go so fast.
- $V_{max}$  is one measure of how active an enzyme is.
- $V_{max}$  is proportional to  $[E]$ .
- The catalytic efficiency of an enzyme is described by a turnover number =  $V_{max}/[E]$ .
- The turnover number is equal to the number of moles of substrate converted or product formed by one mole of enzyme per unit time, under saturating substrate conditions. The higher the turnover number, the more efficient the enzyme.

## Enzyme Kinetics

### Dependence of $V_{max}$ on enzyme concentration



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## Enzyme Kinetics

### Stability of Enzyme-Substrate (ES) Complex

$$K_m = \frac{[E][S]}{[ES]}$$

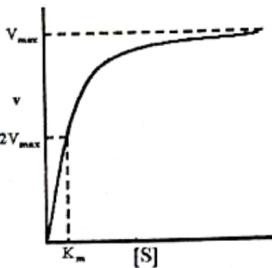
- $K_m$  is like a dissociation constant for the reaction  $ES \rightleftharpoons E + S$
- A large  $K_m$  means S is less tightly bound to enzyme, whereas a small  $K_m$  means S is more tightly bound.
- $K_m$  is also the [S] at  $\frac{1}{2}V_{max}$ .

## Enzyme Kinetics

### Determination of $K_m$ and $V_{max}$ from a Hyperbolic Plot

These values can be estimated from a v versus [S] plot.

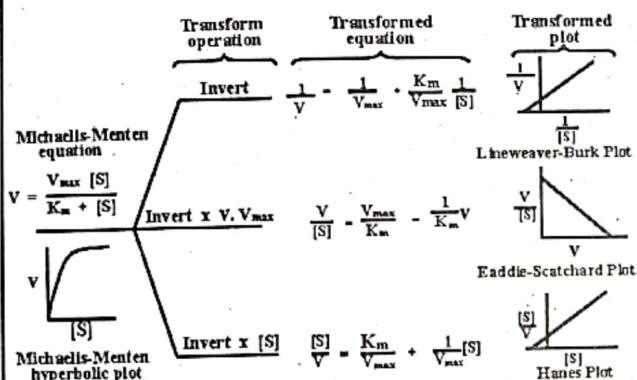
A hyperbolic plot does not give a precise value of  $V_{max}$  and hence  $K_m$ . More accurate measurements are obtained from transformed plots.



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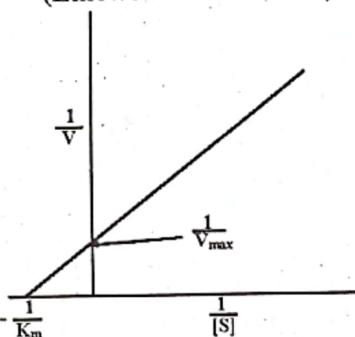
## Enzyme Kinetics

### Linear Transformations of Hyperbolic Plot



## Enzyme Kinetics

### Determination of $K_m$ and $V_{max}$ (Lineweaver-Burk Plot)



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## Enzyme Kinetics

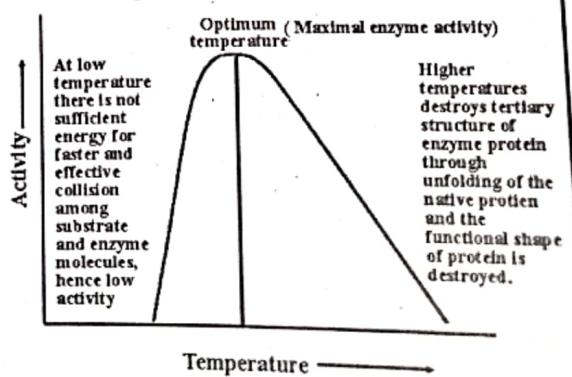
### Stability of Enzyme-substrate Complexes

Enzyme	Substrate	$K_m$ (M)
Chymotrypsin	acetyl-L-tryptophanamide	$5 \times 10^{-3}$
Lysozyme	hexa-N-acetylglucosamine	$6 \times 10^{-6}$
$\beta$ -galactosidase	lactose	$4 \times 10^{-3}$
Threonine deaminase	threonine	$5 \times 10^{-3}$
carbonic anhydrase	carbon dioxide	$8 \times 10^{-3}$
Pyruvate carboxylase	pyruvate	$4 \times 10^{-4}$

Source: Taken from Ouellette, R. J. (1997). Introduction to General, Organic and Biological Chemistry

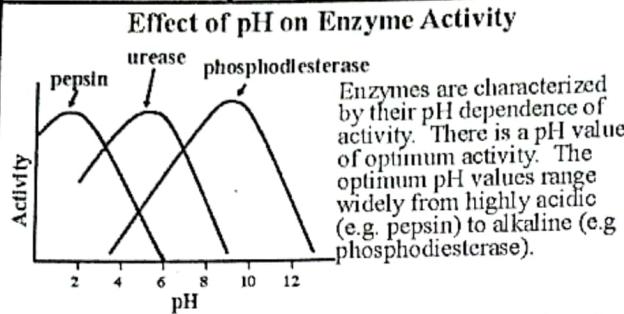
## Enzyme Kinetics

### Effect of Temperature on Enzyme Activity



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## Enzyme Kinetics



pH dependence of enzyme activity is determined by the  $pK_a$  of the ionizable groups of the enzyme, particularly those at or near the active site, that can contribute to changes of the active site through conformational changes of parts of the enzyme protein molecule.

## Self-Test Question

- As you increase the temperature of an enzyme catalyzed reaction, the rate of the enzyme reaction initially increases. It then reaches a maximum and finally dramatically declines. How do you explain the changes in reaction rate associated with the temperature changes?

## Enzyme Kinetics

### Enzyme Inhibition

- Cells regulate reactions within them by slowing down the rate of or shutting off certain reactions.
- An enzyme inhibitor is a compound that decreases the activity of an enzyme.
- There are two types of inhibitors
  - Irreversible and reversible inhibitors
  - Irreversible inhibitors form covalent bonds with the enzyme and cannot be removed without destroying enzyme. They include deadly poisons such as mercury and cyanide.
  - Reversible inhibitors rarely bind covalently and can be removed to restore enzyme activity.

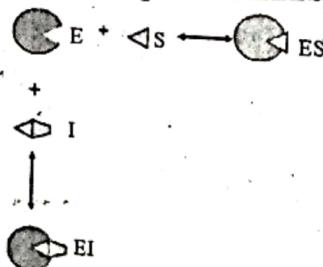
## Enzyme Kinetics

### Reversible inhibition

- There are three types of reversible inhibitors:
  - Competitive inhibitor:** binds to the same site as substrate and thus competes with the substrate for binding.
  - Noncompetitive inhibitor:** binds to a site on the enzyme other than the active site of enzyme.
  - Uncompetitive inhibitor:** binds to a site on the enzyme other than the active site but only does so after the substrate is bound.

## Enzyme Kinetics

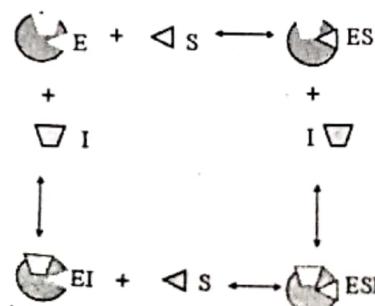
### Competitive Inhibition



Competitive inhibitors are structurally similar to substrate.

## Enzyme Kinetics

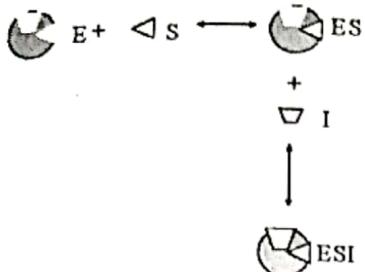
### Noncompetitive Inhibition



Noncompetitive inhibitors bind at a site other than active site of enzyme. They cause a conformational change in the enzyme so that catalytic properties at active site are altered.

## Enzyme Kinetics

### Uncompetitive Inhibition

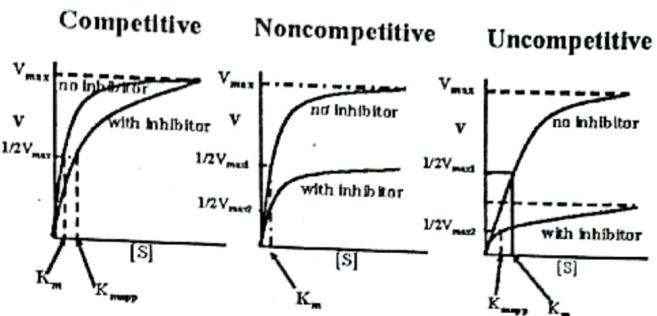


Like noncompetitive inhibitors, uncompetitive inhibitors bind at a site other than the active site, except that they bind only to the ES complex to form an inactive ESI complex. The inhibitor does not bind to the free enzyme.

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## Enzyme Kinetics

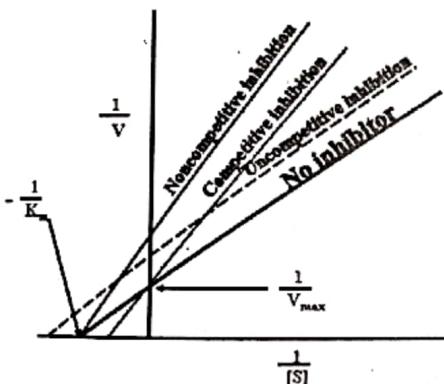
### Detection of Inhibition Type (Hyperbolic Plot)



488

## Enzyme Kinetics

### Detection of Inhibition Type (Transformed Plot: Lineweaver-Burk)



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## Enzyme Kinetics

### Effect of Inhibition Type on K<sub>m</sub> and V<sub>max</sub>

Inhibition Type	Effect on K <sub>m</sub> and V <sub>max</sub>	Michaelis-Menten Rate equation
Competitive	K <sub>m</sub> increases V <sub>max</sub> unchanged	$v = \frac{V_{max}[S]}{K_m(1 + \frac{[I]}{K_i}) + [S]}$
Non competitive	K <sub>m</sub> unchanged V <sub>max</sub> decreases	$v = \frac{V_{max}[S]}{1 + \frac{[I]}{K_i} + \frac{K_n}{K_m} + [S]}$
Uncompetitive	K <sub>m</sub> decreases V <sub>max</sub> decreases	$v = \frac{V_{max}[S]}{\frac{1 + \frac{[I]}{K_i}}{K_m} + [S]}$

490

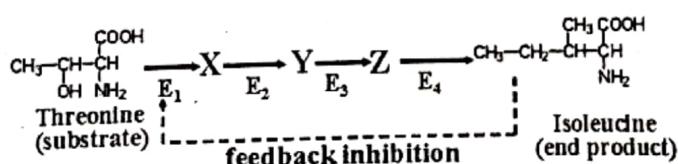
## Enzyme Regulation

- Enzyme activity is regulated through changes in the concentrations of substrates, cofactors, activators and inhibitors. The levels of an enzyme can also be regulated at the gene level.
- Regulation of enzyme activity entails decreasing (or increasing) the activity of an enzyme to conserve resources and /or energy or to produce a needed product faster.
- Methods for regulating enzyme activity include:
  - Substrate/Product effect
    - Feedback inhibition (by product)
    - Feedforward activation (by substrate)
  - Allosteric modulation
  - Covalent modification
  - Zymogen activation
  - Genetic control

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## Enzyme Regulation

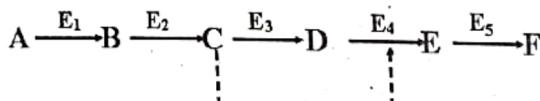
- Feedback inhibition: Is a regulatory process in which a product of a metabolic pathway inhibits an early step in the pathway. In the pathway below, threonine is the substrate, and E<sub>1</sub> is regulatory enzyme. Isoleucine feedback inhibits the enzyme E<sub>1</sub>.



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## Enzyme Regulation

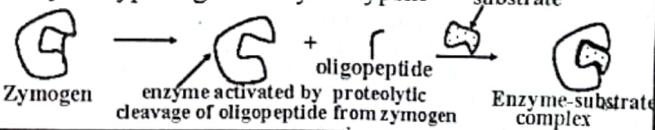
- Feed-forward activation.** This is a regulatory process in which a metabolite produced in an early step in a pathway stimulates an enzyme further down the pathway. In the reaction scheme below substrate C activates the enzyme  $E_4$  on the pathway.



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## Enzyme Regulation

- Zymogen (proenzyme) activation:** A zymogen is an inactive form of an enzyme. It is activated by removing a portion of the polypeptide chain.
- Zymogens are stored until needed and then converted to their active forms by proteolytic digestion.
- Trypsin is stored as inactive trypsinogen and chymotrypsin as chymotrypsinogen in the pancreas.
- If stored in the active form, they would digest pancreas proteins and damage the pancreas.
- The pancreas also has an inhibitor that inhibits the enzyme that converts trypsinogen to trypsin and chymotrypsinogen to chymotrypsin.



Enzyme-substrate complex

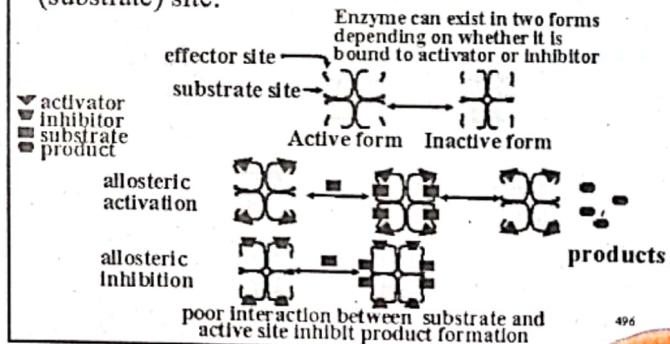
## Enzyme Regulation

- Allosteric modulation:** Allosteric enzymes function through reversible non-covalent binding of regulatory substances called allosteric effectors (activators or inhibitors).
- Allosteric effectors are generally metabolic intermediates or cofactors.
- They are often not structurally related to the substrate or product of the enzyme and bind at a site other than the active site.
- They usually modify the  $K_m$  or  $V_{max}$  of the enzyme reaction.

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## Enzyme Regulation

- Allosteric modulation results in a change in enzyme activity (e.g. inhibition or activation) due to the binding of an effector at a site other than the active (substrate) site.

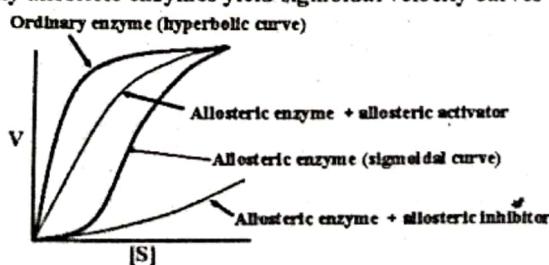


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## Enzyme Regulation

### Allostery and sigmoidal kinetics

Generally allosteric enzymes yield sigmoidal velocity curves



The effect of the interaction between allosteric enzyme subunits, manifested as either positive or negative cooperativity among the subunits, is important in the regulation of enzyme activity since it renders the enzyme more sensitive or less sensitive to changes in  $[S]$ . In the sigmoid curve, the steep part occurs at higher substrate concentration. This allows a small alteration in  $[S]$  to cause a large change in the rate of the reaction.

## Enzyme Regulation

### Covalent Modification

- Involves the reversible covalent binding of a phosphoryl, adenyl, acetyl, methyl or uridyl group to the hydroxyl groups of tyrosine, serine and threonine of an enzyme.
- Regulatory enzymes (both those subject to allosteric modulation and covalent modification) are mostly multi-subunit proteins with the catalytic and regulatory sites in separate subunits in some cases.

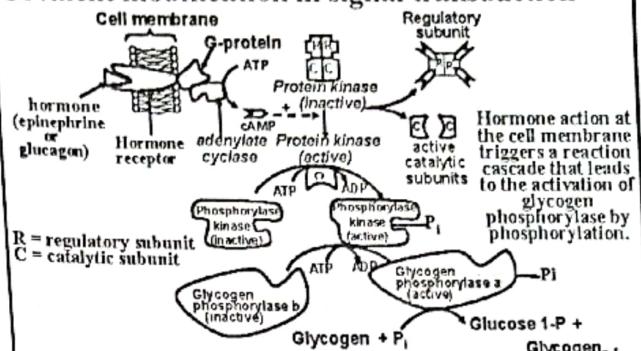
## Enzyme Regulation

### Covalent modification

- The covalent modification of enzymes involved in glycogen breakdown provides an amplification cascade.
- Each step greatly increases the amount of substrate for the subsequent step.
- This results in a large amount of the product being formed.
- The mechanism by which a hormonal effect at the cell membrane is transmitted through a cascade of signaling events from the plasma membrane to produce a metabolic effect inside the cell is referred to as **signal transduction**.<sup>49</sup>

## Enzyme Regulation

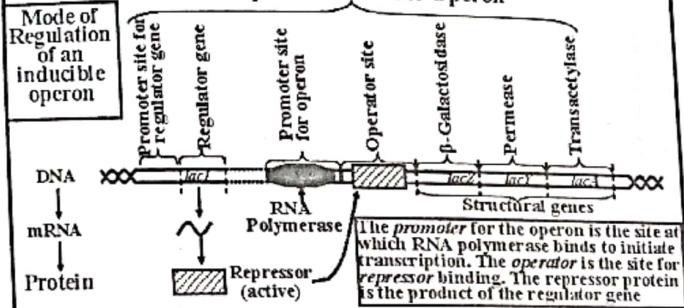
### Covalent modification in signal transduction



Phosphorylase kinase and glycogen phosphorylase are active in their phosphorylated form. Glycogen synthase on the other hand is active in the dephosphorylated form which is triggered by insulin.

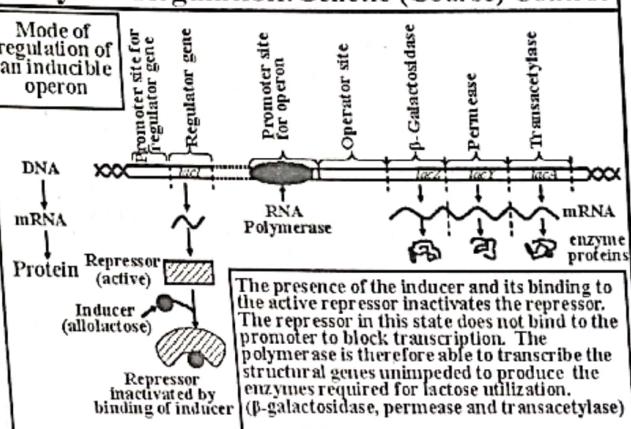
### Enzyme Regulation: Genetic (Coarse) Control

#### Components of the lac Operon



The lac operon is an inducible operon that regulates the expression of the genes for lactose utilization. In the absence of the inducer allolactose, which is an isomer of lactose, the operon is turned off because the repressor which is synthesized in the active form binds to the operator site and prevents (blocks) the RNA polymerase from transcribing the structural genes into mRNA and the translation of the RNA into the three enzymes needed for lactose utilization.<sup>501</sup>

### Enzyme Regulation: Genetic (Coarse) Control



## Enzymes in Disease Diagnosis

Diagnostic Enzyme	Disease
Creatine phosphokinase (CPK), lactate dehydrogenase (LDH), aspartate aminotransaminase (AST), Serum glutamate-oxaloacetate transaminase (SGOT)	Myocardial infarction (heart muscle death)
Alanine aminotransferase (ALT)	Liver disease
Acid phosphatase	Prostate cancer
Alkaline phosphatase (ALP)	Liver or bone diseases
Amylase	Pancreatic diseases or mumps

In most cases cells of the damaged organ release their enzyme content into the serum. High serum enzyme levels are therefore indicative of disease state of organ.<sup>503</sup>

## Blood Proteins

- The five liters of blood of a 70 kg person constitute about 7% of the body's total weight.
- The blood is composed of 52-62% liquid plasma and 38-48% cells. The plasma consists of mainly water (92%), protein (7%) and inorganic electrolytes. It is slightly alkaline (pH = 7.4) and just a little heavier than water (density = 1.06).
- The plasma (which consists of serum and clotting factors) contains 60-80 g/L of proteins, synthesized mostly by the liver.
- There are five main classes of blood plasma proteins. These include:  
**Albumin** (the most abundant ~55%)
  - contributes to osmotic pressure
  - serves as a transport molecule for certain ions ( $\text{Ca}^{2+}$ , organic anions) and poorly soluble molecules (fatty acids, bilirubin)

## Blood Proteins

### $\alpha$ -Globulins ( $\alpha_1$ and $\alpha_2 \sim 13\%$ )

- Include glycoproteins, lipoproteins (HDL, VLDL), haptoglobin (an acute phase protein), hemoglobin (oxygen transport protein), ceruloplasmin (a copper transport protein), prothrombin (blood clotting protein)

### $\beta$ -Globulins ( $\sim 13\%$ )

- Include transferrin (iron storage protein), lipoprotein (LDL)

### $\gamma$ -globulins ( $\sim 11\%$ )

- Include immunoglobulins (IgG, IgM, IgA, IgD and IgE) synthesized by lymphocytes. Protect against diseases.

### Fibrinogen ( $\sim 7\%$ )

- Involved in blood coagulation

## Defense Proteins/Cells

- Humans and other vertebrates have an **immune system** (network) that defends the body against invaders (viruses, bacteria, fungi, parasites, pollen etc.).
- The immune system consists of organs, tissue, cells and cell products that act as the body's armory (weapon) against infection and disease.
- Foreign substances which invade the body are referred to as **antigens**.
- The immune system can;
  - repair damage
  - signal cells to start and stop growing
  - alter cells to prevent viral attack etc.

## Defense Proteins/Cells

### The Innate Immunity System

- Functions as the first line of defence against pathogens.
- Pre-exists an infection or exposure to a foreign agent
- Produces a nonspecific response.
- Acts as a surveillance system that alerts the adaptive system to mount a more specific response.
- It consists of two components:
  - **Cellular components**
    - Phagocytes (monocytes and macrophages), dendritic cells, natural killer cells.
  - **Humoral components**
    - Include cytokines, complement system, acute phase proteins, defensins, lysozyme etc.

## Defense Proteins/Cells

### The Adaptive Immunity (System)

- More specific than the innate system.
- Does not pre-exist an infection or exposure to foreign agents.
- Memory is long-lasting.
- It also consists of two components that are interdependent.
  - **Cellular components**
    - Mainly T cells (helper T cells, cytotoxic cells, suppressor cells)
  - **Humoral components**
    - Consist of antibodies produced by B cells.

### THE IMMUNE SYSTEM (NETWORK)

The system of organs, tissues cells, and cell products that act as natural defences and protect the body against infection and disease. The immune system differentiates "self" from "nonself" and is divided into two arms.

#### INNATE SYSTEM

- Response is non-specific
- Comprise of a set of physical and chemical barriers as well as cellular components.
- Pre-exists infection or exposure to foreign antigen
- Has no memory
- Acts as the first line of defense against pathogens and a surveillance system to alert adaptive immune system to respond

#### ADAPTIVE SYSTEM

- Response is specific
- Does not pre-exist infection or exposure
- Exhibits long-lasting memory
- Alerted by innate system to respond

Cellular components  
Phagocytes (monocytes and macrophages), dendritic cells, natural killer cells

Humoral components  
Complement system, cytokines, acute phase proteins, defensins, lysozyme

Cellular components  
Mainly T-cells (helper, cytotoxic, suppressor)

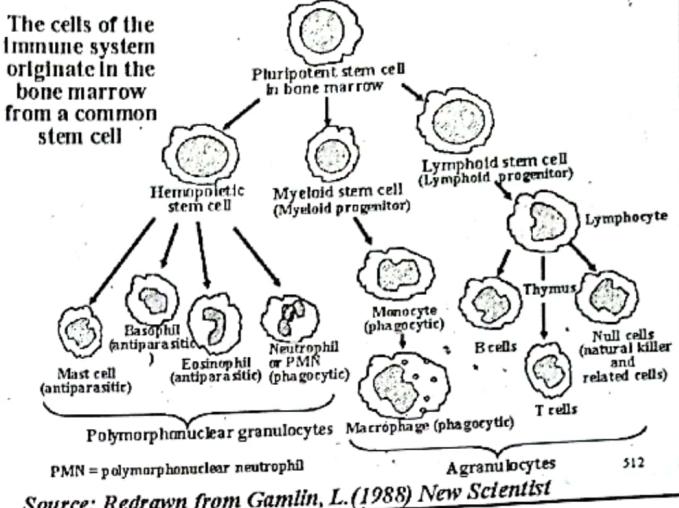
Humoral components  
Antibodies (immunoglobulins)

## Defense Proteins/Cells

### Blood Cells

- All blood cells are manufactured by stem cells in the bone marrow in a process called **hematopoiesis**.
- Stem cells produce hemocytoblasts that differentiate into the different types of blood cells.
- Hemocytoblasts mature into three blood cell types:
  - Erythrocytes** (red blood cells)
  - Leukocytes** (white blood cells)
  - Thrombocytes** (platelets)
- Leukocytes are subdivided into:
  - Granulocytes** (contain large granules in the cytoplasm)
    - » are polynuclear phagocytes
    - » consist of neutrophils (55-70%), eosinophils (1-3%), and basophils (0.5-1.0%)
  - Agranulocytes** (without granules)
    - » are mononuclear phagocytes
    - » consist of lymphocytes (made up of B cells and T cells) which circulate in the blood and lymph systems and finally lodge in the lymphoid organs, and monocytes (limited to the blood) which are the precursors of macrophages (distributed throughout body) and dendritic cells).

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## Defense Proteins/Cells

- Specific responses to pathogens are orchestrated by monocytes, macrophages, granulocytes and dendritic cells that are capable of discriminating between pathogens and self.
- Monocytes and macrophages play the central role of presenting antigens of pathogens to T-lymphocytes leading to the production of **cytokines** thus initiating and regulating both cellular and humoral immune responses

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## Defense Proteins/Cells

### Cytokines

- Belong to the **humoral innate immune system**.
- Are small proteins secreted by cells that affect the behavior of other cells. They carry messages from one cell group to another and mediate hematopoiesis, inflammation, and immunity.
- Are made by many different cells (especially those of the immune system) where and when they are needed.
- Are produced at low concentrations and have short half-lives
- Protect cells from attack by invading agents.
- Interact in a complex network to control immune cells when the cells respond to a "foreign" and potentially harmful invader (antigen).

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## Defense Proteins/Cells

- Cytokines cause many of the symptoms of bacterial and viral infections such as fever, headache, generalized aching, fatigue, weakness etc.
- Some of the cytokines produce dramatic mental and emotional effects.
- Overproduction of one or more cytokines may be responsible for non-specific hypersensitivity.
- Asthma, allergy, rheumatic diseases, autoimmune diseases, multiple sclerosis, diabetes, thyroiditis, psoriasis are examples of hypersensitive diseases which involve humoral and cell-mediated immunity.

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## Defense Proteins/Cells

- In the past, cytokines could be made in the laboratory only by growing body cells in a test tube in the presence of a virus, a bacterium or other harmful agent.
- Recombinant DNA technology now allows scientists to produce cytokines from bacteria.
- Cytokines may help fight infectious diseases by improving the body's response to vaccination by stimulating lymphocytes.
- A feature of the network of cytokines is that one often enhances the function of others.
- Interpretation of message depends on the cytokine, the cell it acts on and other messages being received by the cell.

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## Defense Proteins/Cells

- Cytokines deliver their message by sticking to a cell or binding to a receptor protein on the cell surface.
- Cytokines are extremely potent. For example a picogram ( $10^{-12}$  g) of a cytokine is sufficient to protect one million cells in the test tube from attack by 10 million viral particles.
- Cytokines produced by monocytes are called **monokines**. Those produced by lymphocytes are called **lymphokines**.
- Chemokines** are a group of low molecular weight polypeptides (90-130 amino acids). They selectively and specifically control the adhesion, chemotaxis, and activation of many types of leukocytes.

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## Defense Proteins/Cells

### The cytokines include:

- Interferons (IFN)
- Tumor Necrosis Factor (TNF)
- Interleukins (IL)
- Colony Stimulating Factor (CSF)

Pro-inflammatory cytokines	Anti-inflammatory cytokines
TNF- $\alpha$	IL-2
IL-1	IL-4
IL-6	IL-10
IL-12	IL-13
INF- $\gamma$	

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## Defense Proteins/Cells

### • Interferons

- are cytokines which change the behavior of cells and make them produce other cytokines.  
Humans have three types of interferons ( $\alpha$ ,  $\beta$ ,  $\gamma$ ). The  $\alpha$  and  $\beta$  bind one receptor and the  $\gamma$  to another on the same cell surface.
- were considered as antibiotics of viral infection but have been shown not to act on the virus but on the cells they infect.
- do not only protect cells from viral infection. They can stop growing cells and can also stimulate the cells to produce major histocompatibility complex (MHC) proteins which are important to the human immune system.

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## Defense Proteins/Cells

- Most immune cells (monocytes, macrophages) present antigens to T-cells on MHC
- MHC proteins are produced within the cell to interact with and remove antigens that have found their way into the cell, to the surface for destruction. Self/nonself recognition is achieved by having every cell display a marker based on MHC. Any cell not displaying this marker is treated as non-self and attacked. A breakdown of the process allows the immune system to attack self-cells. This is the cause of autoimmune diseases.

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## Defense Proteins/Cells

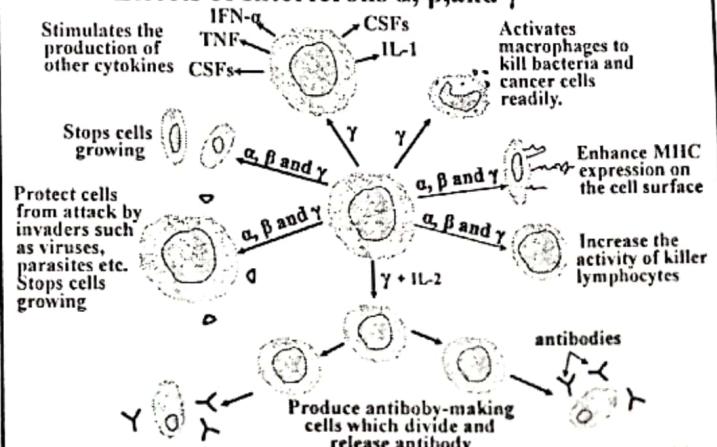
### Interferons

- also change the behavior of cells and make them produce other cytokines.
- have anti-viral and anti-tumor effects and have been used in the treatment of diseases such as:
  - Leukemia (cancer of blood cells),
  - Lymphomas (tumors of lymph nodes),
  - Melanoma (skin cancers),
  - Hepatitis B (a liver disease),
  - Genital warts,
  - Colds etc.
- have been shown to act directly on tumor cells and inhibit growth.
- are more effective in preventing viral infections if given before the viral attack, but are not very effective once an infection is established.

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## Defense Proteins/Cells

### Effects of Interferons $\alpha$ , $\beta$ , and $\gamma$



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Source: Redrawn from Balkwill, F. (1988) New Scientist

## Defense Proteins/Cells

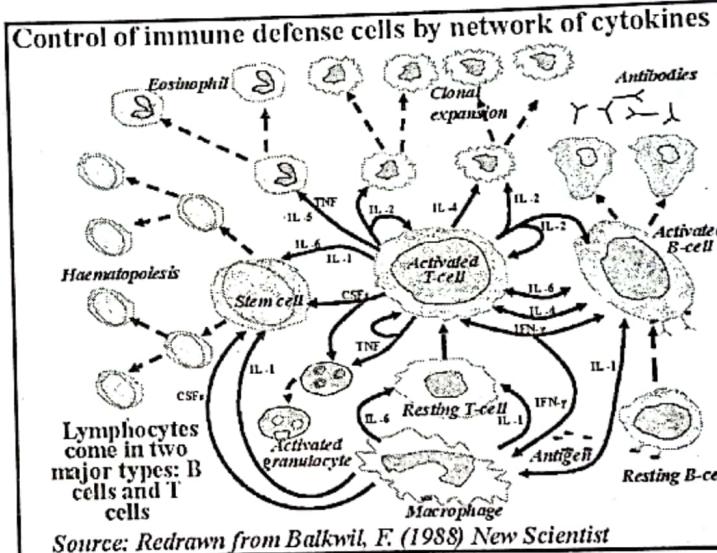
- **Tumor Necrosis Factor (TNF)**
  - Necrosis means death of living tissue.
  - TNF kills some cancer cells, stimulate growth of connective tissue cells, protect some cells against viral infections (but not as many cell types as the interferons).
  - Direct injection of TNF into humans has been found to be effective, though not practical in most cancers)
- **Interleukins (IL)**
- There are about 15 interleukins
  - Interleukin-1 (secreted by activated macrophages)
    - induces fever (an endogenous pyrogen), fatigue, excessive sleepiness,
    - stimulates bone cells,
    - induces breakdown of cartilage,
    - is involved in growth and differentiation of white blood cells.

## Defense Proteins/Cells

- Interleukin-2
  - acts mainly on white blood cells called lymphocytes and makes them grow and divide more rapidly in tissue culture.
  - turns lymphocytes into active "killer" cells, lymphokine activated killer (LAK) cells, that recognize and destroy cancer or damaged cells.
  - may help fight disease by improving the body's response to vaccination by stimulating lymphocytes.
  - produces fever, chills, and mental changes which range from confusion and depression to dementia to somnolence and coma.
- Interleukin-5
  - is very specific and acts only on special white blood cells (eosinophils) which are important in allergic responses and resistance to parasites.

## Defense Proteins/Cells

- **Colony Stimulating Factors (CSF)**
  - First identified by their ability to make blood cells grow in clumps or colonies under laboratory conditions.
  - Boost white cells in certain disease conditions.
  - Stimulate recovery of the bone marrow in patients who have received large doses of radiation to kill tumor cells.
  - Inclusion of CSF in current chemotherapeutic treatments removes the worrying aspects of toxicity due to destruction of patients immune defenses through destruction of white blood cells.



## Defense Proteins/Cells

- Immunoglobulins (antibodies) are key players in the adaptive immune system.
- Making the correct immunoglobulin against an antigen takes a longer time (weeks or months) compared to the signaling by neurotransmitters which take milliseconds and hormones which take seconds, minutes or hours.

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## Defense Proteins/Cells

### Structure of immunoglobulins

- Immunoglobulins are glycoproteins. They contain four peptide chains.
- Immunoglobulins have two types of polypeptide chains (light and heavy).
- Each chain has a constant region at the carboxyl terminal and a variable region at the amino terminal.
- The chains are held together in folds (domains) by a combination of noncovalent interactions and covalent bonds (disulfide linkages) and arranged in a Y-shaped quaternary structure.

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## Defense Proteins/Cells

### Structure of immunoglobulins

- The light chain consists of one variable domain ( $V_L$ ) and one constant domain ( $C_L$ ). The heavy chain consists of a variable domain ( $V_H$ ) and three separate constant domains ( $C_{H1,2,3}$ )
- The variable regions are customized to bind antigen whereas effector functions, such as complement activation, cell membrane receptor interaction and transplacental transfer reside in the constant region.

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## Defense Proteins/Cells

### Structure of immunoglobulins

- The “hinge” region between constant heavy domain one ( $C_{H1}$ ) and constant heavy domain two ( $C_{H2}$ ) is readily accessible to proteolytic attack by enzymes such as papain or pepsin.
- Proteolytic digestion of immunoglobulins produces two Fab fragments and one Fc fragment.
- In vivo* Fab or F(ab') fragments are used as diagnostic and therapeutic agents.

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## Defense Proteins/Cells

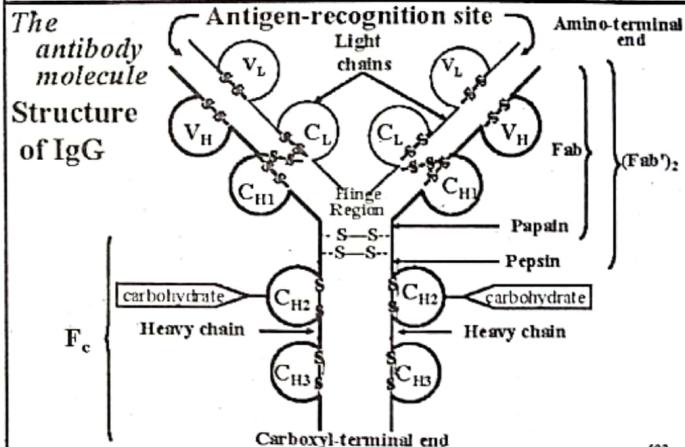
### Structure of immunoglobulins

- The amino end fragment, Fab, retains its antigen binding capabilities but has low nonspecific binding.
- The Fab portion is best suited to those situations where the antigen binding capabilities are desired, without effector functions.
- The Fab fragment is made up of one light chain and the variable region of the heavy chain.

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## Defense Proteins/Cells

### Structure of IgG



Source: Redrawn from Pierce ImmunoTechnology Catalogue and Handbook

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## Defense Proteins/Cells

### Structure of immunoglobulins

- The Fc fragment contains the constant regions of the heavy chain linked by disulphide and noncovalent bonds.
- The Fab portion is used for executing the primary function of immunoglobulins (specific binding to antigen).
- The Fc portion is for the secondary function which is the consequence of the antibody-antigen interaction.

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## Defense Proteins/Cells

- Immunoglobulins are divided into five main types. (IgA, IgD, IgE, IgG and IgM) depending on the amino acid sequence of the constant portion of the heavy chain (Fc).
- Immunoglobulin G is the principal class of antibody found in the serum. It enters tissues freely and can cross membranes and pass from mother to fetus before birth.
- Because of its relative abundance and excellent specificity towards antigens, IgG is used extensively in immunological research and clinical diagnostics.
- The IgG class can be divided into four subclasses in humans (IgG1, IgG2, IgG3 and IgG4).
- IgG1 and IgG3 are cytophilic whereas IgG2 and IgG4 are noncytophilic.

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## Defense Proteins/Cells

- When challenged with an antigen (immunogen), an individual elicits a primary response by producing short-acting IgM antibodies. It is the first antibody produced in response to an infection.
  - IgA and IgG are produced as secondary response immunoglobulins and have longer lasting effects.
  - Antibodies elicit their effect by binding directly with and inactivating the function of an antigen or indirectly by acting in collaboration with monocytes by binding to receptors on the monocytes with their Fc portion.
  - In the latter response the antibodies induce monocytes to secrete cytokines and other soluble factors that inhibit antigen function.
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## Signal Proteins/Molecules

- Hormones are a diverse group of molecules secreted by specific tissues (referred to as the endocrine glands) and travel in the blood stream to their site of action.
- The distinction between a hormone and a neurotransmitter is a physiological and not a chemical one.
- A compound which acts over a short distance ( $10^{-6}$  cm), such as across a synapse, is referred to as a neurotransmitter.
- A significant percentage of neurotransmitter molecules are either amino acids or amino acid derivatives.
- Neurotransmitters are either excitatory or inhibitory.

## Signal Proteins/Molecules

- A compound that acts over a longer distance ( $10^1$  cm) from the source of production and travels through the blood stream to the target site is referred to as a **hormone**.
  - There are three major classes of hormones:
    - steroid hormones which are derived from cholesterol,
    - amino acid derived hormones, and
    - peptide hormones.
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## Signal Proteins/Molecules

Neurotransmitter/ Hormone	Source	Function
Luteinizing hormone (LH)	Anterior pituitary gland	Induces ovulation
Prolactin	Anterior pituitary gland	Stimulates growth of mammary gland
Mamotropin	Anterior pituitary gland	Controls lactation
Vasopressin	Posterior pituitary gland	Regulates reabsorption of water by kidney. Controls blood pressure.
Oxytocin	Posterior pituitary gland	Stimulates contraction of uterus and milk production by mammary gland
Thyroxine	Thyroid	Regulates growth and metabolic rate
Insulin	Beta cells of pancreatic islets	Regulation of carbohydrate metabolism
Glucagon	Alpha cells of pancreatic islets	Regulation of liver glycogenolysis

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## Signal Proteins/Molecules

Neurotransmitter/ Hormone	Source	Function
Parathyroid hormone	Parathyroid gland	Controls excretion of phosphate by kidney. Increases blood calcium.
Progesterone	ovaries	Development of female sex characteristics
Testosterone	Testes	Development of male sex characteristics.
Epinephrine	Adrenal medulla	Regulation of liver and muscle glycogenolysis
Norepinephrine	Adrenal medulla	Regulation of liver and muscle glycogenolysis
Cortisol	Adrenal cortex	Regulation of carbohydrate metabolism
Aldosterone	Adrenal cortex	Regulation of mineral absorption

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## Signal Proteins/Molecules

- The first step in transmitting a signal into a cell involves the binding of the signal to a receptor.
  - For some signals the receptor is inside the cell (e.g. steroid hormones). The hormone crosses the membrane and binds its receptor in or near the nucleus of the cell.
  - For other signals (growth factors and hormones such as insulin), the receptor which is embedded in the cell membrane is an enzyme (e.g. tyrosine kinase).
  - Some neurotransmitters bind to receptors embedded in the cell membrane that function as ion channels.
  - The receptors of photons, certain neurotransmitters and hormones embedded in the membrane are coupled to various enzymes by G-proteins which are key players in intracellular signal transduction mechanism.
- 540

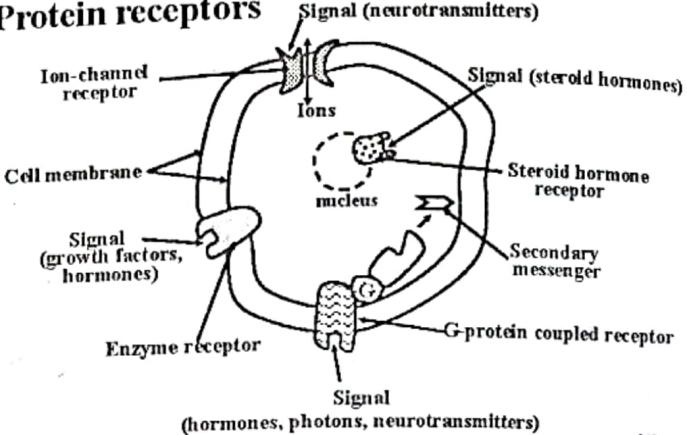
## Signal Proteins/Molecules

- The ligand-binding/G-protein/adenylate cyclase amplification cascade activates proteins through phosphorylation or dephosphorylation
- This cascade has a wide range of targets and effects in the cell including;
  - opening or closing of ion-gated channels
  - activation of enzymes, etc.
- Two G-proteins, called  $G_s$  (for stimulatory) and  $G_i$  (for inhibitory), are involved, each coupled to its own receptor. The two work in opposition to each other to regulate the activity of adenylate cyclase.

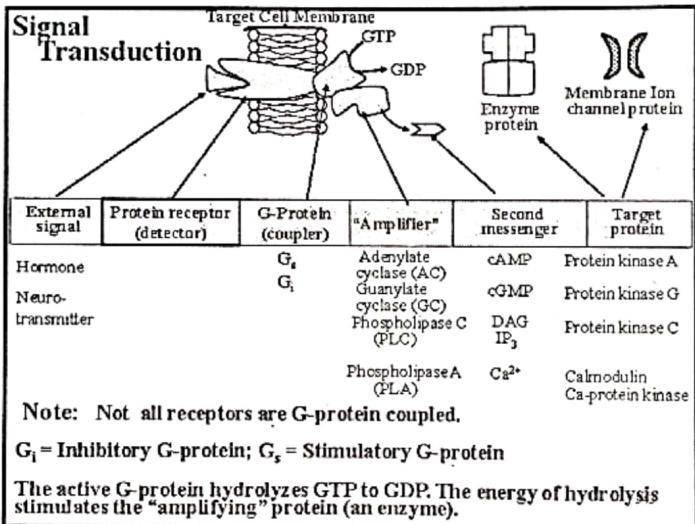
541

## Signal Proteins/Molecules

### Protein receptors



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## Signal Proteins/Molecules

Signal Molecule	Function
Insulin receptor	Induces tyrosine kinase activity
G-protein	Transduces hormone stimulus to adenylate cyclase
$\beta$ -Adrenergic receptor	Binds epinephrine
Adenylate cyclase	Activated by $G_s$ -GTP, converts ATP to cAMP
Phosphodiesterase	Breaks down cAMP to AMP
cAMP	Binds regulatory subunit of protein kinase A
IP <sub>3</sub>	Raises intracellular Ca <sup>2+</sup> levels
Ca <sup>2+</sup>	Activates protein kinases, activates Ca <sup>2+</sup> modulated protein
PIP <sub>2</sub>	Cleaved by phospholipase C
DAG	Activates protein kinase C
Arachidonic acid	Produced from PIP <sub>2</sub> breakdown
Nitric oxide (NO) (both a neurotransmitter and second messenger)	Activates guanyl cyclase

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## Signal Proteins/Molecules

### Target Proteins for Protein Kinases

**cAMP-dependent protein kinases** (e.g. kinase A)

- glycogen phosphorylase (**activated**)
- glycogen synthase (**inhibited**)
- triacylglycerol lipase (**activated**)
- cholesterol ester lipase (**activated**)
- protein phosphate inhibitor-1 (**activated**)

### Protein kinase C

Na<sup>+</sup>/H<sup>+</sup> channel of plasma membrane (**activated**)

### Ca<sup>2+</sup>/Calmodulin-activated protein kinases

- glycogen phosphorylase (**activated**)
- myosin (light chain) (**activated**)

545

## Signal Proteins/ Molecules

### Target Proteins for Protein Phosphatases

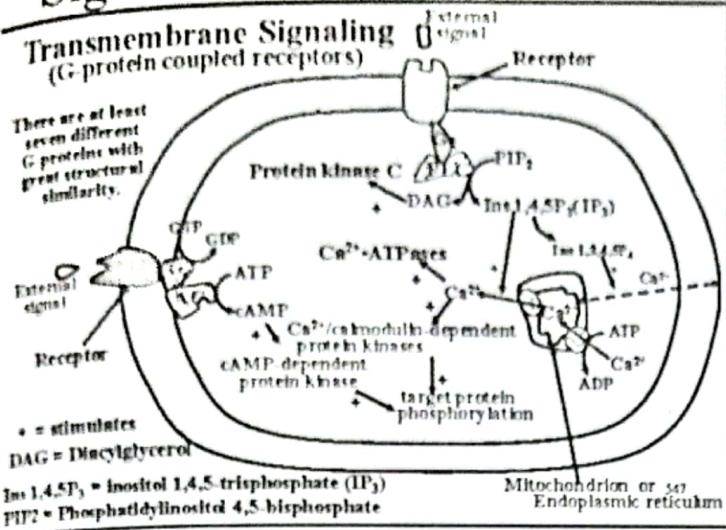
Ca<sup>2+</sup>/ Calmodulin-activated protein phosphatase  
protein phosphatase inhibitor-1 (**inhibited**)

### Other protein phosphatases

- glycogen synthase (**activated**)
- glycogen phosphorylase (**inhibited**)
- pyruvate dehydrogenase (**activated**)

546

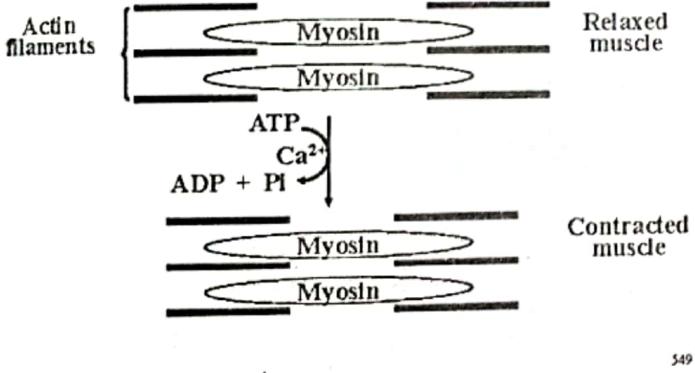
# Signal Proteins/Molecules



# Contraction Proteins

- Muscles consist of a fiber composed of two protein filaments arranged in alternating rows.
- The thick filament is called **myosin** and a thin filament called **actin**.
- During muscle contraction the actin filaments slide towards each other, resulting in the shortening of the muscle fibers.
- The pulling of the actin filaments by myosin filaments during muscle contraction is initiated by calcium ions, released by opening of calcium channels in the muscle cell membrane by a nerve impulse. The energy is provided by ATP hydrolysis.

# Contraction Proteins



# Natural Protein Sweetners

- Thaumatin (1600 x sucrose)
- Monellin (1200 to 3200 x sucrose)
- Brazzein
- Curculin (20,000 x sucrose)
- Miraculin
  - not intrinsically sweet
  - modifies or alters the action of taste receptors to perceive sweet taste when exposed to sour substances.

# Estimation of Protein Amount

- Protein determination is constantly required in biochemical work. Choice of method depends on the nature of the protein, other components present in sample, accuracy and sensitivity. Methods used include:
  - Ultraviolet (UV) absorption** (sensitivity: 50 µg-1mg)
    - A rapid and fairly sensitive method for estimation of protein amount.
    - Most proteins exhibit UV absorption maximum at 280 nm.
    - This is due to the presence of the aromatic amino acids tyrosine, and tryptophan and to a lesser extent phenylalanine.

## Estimation of Protein Amount

### UV Absorbance of Aromatic Amino Acids

Amino acid	$\lambda_{\text{max}}$ (nm)	Molar extinction coefficient ( $M^{-1}$ )
Tryptophan	274	$5.56 \times 10^3$
Tyrosine	275	$1.40 \times 10^3$
Phenylalanine	258	$0.20 \times 10^3$

Quantitatively, it is only the absorption by tyrosine and tryptophan that counts. The UV absorbances at 280 nm of a particular protein depends on its content of tyrosine and tryptophan. Because the combined levels of these amino acids are generally constant in many proteins, the concentration of a pure protein in solution is generally proportional to the absorbance at 280 nm.

## Estimation of Protein Amount

### Ultraviolet absorption

- Nucleic acids absorb strongly at 260 nm and interfere with protein estimation at 280 nm when present.
- Often the 280/260 ratio is used as a criterion for purity.
- For a pure protein, a  $A_{280}/A_{260}$  ratio should have a value of 1.8. Lower values suggest contamination with nucleic acids.
- The extinction coefficient of a 1 mg of pure protein/ml solution at 280 nm is approximately 1.0 when viewed through a 1-cm path length.

554

## Estimation of Protein Amount

### - Folin-Lowry (sensitivity: ~5 $\mu\text{g}$ )

- Assay based on the reaction of the phenolic hydroxyl group of tyrosine with alkaline copper solution to form a Cu-protein complex which reduces a Lowry Folin-Ciocalteau reagent. Other phenolic compounds interfere with assay.

### - Biuret (sensitivity: 1-20 mg)

- Assay based on the ability of compounds with two or more peptide bonds to form a complex with alkaline copper sulfate solution. Name of test comes from the compound biuret. Assay is specific for peptide bonds. Not very sensitive.

- Nitrogen content:** Proteins are composed of about 16% nitrogen. By measuring the nitrogen content as ammonia, one can obtain a rough estimate of protein content using the formula: Grams of protein =  $100/16 \times$  grams of nitrogen.

7

## Nucleic Acids

555

## Chromosomes and Genes

- Self-replication is a central process for the continuance of life.
- Organisms perpetuate their kind through reproduction by;
  - simple duplication (cell division) as in bacteria.
  - sexual reproduction as in plants & animals.
- Reproduction entails the transmission of genetic information from parents to progeny.
- Genetic information is contained in DNA which is stored in the chromosomes in the nuclei of cells.
- Each chromosome contains a large molecule of DNA.

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## Chromosomes and Genes

- Arranged along chromosomal DNA are the basic units of heredity, called GENES (the functional units of the chromosome).
- A gene is a part of the chromosome DNA that codes for a single protein.
- The complete set genes and regulatory elements of an organism is referred to as its GENOME.
- The human genome consists of about three billion base pairs and about 50,000 genes.

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# Chromosomes and Genes

Genetic material of prokaryotes is stored in a single chromosome, in a single molecule of nucleic acid.

A bacterium cell has 3000-4000 genes in the molecule of nucleic acid in one chromosome. *E. coli* has a chromosome length of ~1 mm

Eukaryotic cells have several chromosomes. Each chromosome is present in two (diploid), each with one giant DNA molecule.

The haploid chromosome set (23) of humans contains approximately 1m DNA.

Each human chromosome has between 15 to 85 mm DNA.

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# Chromosomes and Genes

- Chromosomes, the functional units of the genome, are complexes of nucleic acids, proteins, and other molecules located in nucleus of eukaryotic cells.
- Despite obvious differences between people greater than 99% of DNA are identical in everybody.
- Differences (hair colour, height, susceptibility to cancer, tendency towards obesity etc.) lie in the remaining fraction of a percent.
- All mammals from mice to humans have similar genes and make similar proteins. They turn out so different because they switch genes on and off in different ways.

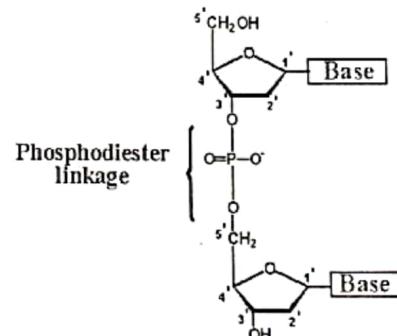
## Nucleic Acids

- Are important, high molecular weight polymeric cell constituents.
- The monomeric units are called **nucleotides**. Hence nucleic acids are also called **polynucleotides**.
- Nucleotides in a polynucleotide are linked by **phosphodiester** bonds.
- Each nucleotide consists of a purine or pyrimidine base linked to a pentose sugar esterified with phosphoric acid between the 5'-C of the sugar of one nucleotide and 3'-OH of next nucleotide.

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## Nucleic Acids

### Phosphodiester Linkage



Sugars in DNA and RNA are linked by phosphate through phosphodiester bonds

## Nucleic Acids

Source	Mol. Wt	Number of bases
tRNA	$2 \times 10^4$	90
mRNA	$5 \times 10^5$	1500
pBR322 plasmid	$3 \times 10^6 *$	4,500
Polio virus	$3 \times 10^6$	4,500
Bacteriophage λ	$3 \times 10^7$	45,000
Yeast chromosome	$6 \times 10^8$	$9 \times 10^5$
Bacterial chromosome	$2 \times 10^9$	$3 \times 10^6$
Human chromosome	$8 \times 10^{10}$	$1 \times 10^8$

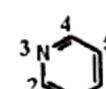
Some amphibia, fishes and algae contain more DNA than mammals

\* upper limit for proteins

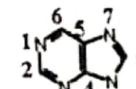
563

## Nucleic Acids

- DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) are polymers of nucleotides.
- Nucleotides are glycosides made up of;
  - a pentose sugar (ribose or deoxyribose),
  - a nitrogenous base,
    - the purines: adenine (A) and guanine (G) and
    - the pyrimidines: cytosine (C), thymine (T), and uracil (U)
  - phosphate



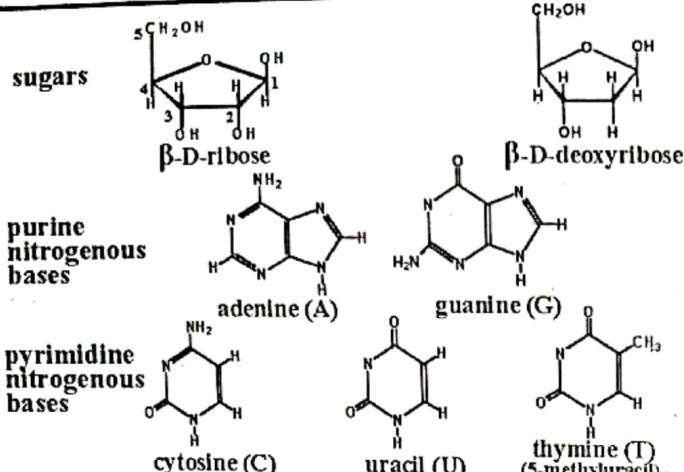
pyrimidine



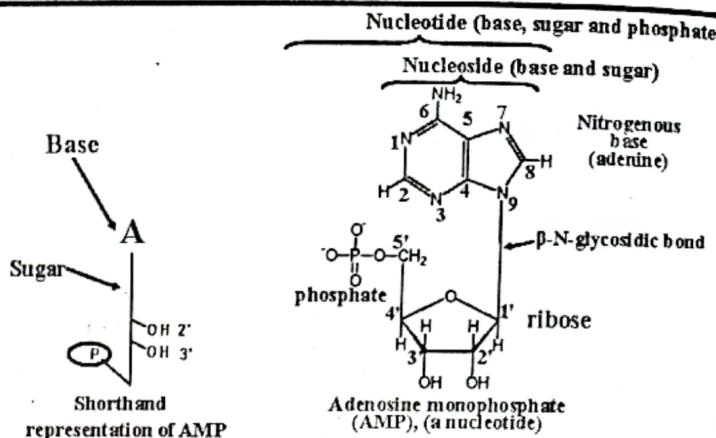
purine

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## Nucleic Acids



## Nucleotides



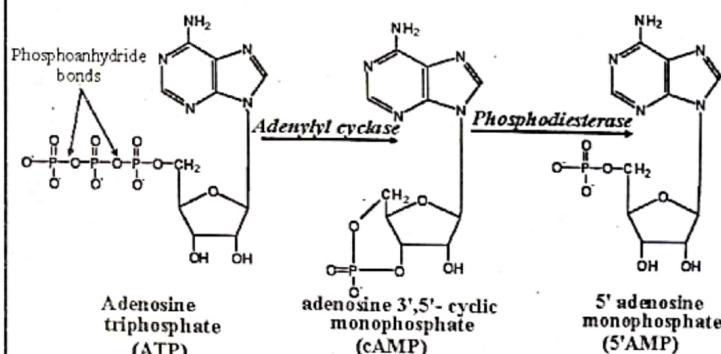
In order to differentiate between carbon atoms in the sugar and the carbon and nitrogen atoms in the bases, the carbon atoms in the sugars are numbered 1', 2', 3' and so on.

## Nucleosides and Nucleotides

Base	Nucleoside	Nucleotide
Adenine (A)	Adenosine (A do)	A denyllic acid A deoxyadenosine monophosphate (AMP)
Guanine (G)	Guanosine (Guo)	Guanylic acid Guanosine monophosphate (GMP)
Cytosine (C)	Cytidine (Cyd)	Cytidylic acid Cytidine monophosphate (CMP)
Uracil (U)	Uridine (Urd)	Uridylic acid Uridine monophosphate (UMP)
Adenine	Deoxyadenosine (dAdo, dA)	Deoxyadenylic acid Deoxyadenosine monophosphate (dAMP)
Guanine	Deoxyguanosine (dGuo, dG)	Deoxyguanylic acid Deoxyguanosine monophosphate (dGMP)
Cytosine	Deoxycytidine (dCyd, dC)	Deoxycytidylic acid Deoxycytidine monophosphate (dCMP)
Thymine (T)	Deoxythymidine (dTd, dT)	Deoxythymyldic acid Deoxythymidine monophosphate (dTMP)

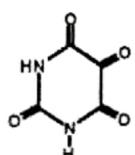
567

## Nucleotides

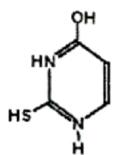


## Pyrimidine Bases

- Two pyrimidine derivatives are not found in nucleic acids but are of physiological interest.
  - Alloxan:** causes glycosuria when administered to experimental animals. Produces diabetes by selective necrosis of pancreatic islet  $\beta$ -cells.
  - Thiouracil:** derivatives used in treatment of hyperthyroidism.



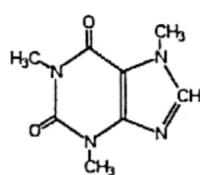
Alloxan



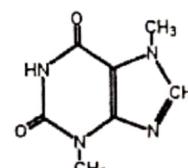
2-thiouracil

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## Purine Bases



1,3,7-trimethylxanthine (caffeine)

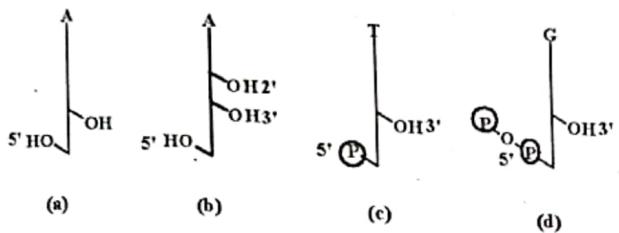


3,7-dimethylxanthine (theobromine in cocoa)

Caffeine is an alkaloid present in coffee, tea, chocolate, cocoa etc. It is a stimulant of the central nervous system. It increases alertness, but may cause nervousness and insomnia. It is used in certain pain relievers to counteract the drowsiness caused by antihistamine.

## Self-Test Question

Name the following nucleosides and nucleotides



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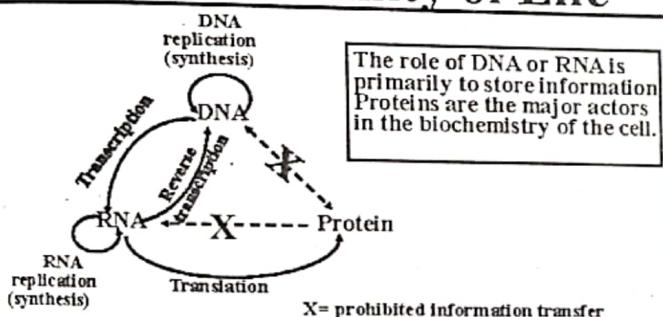
## Self-Test Question

1. Draw molecular structures of the following compounds.

- 2'-deoxythymidine 5'-monophosphate
- Guanosine
- Uridine 5'-diphosphate
- Adenosine 2',3'-cyclic monophosphate

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## Molecular Trinity of Life



The general statement that information is transferred from DNA to RNA to protein is referred to as the *CENTRAL DOGMA OF MOLECULAR BIOLOGY*

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## DNA Structure

### Organization of DNA in Chromosomes

- Chemical analysis, electron microscopy and X-ray diffraction studies provided a picture of chromosome structure in eukaryotes.
- The nucleoprotein material (substance) which chromosomes are made of is referred to as **chromatin**.
- Chemical analysis of chromatin shows the presence of DNA, Protein, RNA (in lesser amount)

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## DNA Structure

### Organization of DNA in Chromosomes

- Two major classes of proteins are found in chromosomes; **histones** and **non-histone proteins**
  - Histones**
    - are positively charged at neutral pH.
    - function in the organization of DNA into compact forms through electrostatic interaction between the positively charged histone proteins and the negatively charged phosphate of DNA.
    - histones are present in chromatin of higher eukaryotes in amounts equivalent to DNA (i.e. weight by weight)

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## DNA Structure

### Organization of DNA in Chromosomes

- Histones consists of 5 different proteins (H1, H2a, H2b, H3, H4) classified according to size, charge and amino acid composition.
- They are present in molar ratios of 1H1:2H2a:2H2b:2H3:2H4
- Non-histone proteins**
  - A heterogenous group of phosphorylated acidic proteins
  - Composition not fully known. Varies among different cell types of the same organism.

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## DNA Structure

### Nucleosomes

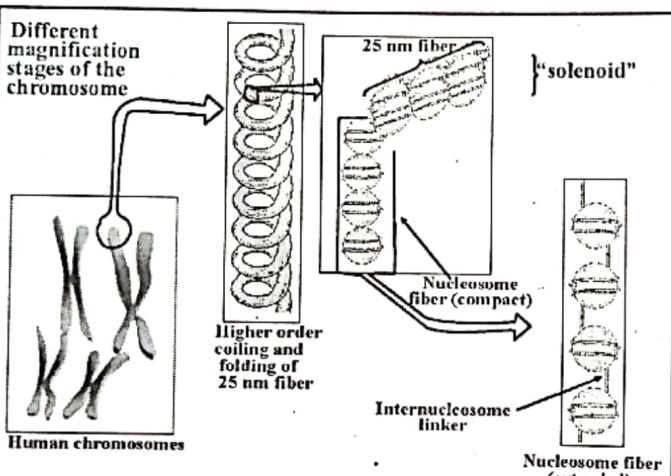
- The DNA duplex wraps around a core of histones proteins to form the basic structural subunit of chromatin called **Nucleosomes**.
- Nucleosomes consists of about 140 nucleotide pairs of DNA wound around a core composed of an octamer of histone molecules.
- The octamer consists of two molecules each of four of the histone proteins (H2a, H2b, H3, and H4).
- An "internucleosome" thread or "linker" (15 to 100 nucleotide pairs) connects the core particles to give a chain of nucleosomes.
- Packaging DNA into nucleosomes reduces the length of DNA by a factor of about 7.<sup>577</sup>

## DNA Structure

### Nucleosomes

- Linker regions are sites of nuclease attack.
- The chain of nucleosomes are packed, without a detectable linker, to yield a 10 nm diameter nucleosome fiber.
- The 10 nm fiber is wound into a higher order to form a highly compact, highly supercoiled ("super supercoil") solenoid fiber (a 25 nm fiber).
- The solenoid fiber generated is then packed into chromatin threads, which are then condensed into the chromosome.
- Usually, two chromatid threads joined at the centromere to form a chromosome.

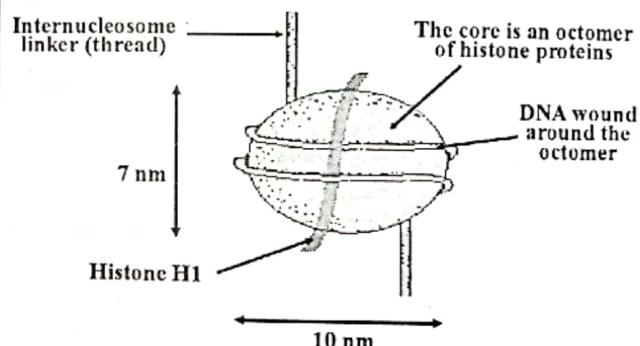
578



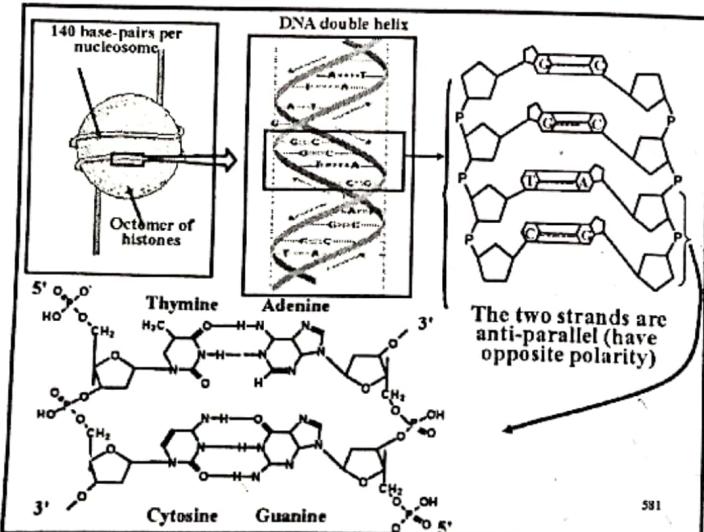
Source: Redrawn from Gardner, E.J. and Snustad, D. P. (1981) Principles of Genetics

## DNA Structure

### Nucleosome



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## Elucidation of DNA Structure

- The structure of DNA was elucidated by Watson and Crick in 1953.
- This was a significant event in the history of science.
- Neither was working on DNA at the time.
- Motivation:
  - Provide a solution to basic secrets of life
  - Win Nobel Prize
- Won Nobel Prize in Physiology & Medicine in 1962.

582

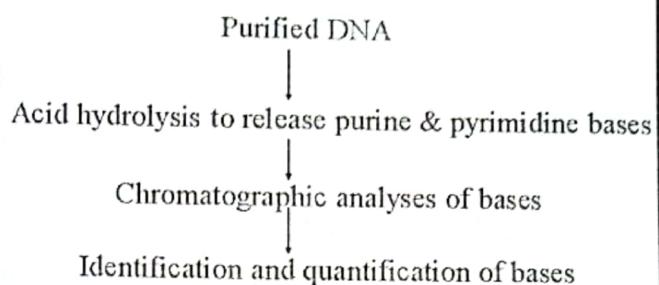
## Elucidation of DNA Structure

- Watson and Crick decided to build a model based on available information and reasoned that the model must be compatible with the biological properties of the genetic material namely;
  - accurate duplication
  - ability to carry genetic information
  - potential for mutation
  - satisfy the chemical requirements of Chargaff's rule
- Chargaff's experiment consisted of the analysis of purine and pyrimidine contents of DNA in different cells.

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## Elucidation of DNA Structure

### Chargaff's Experiment



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## Elucidation of DNA Structure

### Results of Chargaff's Experiment

Organism	Tissue	Adenine	Thymine	Guanine	Cytosine
Man	Sperm	1.00	1.07	0.62	0.62
	Thymus	1.00	1.00	0.68	0.57
Yeast	—	1.00	0.97	0.60	0.50
Avian tubercle	—	1.00	0.92	2.32	2.16

Source: Adapted from Woods, R.A. (1980) Biochemical Genetics

585

## Elucidation of DNA Structure

### Deductions from Chargaff's Experiment

- molar ratio of

$$\frac{\text{Total pyrimidines}}{\text{Total purines}} = \frac{C + T}{A + G} = 1$$

- molar ratio of

$$A/T = 1 \text{ and } G/C = 1$$

$$\%C = \%G \text{ and } \%A = \%T$$

This phenomenon of molar equivalence is referred to as **Chargaff's rule**

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## Elucidation of DNA Structure

- Chargaff's rule was critical in the elucidation of DNA structure.
  - data indicated complementarity in molecule.
- X-ray analysis of DNA by Franklin and Wilkins;
  - demonstrated helical configuration of DNA with repeat patterns at 3.4 nm and 0.34 nm.
- Analysis of available data suggested that,
  - the structure consisted of a helix, with 10 bases per twist or turn of helix.
  - the helix was made up of purine and pyrimidine bases that were 0.34 nm apart.

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## Elucidation of DNA Structure

### Model building

- Watson and Crick reasoned that the most fruitful approach was to build models to satisfy the **physical** and **chemical** requirements of DNA.
- The most plausible scheme they thought was to have a double helix with two sugar phosphate backbones connected by pairs of H-bonded bases.
- The **Chargaff's rule**, **physical**, and **chemical** requirements were satisfied only by
  - purine:pyrimidine** pairs
- Molecular compatibility (H-bonding) required base pairing between,
  - adenine:thymine** and
  - guanine:cytosine**

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## Elucidation of DNA Structure

### Features of Watson and Crick Model of DNA

- DNA has a double helical structure with sugar phosphate backbones.
- The nucleotides of the backbones are linked by phosphodiester bonds.
- The two polynucleotide chains are held together by H-bonded bases.
- Base pairing is specific,
  - A-T and G-C
- Strands of the double helix are complementary (complementarity makes DNA suited to store and transmit genetic information).

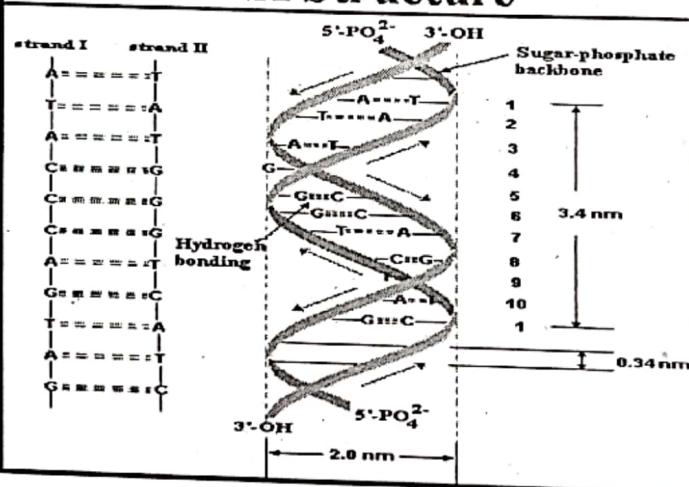
## Elucidation of DNA Structure

### Features of Watson and Crick Model of DNA

- Base pairs are stacked at 0.34 nm apart.
- There are 10 base pairs per turn of helix.
- Bases lie almost perpendicular to longitudinal axis of the double helix.
- Sugar-phosphate backbones are anti-parallel (has opposite chemical polarity).
  - polarity important in the mechanism of DNA replication.

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## DNA Structure



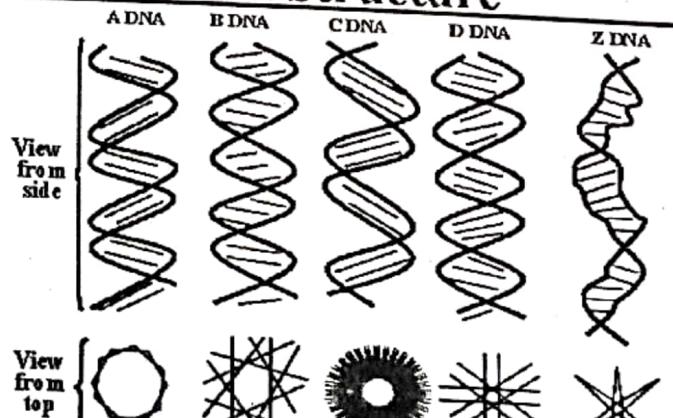
## DNA Structure

### Variants of secondary structure

- The Watson and Crick structure (called B-DNA) is just one of the many alternative conformations of the double helix.
- Although most natural DNA is in the B form, other forms (A, C, D and Z) have been characterized.

592

## DNA Structure



Source: Redrawn from Scott, A. (1984). New Scientist

## DNA Structure

	A-DNA	B-DNA (Watson & Crick DNA)	Z-DNA
Helix sense	Right handed	Right handed	Left handed
Nucleotide pairs per turn (pitch)	11	10	12
Rise per nucleotide	0.26 nm	0.34 nm	0.37 nm
Helix pitch (twist)	2.8 nm	3.4 nm	4.5 nm
Base pair tilt	20°	6°	7°
Rotation per nucleotide	33°	36°	60°
H <sub>2</sub> O in environment	<40 %	>40 %	—

A, B, C and D are right-handed forms  
Zubay, G. (1983). Biochemistry. Addison-Wesley Publishing Co., 3<sup>rd</sup> Massachusetts, USA

## DNA Structure

### Tertiary structure

- Nucleic acid tertiary structure is not well characterized.
- It is significant for tRNA, rRNA, chromatin and ribozyme structure, and hence plays an important role in the regulation of replication, transcription and translation.

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## Self-Test Questions

1. If a virus particle contains double-stranded DNA with 500,000 base pairs
  - (i) How many nucleotides would be present?
  - (ii) How many complete turns would occur on each strand?
  - (iii) How many atoms of phosphorus would be present?
  - (iv) What would be the length of the DNA configuration in the virus?
2. What is the difference, if any, between chromosomes, DNA double helix, and genes?

396

## Self-Test Questions

3. The base composition of one of the DNA chains of a DNA double helix contains 18 mol-%A, 35 mol-%T, 26 mol-%C, and 21 mol-%G
  - (a) What is the base composition of the complementary DNA chain?
  - (b) Is the total amount of purine bases equal to the total amount of pyrimidine bases for the DNA double helix?

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## Physical Properties of Nucleic Acids

### • Stability

- Double stranded(ds) DNA are more stable than single stranded (ss) DNA.
- Stability of DNA double helices is due to;
  - high number of H-bonds between base pairs
    - »Greater for G-C (3 H-bonds) than
    - »A-T (2 H-bonds)
  - hydrophobic interactions,
  - stacking interactions between bases.

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## Physical Properties of Nucleic Acids

### Melting of DNA

- Melting is the separation of double-stranded DNA by heating.
- The melting temperature ( $T_m$ ) is the temperature at which the two DNA strands unwind or the temperature corresponding to the midpoint of optical density increase caused by heat denaturation.

399

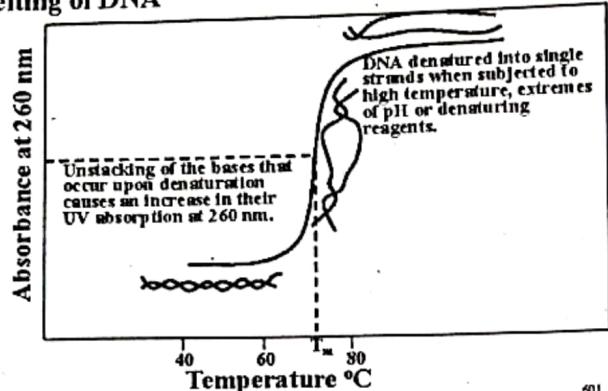
## Physical Properties of Nucleic Acids

- Factors which affect the melting temperature include:
  - Composition (GC pairs increase  $T_m$ ).
  - Salt (increasing salt increases  $T_m$ ).
  - Denaturants (high formamide or urea concentrations decrease  $T_m$ )
  - Length (for short strands,  $1/T_m$  is approximately linear in the inverse of the nucleotide length).
  - Concentration: (for short strands,  $1/T_m$  is approximately linear in the log of strand concentration)

400

## Physical Properties of Nucleic Acids

### Melting of DNA



## Physical Properties of Nucleic Acids

### Hyperchromic effect:

- Is the increase in absorbance when DNA is heated.
- Results from unwinding of double helix so that the bases no longer interact.
- Hydrogen bonding and hydrophobic interaction depress UV absorption.

### Hypochromic Effect

- The decrease in absorbance when DNA is cooled.
- Chains come together and complementary strands anneal on cooling.

602

## Self-Test Questions

1. Order the following DNA molecules from the lowest to the highest melting temperature. Give reasons for order.

- AAGTT CTCTGAA  
T TCAAGAGACTT
- AGTCGTCAATGCAC  
TCAGCAGTTACGTG
- GGACCTCTCAGC  
CCTGGAGAGTCC

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## Self-Test Questions

2. List the forces that are primarily responsible for the stability of the double-helical structure of DNA.

What is hyperchromic effect? What is the basis of this effect?

List four differences that you could use to distinguish DNA from RNA.

3. At high temperatures, deoxyribonucleic acids become denatured. They unwind from double helices into disordered single strands. Account for the fact that the higher the content of G-C base pairs, the higher the temperature required to denature the DNA double helix.

604

## Isolation of Nucleic Acids

### Separation from proteins

- By digestion with protease or extraction with phenol.
- Phenol denatures and solubilizes proteins. The proteins stay in the phenol phase and the more polar nucleic acids remain in the aqueous phase. DNA can be precipitated with isopropanol.

### Separation of DNA and RNA

- Treat with DNase-free RNase (yields DNA)
- Treat with RNase-free DNase (yields RNA)
- High speed centrifugation separates high molecular weight DNA from smaller plasmid DNA or RNA.
- By anion exchange chromatography.

605

## Isolation of Nucleic Acids

### Electrophoresis

- Separates different sizes and types of nucleic acids.
- Agarose gel preferred due to large size of nucleic acids.
- Denaturing agents such as formamide, urea, glyoxal are added to eliminate secondary structure that could interfere with size.

606

## Estimation of Nucleic Acids

- A very sensitive method for detecting nucleic acids is UV absorption at 260 nm.
- This is due to the conjugated double bonds in the bases.
  - Unlike proteins where only a few amino acids (tyr, trp, and phe) absorb, all nucleotides absorb significantly in the UV region.
  - This results in a higher extinction coefficient for nucleic acids. A mg/ml solution of pure nucleic acid has an extinction coefficient of approximately 20, whereas that for a solution of pure protein at the same concentration is approximately 1.0

607

## Estimation of Nucleic Acids

### Spectrophotometric determination of DNA and RNA

- To check for contamination from proteins, the value of the absorbance ratio measured at 280 nm and 260 nm is used since both nucleic acids and proteins absorb to some extent at both wavelengths.
  - For nucleic acids an absorbance of 1 at 260 nm corresponds to;
    - 50 µg/ml of double-stranded DNA (ds DNA);
    - 40 µg/ml of single-stranded (ss) DNA or RNA

608

## Estimation of Nucleic Acids

- Often the 260/280 ratio is used as a criterion for purity.
  - For pure DNA
    - $A_{260}/A_{280} = 1.8$  (lower values suggest contamination with protein)
  - For pure RNA
    - $A_{260}/A_{280} = 2.0$  (lower values suggest contamination with protein)
  - Pure proteins
    - $A_{260}/A_{280} = 1.8$  (lower values suggest contamination with nucleic acids)

609

## Detection of Nucleic Acids

- Fluorescent dye binding
  - RNA and DNA bind tightly to dyes to activate their fluorescence.
- Ethidium bromide
  - Intercalate stacked bases of DNA
  - Used in the detection of both single and double stranded nucleic acids
- Acridine orange
  - For detecting RNA
- Both dyes are extremely potent mutagens and should be used with care.

610

## Detection of Nucleic Acids

- Radioisotope incorporation
  - Nucleic acids can be labeled *in vivo* with  $^3\text{H}$ ,  $^{14}\text{C}$  or  $^{32}\text{P}$  nucleotides or bases
  - Radioactive thymine for labeling DNA
  - Radioactive uracil for labeling RNA

611

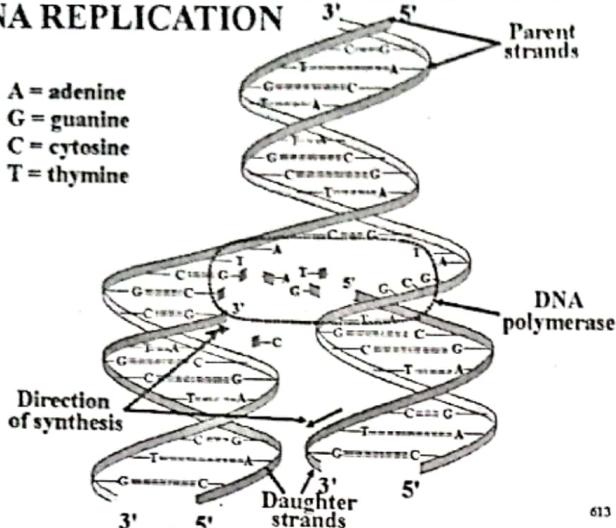
## DNA Replication (Synthesis)

- Watson and Crick double helical structure, with complementary base pairing specificity, provided the basis for the mechanism of DNA replication.
- During replication, the parental strands separate by the breaking of H-bonds.
- A complementary strand is synthesized by specific base pairing requirements.

612

## DNA REPLICATION

A = adenine  
G = guanine  
C = cytosine  
T = thymine



613

## DNA Replication

### Mechanism

- Theoretically 3 mechanisms were proposed:
- **Semiconservative replication**

In which the double helix is half conserved and each parental strand serves as a template for the synthesis of a new DNA strand.

### -Conservative replication

In which the parental strand is totally conserved and directs the synthesis of two new daughter strands.

614

## DNA Replication

### Mechanism

#### -Dispersive Replication;

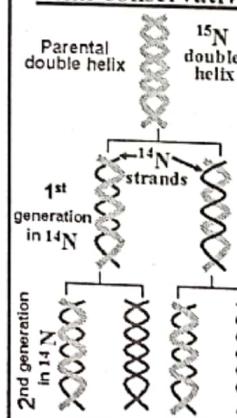
- In which segments of the parental strand and progeny strand are interspersed by a fragmentation and rejoining process during new DNA synthesis.

615

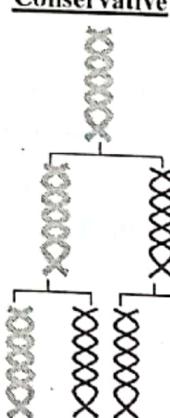
## DNA Replication

### Proposed Mechanisms

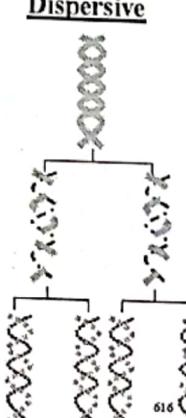
#### Semi-conservative



#### Conservative



#### Dispersive



616

## DNA Replication

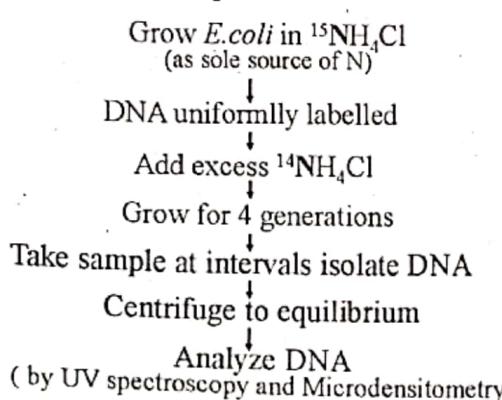
### Evidence for semi-conservative replication

- Data was provided by Meselson & Stahl (1958)
- Evidence was based on the hypothesis that;
  - molecules of DNA labelled with  $^{14}\text{N}$  (normal isotope) and  $^{15}\text{N}$  (heavy isotope) can be separated by density gradient centrifugation, by layering the DNA on a concentrated solution of CsCl.
  - the DNA sediments until its buoyant density is in equilibrium with the buoyant density of the CsCl.

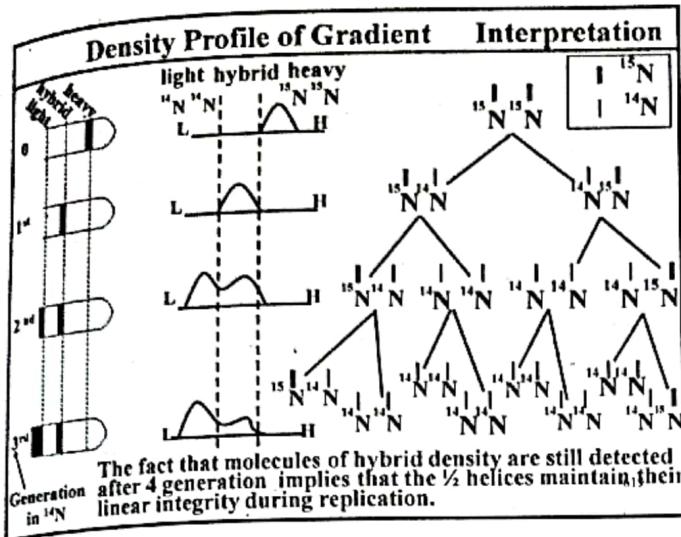
617

## DNA Replication

### Meselson and Stahl Experiment



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## DNA Synthesis

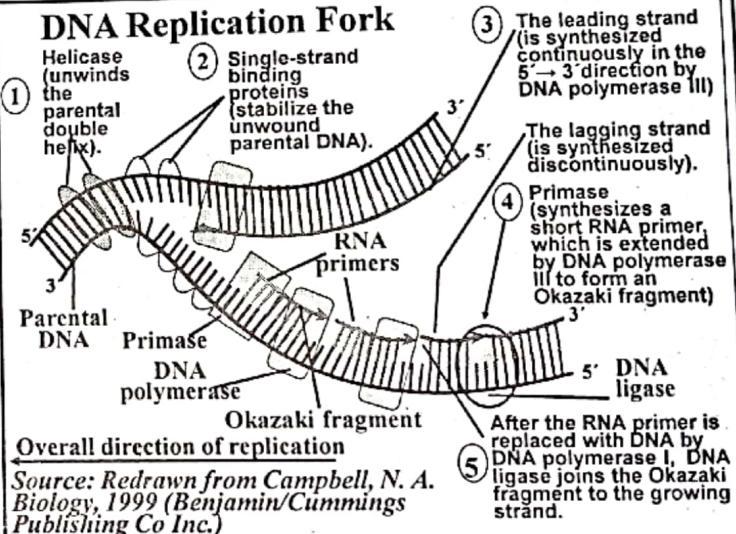
- In vitro synthesis of DNA was first accomplished by Arthur Kornberg (Nobel laureate 1959)
- He isolated an enzyme from *E. coli* initially called DNA polymerase or Kornberg enzyme, now DNA polymerase I
- Requirements for DNA polymerase I include:
  - free 3'-OH on a pre-existing DNA chain (called primer)
  - an intact DNA chain (called template)
  - four deoxyribonucleotide triphosphates (dATP, dCTP, dTTP, dGTP) and  $Mg^{2+}$
- Replication can be divided into three steps: <sup>620</sup>
  - Initiation, Elongation, and Termination

## DNA Replication

### Replication Factors in *E. coli*

Protein	Function
DNA polymerase III	1. Catalyzes $5' \rightarrow 3'$ polymerization 2. Has $3' \rightarrow 5'$ exonuclease activity. (Performs proofreading function).
DNA polymerase I	1. Has $5' \rightarrow 3'$ exonuclease activity. (Removes RNA primers) 2. Has $5' \rightarrow 3'$ polymerizing activity. Adds new strands
DNA ligase	Catalyzes covalent joining of Okazaki fragments
Helicase (unwinding protein)	Unwinds DNA at replication fork
DNA gyrase	Precesses replication fork and introduces negative supercoils
Single-strand binding protein (SSB)	Stabilizes single-stranded DNA <sup>621</sup>
Primase	Synthesizes primers

## DNA Replication Fork



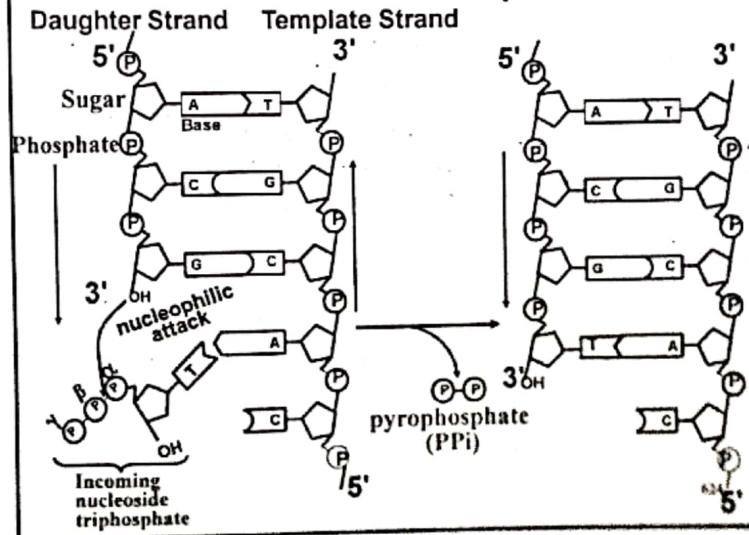
## DNA Replication

### Mechanism

- The mechanism of DNA synthesis occurs by a nucleophilic attack by the 3'-OH of the terminal nucleotide residue on the  $\alpha$ -phosphate group of an incoming nucleoside-5'-triphosphate.
- This results in the displacement of  $PP_i$  and the formation of an internucleotide linkage (a phosphodiester bond).
- The same mechanism is used for strand extension during RNA synthesis (transcription)

623

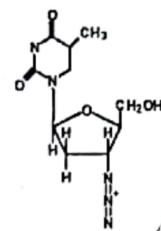
## Mechanism of DNA Replication



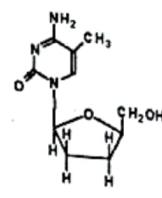
## DNA Synthesis and Repair

- DNA polymerase I has associated exonuclease activities with the following functions:
  - **3' → 5' exonuclease activity**
    - removal of terminal mismatched or unpaired nucleotides from 3' ends.
    - critical "proofreading" or "editing" function (error <1/10,000)
  - **5' → 3' exonuclease activity**
    - removes UV damaged DNA
    - removes RNA primers after DNA synthesis.
    - more effective on base-paired regions

## Inhibitors of DNA Synthesis



3'-azido-2'-deoxythymidine (AZT)



2',3'-dideoxyctydine (DDC)

These nucleosides are important drugs for treating patients with Acquired Immune Deficiency Syndrome (AIDS). AZT and DDC resemble normal nucleosides but neither AZT nor DDC has the 3'-hydroxyl group needed to form the phosphodiester bond in RNA or DNA synthesis. Therefore RNA and DNA chain extension is blocked after AZT or DDC incorporation in a growing nucleotide chain.

## Self-Test Question

1. *E. coli* cells were grown on a medium with only the heavy isotope of nitrogen ( $^{15}\text{N}$ ). If an excess of the ordinary isotope ( $^{14}\text{N}$ ) is added to the medium, what relative contents of  $^{15}\text{N}$  and  $^{14}\text{N}$  and what arrangement in DNA strands would be expected after one generation assuming:
- (i) conservative
  - (ii) semi-conservative replication.

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## Transcription (RNA Synthesis)

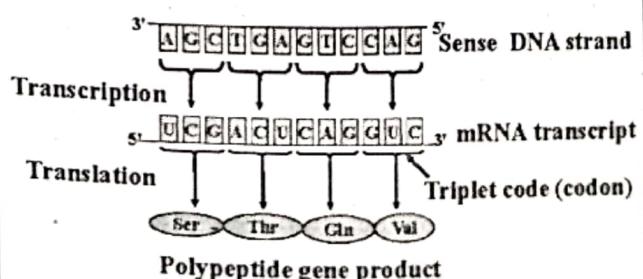
- In eukaryotes, genes remain in the nucleus whilst protein synthesis takes place in the cytosol.
- The DNA cannot therefore serve directly as a template for protein synthesis.
- Information stored in the nucleotide-pair sequence in DNA is transferred by transcription to a nucleotide sequence in mRNA.
- mRNA, therefore, carries information from genes in the chromosomes to ribosomes in cytosol for protein synthesis.

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## Transcription

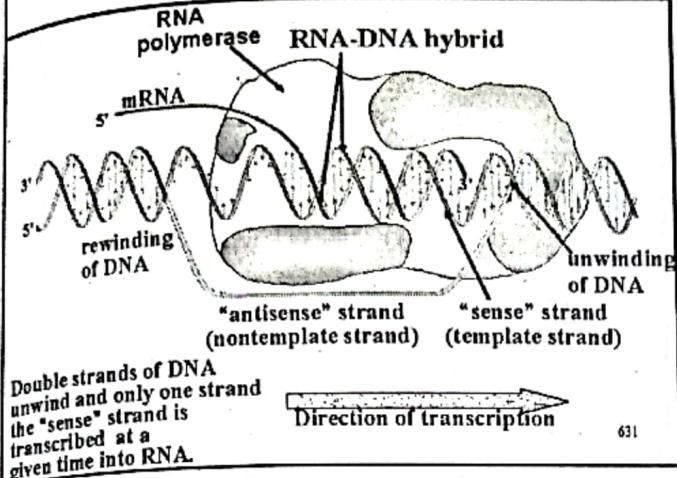
- DNA codes for several types of RNA
- **Sense strand:** the strand of DNA that contains the code that is transcribed into mRNA
- **Ribosomal RNA (rRNA):** RNA in the structure of the ribosome (site of protein synthesis)
- **Messenger RNA (mRNA):** carries instruction for protein synthesis in codes called **codons** (3-nucleotide sequences in mRNA that code for one amino acid)
- **Transfer RNA (tRNA):** binds amino acid and carries (transfers) it to site of protein synthesis.

## Transcription and Translation



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## Transcription



## Types of RNA in *E.coli*

Type	Molecular Shape	Function	# of different kinds	# of nucleotides	% of total RNA in cell	Stability ( $T_{1/2}$ )
mRNA	extended	messenger	thousands	500-6000	3	1 to 3 min
rRNA	extended to compact	structure and function of ribosomes	23S 16S 5S	2800 1540 120	90	stable
tRNA	clover leaf	Adaptor	50-60	75-90	7	stable
RNA primers		DNA replication	?	< 50	< 1	
Ribozymes		?	1 or 2	250-350	< 1	?

Source: Adapted from Zubay, G. (1983). Biochemistry

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## Reverse Transcription

- Some RNA viruses (**retroviruses**) contain an unusual DNA polymerase (**Reverse Transcriptase** or **RNA-dependent DNA-polymerase**).
- Reverse transcriptase also contains a DNA-dependent DNA polymerase activity which is responsible for second-strand formation in cDNA (complementary DNA) synthesis.
- The virus is therefore able to transcribe its RNA into cDNA which can be integrated into the host chromosome.

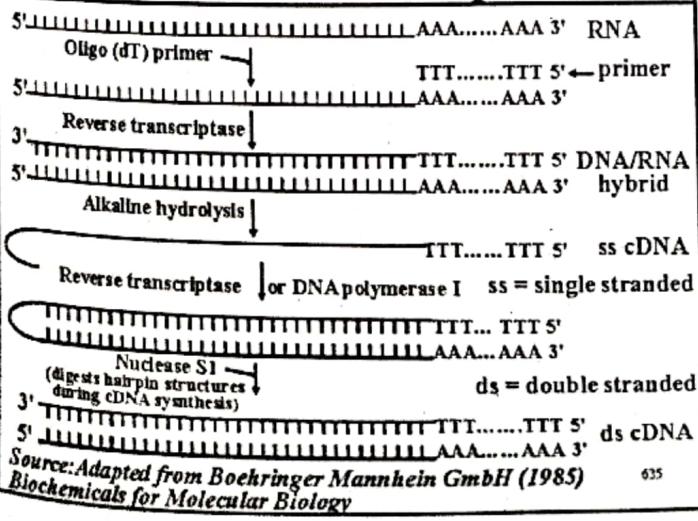
633

## Reverse Transcription

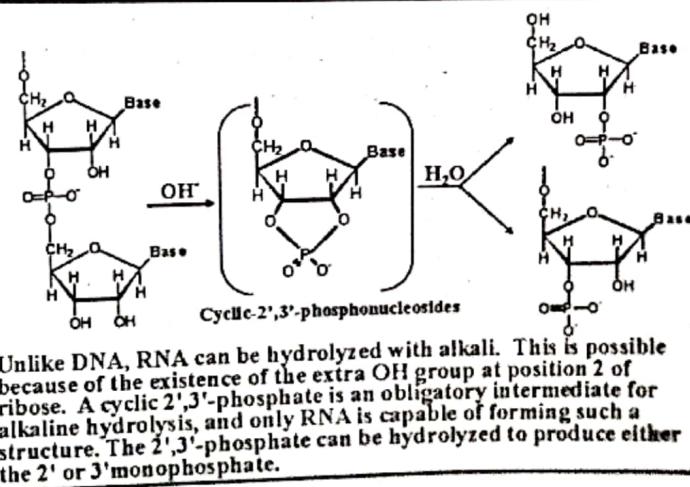
- Synthesis of cDNA is an important tool for the isolation of functional DNA sequences.
- The cloning of cDNA copies of the genomes of RNA viruses has been used to study the genomic structure of the viruses.
- cDNA transcripts are used for analyses of eukaryotic gene structure, organization, and expression.
- Comparison of cDNA and genomic DNA is used in the study of intervening sequences and gene splicing events.

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## Reverse Transcription



## Alkaline Hydrolysis of RNA



## Inhibitors of Transcription

- Actinomycin D:** - an antibiotic that binds DNA and blocks template function.
- Rifampicin:** - a synthetic antibiotic that inhibits bacterial polymerases.
- $\alpha$ -amanitin:** - a toxin in poisonous mushroom (*Amanita phalloides*). It Inhibits eukaryotic RNA polymerase but not the bacterial enzyme.
- Cordycepin:** - a 3'-deoxy substrate analog. Causes chain termination.

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## Self-Test Question

1. Assume that the short polynucleotide strands below are part of a DNA or RNA molecule. Draw a complementary strand for each.

(a) 5'-AGCTTACGTC- 3'

(b) 5'-UAGGUACUUCG- 3'

638

## Translation (Protein Synthesis)

- Genetic information stored in sequence of nucleotides in mRNA is translated following the dictates of the **Genetic Code** into a sequence of amino acids in a polypeptide gene product.
- Translation involves 3 RNAs (tRNA, rRNA, mRNA), all transcribed from DNA.
- Other macromolecules involved in the process include:
  - aminoacyl-tRNA synthetases (amino acid activating enzymes).
  - soluble protein factors involved in chain initiation, elongation and termination.
- Proteins are synthesized in the cytosol on the ribosomes.

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## The Genetic Code

- Is a catalogue of base sequences in the mRNA that specify amino acids in proteins.
- Code is a **triplet codon**
  - a sequence of 3 nucleotides specify one amino acid.
- Code is **non-overlapping and comma free**
  - triplets follow in immediate sequence, no intervening sequences.
- Code is **almost universal**
  - same or nearly so in all organisms
  - Termination codon "UGA" directs tryptophan incorporation in yeast and human mitochondrion. In some protozoa, UAA and UAG are not stop signals.

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## The Genetic Code

- Code is **degenerate**
  - more than one triplet code for the same amino acid.
- The genetic code has 64 codons. 61 of which specify amino acids. The other 3 specify stop codons.
- The 1<sup>st</sup> and 2<sup>nd</sup> nucleotides are often sufficient to specify an amino acid, the 3<sup>rd</sup> nucleotide is redundant. This is designed to minimize effects of mutations.
- The flexibility in base pairing at the 3<sup>rd</sup> nucleotide position is responsible for the degeneracy of the Genetic Code.

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## The Genetic Code

	U	C	A	G	
U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Try STOP STOP	Cys Cys STOP Trp	U C A G
C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G
A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G
G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G

642

## The Genetic Code

- There are now 22 known amino acids that are found in proteins. Two additional amino acids, selenocysteine and pyrrolysine, have been discovered in the past 40 years.

643

## Wobble Hypothesis

- Explains the basis for degeneracy of Genetic Code.
- From the codon to amino acid assignments one will predict 61 different tRNA species/cell
- But *E. coli* has ~ 50 tRNA
- It was concluded that there must be either,
  - different tRNAs that recognize different codons for a given amino acid, or
  - the anticodon of a given tRNA base pairs with different codons for a given amino acid (wobble hypothesis)
- Both situations do occur.

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## Wobble Hypothesis

- On the basis of molecular distances and steric (3-D structures) considerations, Crick proposed that a wobble would allow several but not all types of base pairing at the third base in codon-anticodon interaction.
- The proposal was supported by experimental data.

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## Wobble Hypothesis

Base-pairing predicted by wobble hypothesis

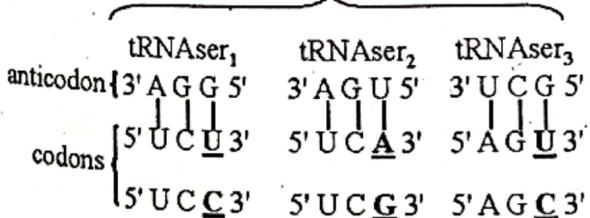
1 <sup>st</sup> base in anticodon	3 <sup>rd</sup> base in codon
U	A or G
C	G
A	U
G	C or U
I	A, C or U

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## Wobble Hypothesis

Degeneracy explains 6 codons for 3 tRNAs for serine

Different tRNAs recognize different codons for a given amino acid



Anticodon of a given tRNA base pairs with different codons of a given amino acid

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## Transfer RNA (tRNA)

- Is an adaptor molecule with a triplet base sequence, **anticodon**, that recognizes the codon of the mRNA.
- The tRNA binds amino acids and carries them to the ribosomes.
- One to four tRNAs are known for each amino acid.
- A mature tRNA contains several unusual bases not present in the primary tRNA transcript.
- The unusual bases (about 30/molecule of tRNA) are produced by post-transcriptional modification.
- Unusual bases render tRNA resistant to nucleases and support formation of tertiary structure by preventing double strand formation in certain regions of tRNA (this results in looping in tRNA).

## Protein Synthesis

- Protein synthesis takes place in several steps:
  - Activation of amino acids by specific aminoacyl-tRNA synthetases and their binding to tRNA.
  - Initiation of peptide chain in the presence of initiation factors (IF1, IF2, IF3), mRNA, GTP, Mg<sup>2+</sup>, ribosomal subunits, and initiator tRNA.
  - Elongation of chain in the presence of elongation factors (EF-Ts, EF-Tu and EF-G). Elongation is divided into 3 stages;
    - Binding
    - Transpeptidation
    - Translocation
  - Termination

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## Protein Synthesis

- The first step in protein synthesis is the activation of amino acids.
- Amino acids are activated and attached to tRNA by a "high energy" bond between -COOH of amino acid and 3'-OH of tRNA to form aminoacyl-tRNA.
- The reaction is catalyzed by an aminoacyl-tRNA synthetase.
- Synthetases are specific for individual amino acids.

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## Activation of Amino Acid

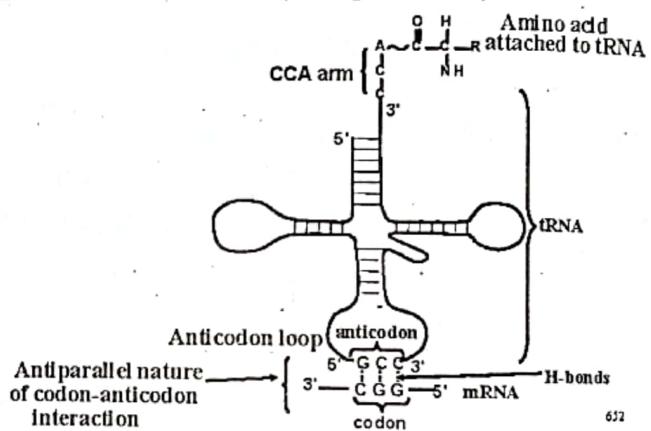
- The activation reaction proceeds in two steps:
    - Formation of aminoacyladenylyl (AA-AMP)
    - Formation of aminoacyl-tRNA (AA-tRNA)
  - Both reactions are catalyzed by a single enzyme with two catalytic sites. One for reaction 1 and the other for reaction 2 below.
1. AA + ATP → AA-AMP + PPi
  2. AA-AMP + tRNA → AA-tRNA + AMP
- AA + tRNA + ATP → AA-tRNA + AMP PPi  
(charged tRNA)

The AA-AMP intermediate is not released from the enzyme before undergoing the second reaction.

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## Aminoacyl tRNA

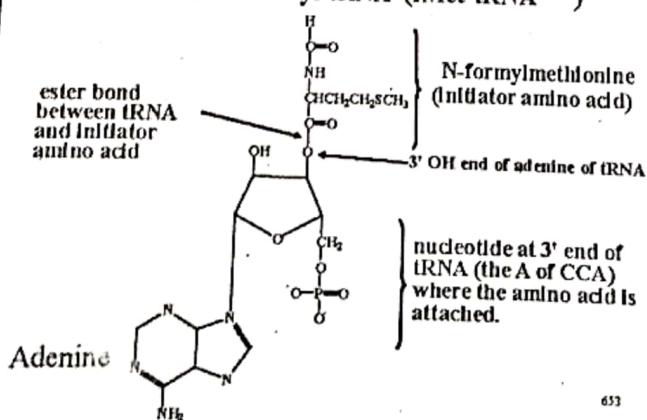
Activated amino acid (charged tRNA)



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## Aminoacyl tRNA

Initiator aminoacyl-tRNA (fMet-tRNA<sup>fMet</sup>)

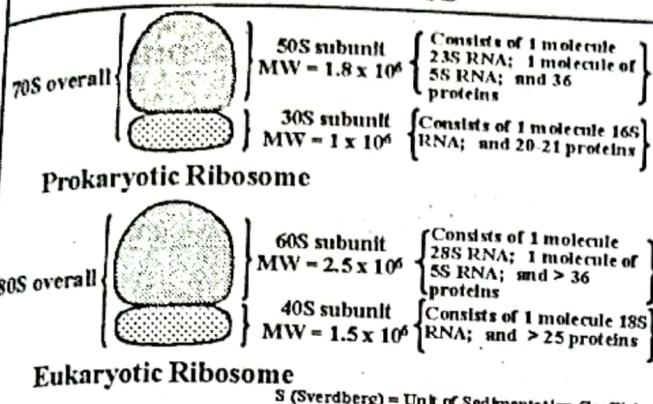


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## Protein Synthesis

- Ribosomes are the "workbenches" on which proteins are synthesized and contain the enzymes for protein synthesis.
- Ribosomes consist of two subunits.
- In *E. coli*, the smaller subunit is the 30S subunit. It contains about 21 proteins and a molecule of 16S rRNA.
- The larger subunit, the 50S subunit, contains about 36 proteins and two rRNA molecules (of sizes 5S and 23S).
- The two subunits interact during protein synthesis to give a 70S complex ribosome.
- Ribosomes of eukaryotes are larger (80S) than those of prokaryotes and consist of a smaller 40S subunit and a larger 60S subunit.

## Ribosomes



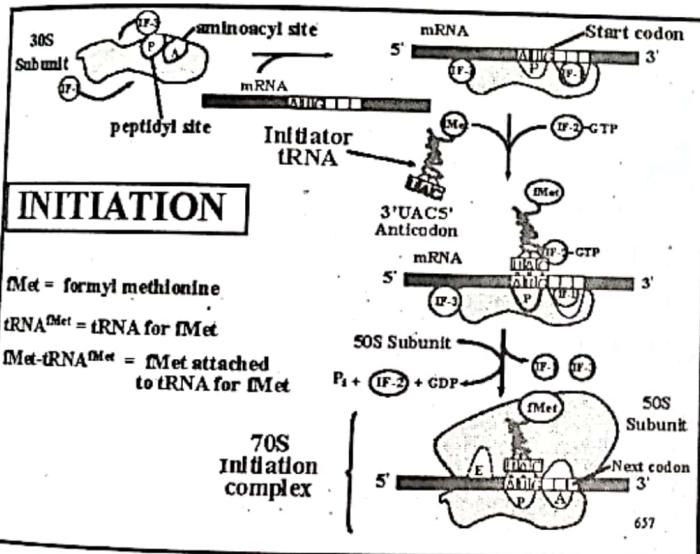
Source: Redrawn from Clark, J. M. Jr., Switzer, R. L. (1964) Experimental Biochemistry. W. H. Freeman and Company

## Protein Synthesis

### Initiation.

- A 70S initiation complex is formed in bacteria between the mRNA, the 30S subunit, an initiator aminoacyl-tRNA (fMet-tRNA<sup>fMet</sup>) and the 50S subunit.
- The initiation codon is registered at the P site and interacts with the anticodon of the tRNA.
- The formation of the initiation complex is promoted by initiation factors (IF1, IF2 and IF3).
- The process requires 1 GTP molecule.

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## Protein Synthesis

### Elongation involves:

- Binding of incoming aminoacyl-tRNA
  - the incoming activated tRNA binds to the A site. One GTP molecule is used.
  - two elongation factors (EF-Tu, EF-Ts) are involved.
- Transpeptidation (peptide bond formation)
  - A peptide bond is formed between the amino acid attached to tRNA at the P site and the amino acid attached to the tRNA at the A site. The bond formation is catalyzed by peptidyl transferase, a 50S associated enzyme. Transpeptidation requires no GTP.

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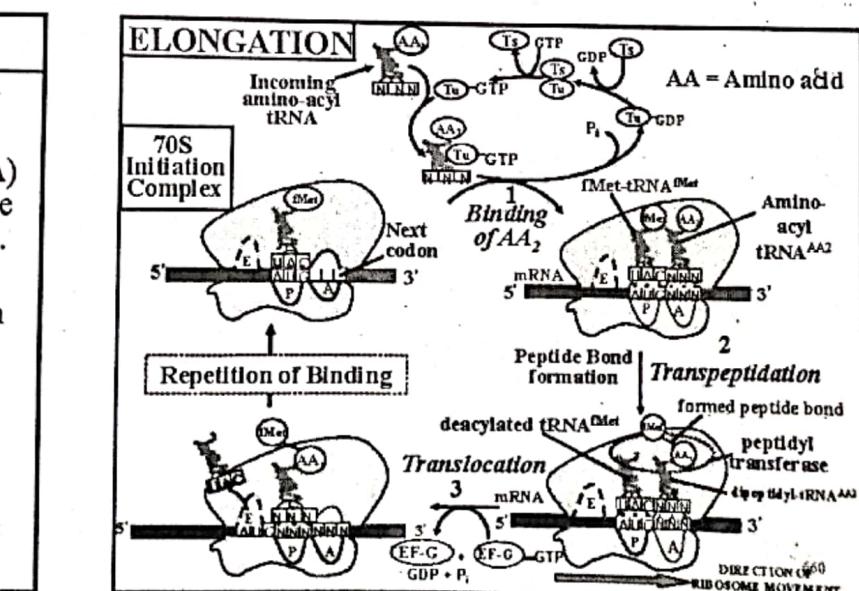
## Protein Synthesis

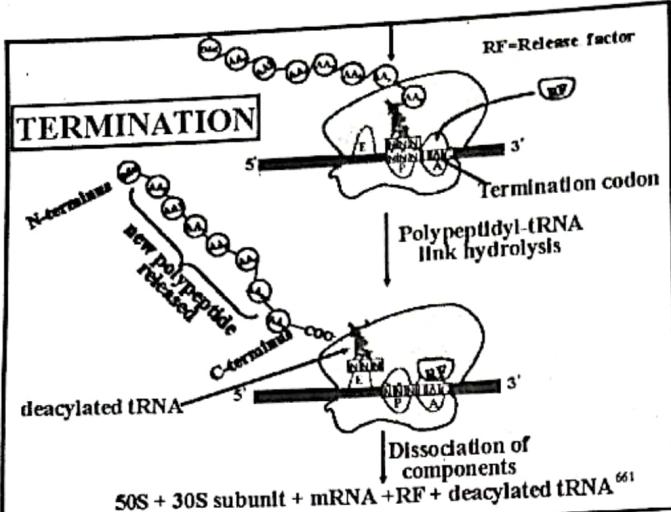
### - Translocation

- Involves the movement (translocation) of the tRNA with the bound peptide (peptidyl-tRNA) from the A to the P site and the removal of the deacylated tRNA (free tRNA) from the E site.
- This process requires the participation of the elongation factor G (EF-G) and the utilization of one molecule of GTP.

### - Termination

- The termination of protein synthesis is initiated when a termination codon is registered at the A site. It involves the participation of release factors (RFs) and the use of one GTP molecule.





## Self-Test Questions

- Calculate the number of GTP (or ATP) molecules required for the synthesis of a polypeptide with 600-amino acid residues.  
 (a) beginning with free amino acids  
 (b) beginning with activated amino acids.
- The use of ATP in energy metabolism sometimes results in the formation of AMP and PP<sub>i</sub> rather than the more common ADP + Pi. Give examples of reactions in which there is pyrophosphate cleavage of ATP and describe advantages of this cleavage, if any.

## Self-Test Questions

- How many activation cycles are needed for a protein with 50 amino acids?
- How many initiation cycles are needed for a protein with 150 amino acids?
- How many elongation cycles are needed for a protein with 150 amino acids?
- How many termination cycles are needed for a protein with 150 amino acids?

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## Self-Test Questions

- Name the type of bond that links the following molecules.  
 (a) Amino acid to amino acid in proteins  
 (b) Nucleotide to nucleotide in DNA  
 (c) Amino acid to tRNA  
 (d) Nucleotide to nucleotide in RNA  
 (e) Codon in RNA to anticodon in aminoacyl-tRNA
- Briefly describe the function of each of the following in protein synthesis. (a) Ribosomes  
 (b) Codon (c) Anticodon (d) Aminoacyl-tRNA synthetases (e) Peptidyl transferase.



## 8

# Recombinant DNA Technology (Genetic Engineering)

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## Recombinant DNA Technology

- Genetic engineering is the construction or alteration of DNA to make or change genes.
- Discovery of site specific DNA "cutting" enzymes (Restriction Endonucleases, REs) led to the development of recombinant DNA technique.
- Technique allows DNA fragments from any source to be joined artificially with prokaryotic or eukaryotic DNA that replicates autonomously.
- Microorganisms with recombinant DNA are cloned in large numbers, and the exogenous fragment isolated in quantities sufficient for size and sequence analysis.

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## Recombinant DNA Technology

- Recombinant techniques have permitted analyses of
  - gene structure
  - gene organization
  - gene expression
  - gene evolution
- Genetic engineering of microorganisms has been used to produce protein products (e.g. insulin) of artificially introduced genes.

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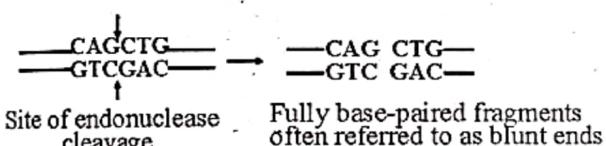
## Restriction Endonucleases

- Large numbers of restriction endonucleases with different specificities have been purified from a variety of bacteria.
- Restriction endonucleases are named by,
  - the first letter of bacterial genus (e.g. E for *Escherichia*).
  - the first two letters of the species name (co, for *coli*) followed by the serotype or strain designation if any.
  - A Roman numeral designation is added if bacterium contains more than one such endonuclease e.g EcoR I.
  - For example Hinf I and Hinf II are obtained from *Hemophilus influenzae*, serotype f

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## Restriction Endonucleases

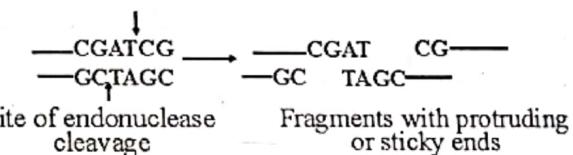
Some restriction endonucleases catalyze coincident cleavage of double-stranded DNA resulting in fully base-paired fragments.



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## Restriction Endonucleases

- Other restriction endonucleases catalyze a staggered cleavage of two DNA strands, to give fragments with protruding ends which consist usually of one to four nucleotides in length, referred to as sticky ends.
- These can re-associate by annealing through complementary base pairing.



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## Restriction Endonucleases

- Restriction enzymes are being used to cleave DNA molecules into specific fragments that are easily analyzed and manipulated than the parent molecule.
- Chromosomes can be mapped by using a series of restriction enzymes.
- The pattern of restriction fragments can serve as a fingerprint of the DNA molecule.
- Small differences between related DNA molecules can be readily detected by separating and displaying their restriction fragments by gel electrophoresis (Restriction Fragment Length Polymorphism, RFLP or DNA finger printing).

## Restriction Endonucleases

DNA finger printing (Restriction Fragment Length Polymorphism, RFLP)

- The technique exploits the ability of a single stranded DNA to anneal to another strand of DNA with a complementary sequence.
- RFLP markers are used to detect variations in DNA sequences at a given loci in different individuals.
- Any differences in the DNA sequence due to base additions, deletions, substitutions, etc. result in differences restriction fragment sizes.
- The presence or absence of fragments due to changes in recognition sites provides a pattern used in species identification.

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## Restriction Endonucleases

### Factors Affecting Optimum Activity of REs

- Temperature
- Buffer systems
  - Tris HCl is the most commonly used buffer system. pH of Tris buffer is temperature dependent.
- Ionic conditions
  - $Mg^{2+}$  is an absolute requirement for all REs.
  - Addition of other salts depend on different endonucleases.
- Methylation of DNA
  - Digestion of DNA strongly affected (inhibited) by methylation of residues within recognition sequence.

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## Restriction Endonucleases

### Factors Affecting Optimum Activity of REs

- **DNA preparation**
  - Efficiency of restriction reaction is dependent on the purity of DNA.
  - Contamination with protein, phenol, chloroform, ethanol, EDTA, SDS and high salt concentration, etc., may inhibit enzyme activity.
  - Factors such as high glycerol concentration, high enzyme/DNA ratio, prevalence of other divalent ions may alter properties of REs.
  - Inability of REs to cut DNA into fragments may be due to presence of nucleases or other proteins.

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## Restriction Endonucleases

### • Restriction fragment

- Restriction endonucleases catalyze cleavage of large DNA into population of specific restriction fragments, with different lengths.

### • Restriction map

- Map constructed for a DNA fragment by determining the number and locations of sites for various endonucleases



Numbers indicate nucleotide pairs between restriction sites

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## Construction of Recombinant DNA

- Recombinant DNA is formed by enzymatically joining two DNA fragments.
- Fragments can be from any source.
- Three general methods used;
  - Non covalent annealing of complementary base-pairing of sticky ends, followed by covalent nick closure with DNA ligase.

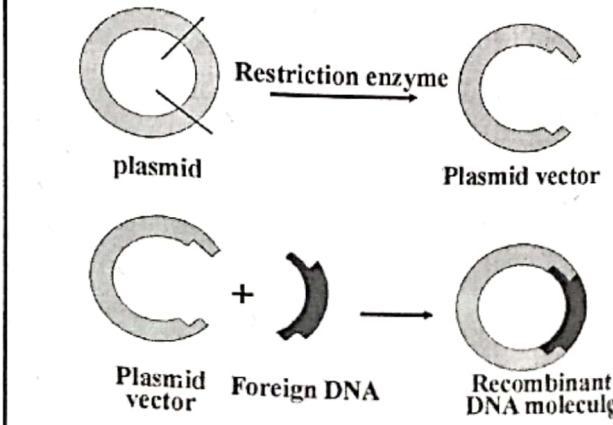
676

## Construction of Recombinant DNA

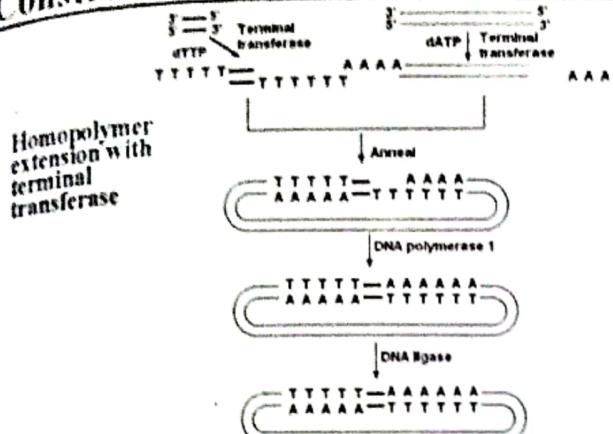
- Addition of homopolymer extensions (tails) at 3' ends using **Terminal Transferases**.
  - Does not require template.
  - Gaps filled with DNA polymerase.
  - Fragments joined with DNA ligase
- Joining of fully base-paired duplex fragments
  - by directly using high concentrations of bacteriophage T4 DNA ligase.
  - alternatively, by **linker adaptation**, where blunt ends are converted to sticky ends by joining to short chemically synthesized linker sequences which carry a recognition site for REs that makes staggered breaks.

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## Construction of Recombinant DNA



## Construction of Recombinant DNA



## Vectors (Vehicles)

- A crucial step in genetic engineering is the introduction of DNA recombined *in vitro* into a living cell (host).
- Recombinant DNA can be introduced into cells and replicated *in vivo* using an autonomously replicating molecule known as VECTOR or VEHICLE.
- The fragment carried by a vector is called an INSERT.
- The cell used for vector propagation is called a HOST
- Propagation of inserted DNA by this technique is called MOLECULAR CLONING.

650

## Vectors

### Plasmid vectors

- Are circular extrachromosomal bacterial DNA molecules that can replicate autonomously (independent of chromosomes).
- Recombinant DNA techniques have been used to develop *E. coli* plasmids such as pBR322, that carry drug resistance markers as well as single recognition sites for restriction endonucleases.

### Viral vectors

- Used for propagating foreign DNA sequences, not only in bacteria but also in eukaryotic cells. Viral vectors have almost 100 % probability of infection whereas plasmids have about 0.1 % probability of transformation.

## Vectors

### Cosmid vectors

- Cloning vectors designed for the cloning of large fragments (up to 45kb in size) of DNA into *E. coli* at high frequency.
- They contain the *cos* region from phage which allows DNA of correct size to be packaged into lambda ( $\lambda$ ) phage head during maturation for efficient introduction into bacteria.

### Yeast-plasmid vectors

- These are yeast-*E. coli* hybrid plasmids which can replicate in either host.
- Provide useful vehicles for recombinant DNA propagation in yeast.

## Vectors

### Bacteriophage vectors

- These have been used as gene delivery vectors *in vitro* in mammalian and bacterial cells.
- Bacteriophages hold good promise as phage-based vectors in gene therapy.

### Yeast artificial chromosome (YAC)

- These are high capacity vectors that can accommodate up to  $10^6$  nucleotides of foreign DNA.
- They replicate like yeast chromosome when introduced into yeast cells.
- There is a difficulty in isolating YAC insert DNA. Transformation efficiency is also low.

## Transformation

- Transformation is the re-introduction of vector with inserted DNA into bacterium.
- Cells to be transformed are made competent by treatment with calcium chloride or by electroporation.
- The frequency of transformation is low (about 1/1000)
- Many but not all progeny of transformed cells acquire plasmid to produce clone of transformed cells.
- Also not all reconstituted plasmids carry the desired insert.

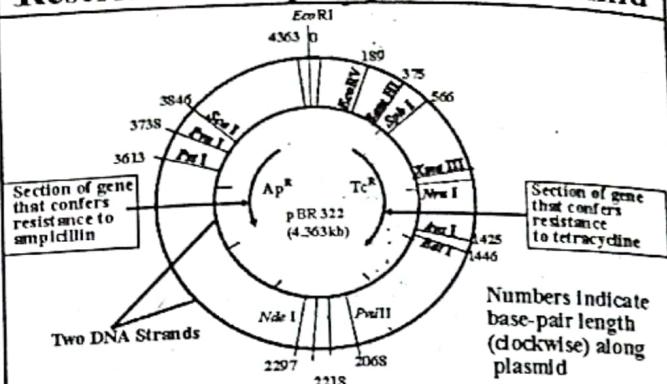
## Screening

### Under Antibiotic Selection

- It is convenient to use conditions that select for cells that have plasmid with insert.
- A screening procedure is used to determine which transformed clones carry recombinant plasmids.
- For example, *E. coli* plasmid pBR322 carries two genes that confer tetracycline resistance ( $Tc^R$ ) and ampicillin resistance ( $Ap^R$ ).
- The plasmid has a single recognition site for the restriction enzyme Bam H1 located within the ( $Tc^R$ ) gene.

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## Restriction Map of pBR322 Plasmid



Source: Adapted from Biochemicals for Molecular Biology Catalogue. 685  
Boehringer Mannheim GmbH, Munich, Germany.

## Screening

- To clone foreign DNA at Bam H1 site, cut both plasmid and exogenous DNA with Bam H1, anneal and ligate.
- The untransformed bacteria is sensitive to both ampicillin and tetracycline.
- Transformed bacteria is incubated on agar plates in the presence of ampicillin. Only cells carrying plasmid can grow.
- Those that form colonies are tested with tetracycline.
- Cells with plasmid that carry insert at Bam H1, will die in the presence of tetracycline because the  $Tc^R$  gene is interrupted by insert.

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## Screening

- If plasmid carries no insert, transformed cells will be resistant to tetracycline because the  $Tc^R$  gene is intact.
- Therefore ampicillin resistant tetracycline sensitive cells are likely to carry the plasmid with desired insert.

688

## Self-Test Questions

- State the function of the following enzymes in recombinant DNA technology

T4 DNA ligase \_\_\_\_\_

Alkaline phosphatase \_\_\_\_\_

Reverse transcriptase \_\_\_\_\_

DNA polymerase I \_\_\_\_\_

*Thermus aquaticus* (*Taq*) polymerase \_\_\_\_\_

Terminal transferase \_\_\_\_\_

Polynucleotide kinase \_\_\_\_\_

- Distinguish among vector DNA, donor DNA, and recombinant DNA in Recombinant DNA technology.

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## DNA Amplification

### Polymerase Chain Reaction (PCR)

- PCR technology is a powerful tool for amplifying selected DNA sequences (a DNA "copy" machine).
- The reaction is carried out in a small tube in a thermocycler machine.
- The reaction mixture consists of;
  - a target (template) DNA,
  - a primer complementary to the region of DNA to be amplified.
  - the four deoxyribonucleoside triphosphates (dNTP)
  - a thermostable DNA polymerase (*Taq* polymerase).

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## DNA Amplification

- A PCR reaction cycle consists of three steps:
  - Denaturation
  - Primer annealing
  - Chain extension
- Each step is carried out at an appropriate temperature.
  - In the **denaturation** step,
    - the temperature of reaction mixture is raised to about 90 °C (90-95 °C).
    - the double-stranded DNA separates into single strands.
  - In the **annealing** step
    - the temperature is lowered to about 60°C (40-60°C) to allow the primer to anneal to the target (template) DNA.
    - annealing temperature depends on base composition and length of DNA.
  - In the **extension** step
    - the temperature is raised to about 72°C (72-75°C) to catalyze polymerization.
    - the process of heating to denature, cooling to allow primer binding, and heating to extend primer represents a PCR cycle.

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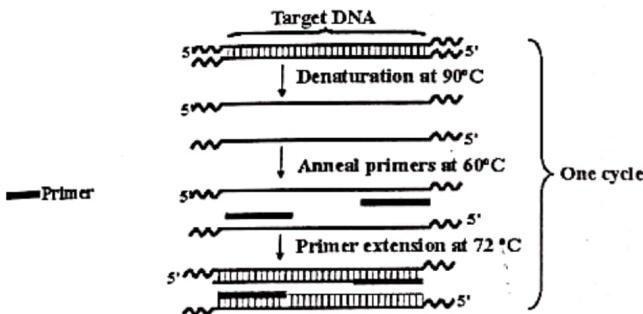
## DNA Amplification

- In the **chain extension** step
  - the temperature is raised to about 72°C(72-75°C) to catalyze polymerization.
- The process of heating to denature, cooling to allow primer binding, and heating to extend primer represents a PCR cycle.
- The PCR cycle is repeated a number of times.
- 20 to 50 amplification cycles are carried out normally.
- In each cycle the amount of DNA doubles.
- The amount of target DNA produced is  $2^n$  times the starting DNA (where "n" is the number of cycles)
- Amplification of DNA is extremely rapid (exponential). 25 cycles can be carried out in one hour.

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## DNA Amplification

### Polymerase Chain Reaction



Repeat sequence of events for 20 to 50 amplification cycles

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## DNA Amplification

- Successful PCR does not require perfect complementarity of primer and target DNA.
- A primer with a base pair **addition, substitution or deletion** can be used and the change incorporated into the PCR product (**side-directed mutagenesis**).
- PCR has been used to simplify techniques in molecular biology such as:
  - Engineering changes in target DNA sequence
  - Cloning and
  - Sequencing
- The study of RNA molecules has been improved through application of PCR.
  - RNA can be converted into double-stranded complementary DNA (cDNA) by reverse transcription.
  - The cDNA can then be amplified by PCR.

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## DNA Amplification

### Uses of Cloned or Amplified DNA

- Transcriptional analysis
- Expression into protein
- Sequence analysis
  - Sequencing methods include:
    - Maxam and Gilbert chemical sequencing
    - Sanger chain termination sequencing
    - Pyrosequencing
    - 454 sequencing
    - Illumina sequencing

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## DNA Sequencing

- Involves the generation of shorter restriction fragments.
- Fragments are multiplied by cloning or polymerase chain reaction.
- Effective sequencing methods depend on,
  - the generation of a continuous set of DNA fragments that differ in length by one nucleotide in four reaction mixtures.
  - separation of fragments in the four reaction mixtures by gel electrophoresis,which can resolve oligonucleotides that differ by one nucleotide.
  - observation of the fragmentation pattern by autoradiography or fluorescence
  - identifying the chemical nature of the end nucleotide by inspection of the gel autoradiogram.

## DNA Sequencing

Two methods for sequence analysis of DNA fragments are considered easy and reliable.

### 1. Base-specific chemical cleavage method (Maxam and Gilbert method).

- This was an early sequencing method that is no longer used.
- It involves radioactive labeling of the 5' termini of DNA fragment with  $^{32}\text{P}$  using polynucleotide kinase.
- This is followed by the generation of base-specific 3'-termini by base-specific cleavage of the terminally labeled DNA.
- The procedure is good for single-stranded or double-stranded DNA.

### 2. Sanger's method (also known as chain termination method or the dideoxy method).

- Involves sequence analysis by partial enzymatic synthesis.

## DNA Sequencing

### Maxam and Gilbert Sequencing Method

#### • Base specific reactions

- DNA is treated with specific chemical agents that react with 1 of 4 bases.
- The four chemical treatments differentially remove bases and cause fragmentation of the DNA.
- A limited period of reaction is employed, so that only a single nucleotide residue reacts with and is cleaved by the reagent.

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## DNA Sequencing

### Maxam & Gilbert Sequencing Method

- The four base-specific reactions are:

- Reaction with dimethyl sulfate at pH 7.0 and heating.  
• G is removed but not A (G>A)
- Reaction with dimethyl sulfate in acid at 0 °C  
• A is removed but not G (A> G)
- Reaction with hydrazine and piperidine  
• Removes both T and C with equal frequency (C+T)
- Reaction with hydrazine (in 5M NaCl) and piperidine  
• Removes only C

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## DNA Sequencing

### Fragments from Base-specific Reactions

DNA strand to be sequenced = 5'  $\text{TCAGGGTTAACG}$

#### 1. A>G (removes A)

$\text{*}_\text{P} \text{TC}$   
 $\text{*}_\text{P} \text{TCAGGGTT}$   
 $\text{*}_\text{P} \text{TCAGGGTTA}$

#### 2. G>A (Removes G)

$\text{*}_\text{P} \text{TCA}$   
 $\text{*}_\text{P} \text{TCAG}$   
 $\text{*}_\text{P} \text{TCAGGGTTAAC}$

#### 3. Removes C + T

$\text{*}_\text{P} \text{T}$   
 $\text{*}_\text{P} \text{TCAGG}$   
 $\text{*}_\text{P} \text{TCAGGT}$   
 $\text{*}_\text{P} \text{TCAGGGTTAA}$

#### 4. Removes C

$\text{*}_\text{P} \text{T}$   
 $\text{*}_\text{P} \text{TCAGGGTTAA}$

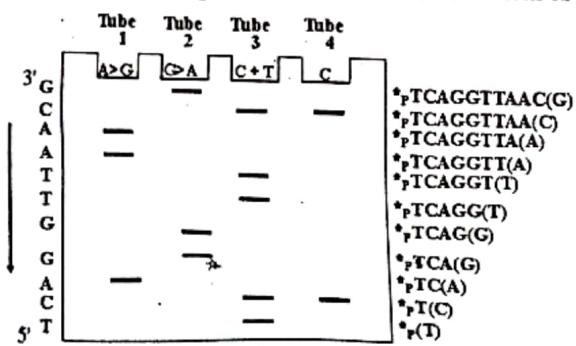
\* = labeled phosphate at 5' end

700

## DNA Sequencing

### Maxam and Gilbert Sequencing Gel

#### Gel autoradiogram of four reaction mixtures



The base cleaved off is shown in brackets

701

## DNA Sequencing

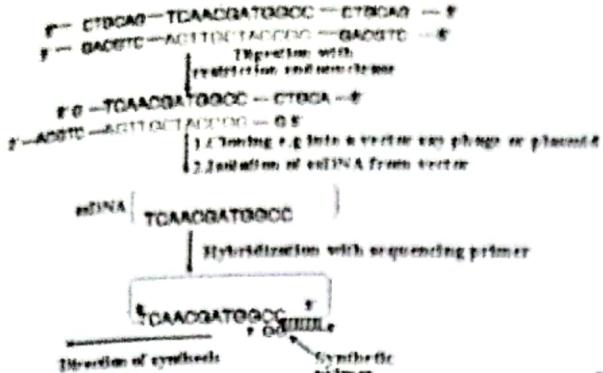
### Chain Termination Method

- Is a primed enzyme synthesis method that is fast and simple to perform.
- Unlike the Maxam and Gilbert method in which the DNA fragments are terminally labeled, the fragments are internally labeled in the dideoxy method.
- Base-specific termini are produced enzymatically based on the incorporation of 2',3'-dideoxy nucleotide analogues into growing DNA chain.

702

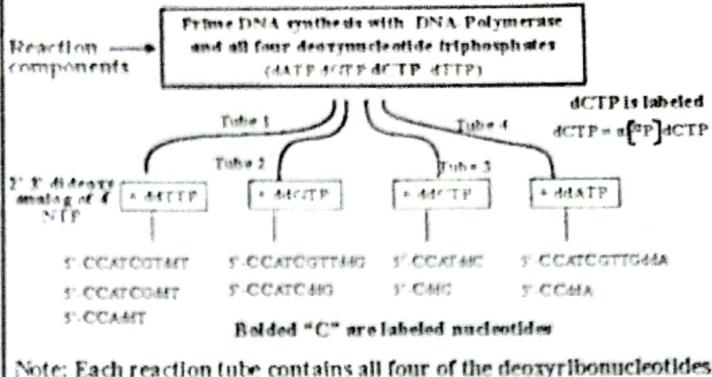
## DNA Sequencing

### Chain Termination Method



## DNA Sequencing

### Chain Termination Method



## DNA Sequencing

### Chain Termination Sequencing Gel

	ddT	ddG	ddC	ddA	
A					5'-CCATCGTTGddA
G					5'-CCATCGTTddG
T	—				5'-CCATCGTddT
T	—				5'-CCATCGddT
G		—			5'-CCATCddG
C			—		5'-CCATddC
T	—				5'-CCAddT
A				—	5'-CCddA
C			—		5'-CddC
					5'-ddC

Sequence: 5'-CATCCGTTGA-3'

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## DNA Sequencing

### Limitations of Sequencing Methods

- Methods of choice depends on the resolving capabilities of gel.
- Fragments > 200 residues difficult to sequence directly.
- Long stretches sequenced as overlapping DNA fragments.

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## DNA Sequencing

**Comparison of Chemical and Enzymatic Methods**

- Chemical method suitable for ds- and ssDNA
- Primed synthesis,
  - requires ssDNA
  - is rapid and less laborious
  - is less susceptible to artifacts due to small changes in reaction conditions.
  - gives orientation of sequence (not always the case in chemical method)
  - uses fewer hazardous reagents
  - misses first 10 nucleotides (chemical method can sequence within 2-3 nucleotides)
  - reactions can be analyzed on thin gels.

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## DNA Sequencing

### Automation of DNA sequencing

- Strategies have evolved for automation of DNA sequencing based on the dideoxy enzymatic synthesis method.
- This involves the linking of each of the four dideoxynucleotides to a fluorescent molecule.
- DNA fragments that terminate with specific colors are synthesized.

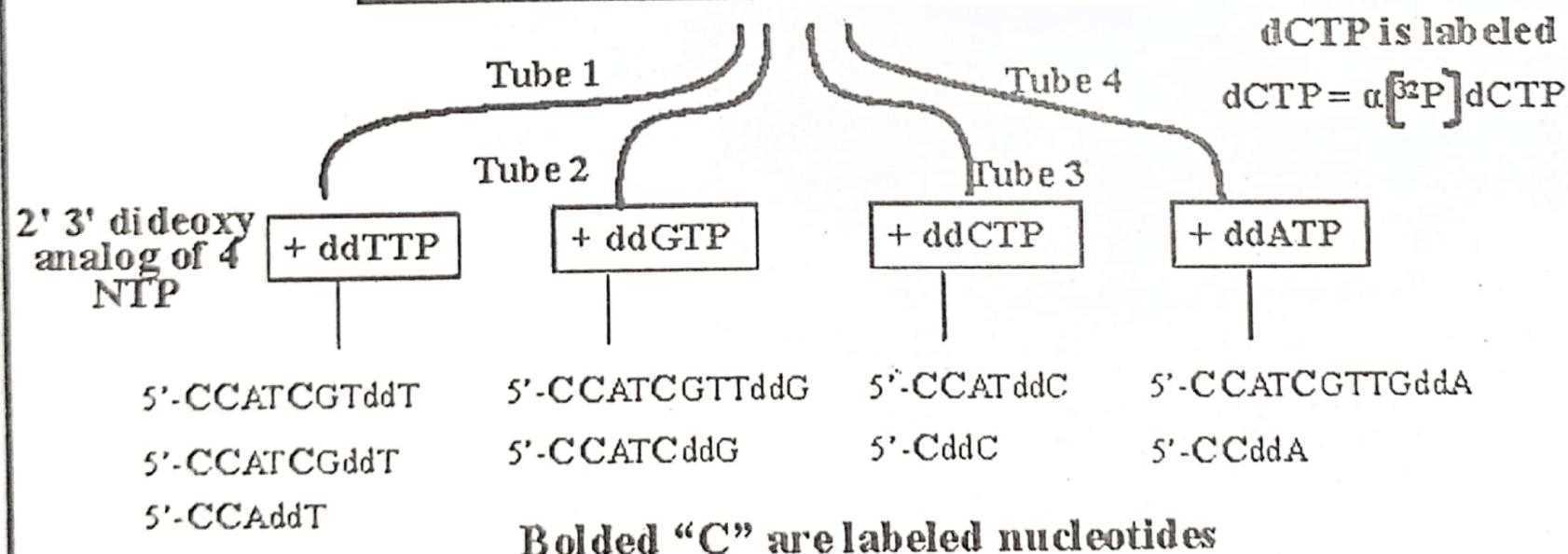
708

# DNA Sequencing

## Chain Termination Method

Reaction components →

Prime DNA synthesis with DNA-Polymerase  
and all four deoxynucleotide triphosphates  
(dATP dGTP dCTP dTTP)



Note: Each reaction tube contains all four of the deoxyribonucleotides but only one of the dideoxy analogues.

## DNA Sequencing

### Automation of DNA sequencing

- Since the terminator nucleotide of the synthesized fragment can be distinguished by color, all four reactions can be performed in a single reaction tube.
- The color specific DNA fragments are then resolved in a one gel lane or in a single capillary tube by electrophoresis
- The color associated with each fragment band, as it migrates through the capillary tube, is detected using a laser beam.

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## DNA Sequencing

- DNA sequence determination is done by reading the sequence of the specific colors as they pass a detector connected to a computer.
- A software for sequence analysis is used to provide the sequence in text and electropherogram.

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## Self-Test Question

1. The oligonucleotide d-ATGCCTGACT was subjected to sequencing by (a) Sanger's method and (b) Maxam-Gilbert's chemical cleavage method in which the label was in the 3' in one instance and in the 5' in another instance, and the products were analyzed by electrophoresis on a polyacrylamide gel. Draw diagrams of the gel banding patterns obtained for (a) and (b).

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## 9

## Mutation

712

## Mutation

### Importance of mutation

- Provides the ultimate source of genetic variation.
- Provides new material for evolution, without which living organisms will not evolve and adapt to changes in their environment.
- Some level of mutation is considered good, however too frequent a mutation disrupts transmission of genetic material.

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## Mutation

- Types of genotypic changes include:
  - changes in chromosome number,
  - gross changes in chromosome structure,
  - changes in individual genes
- The term mutation now frequently refers to changes in individual genes.

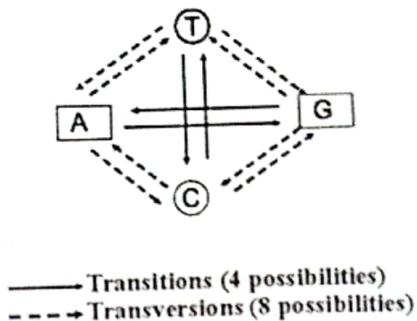
714

## Mutation

- **Point mutations**:- are mutations involving changes in single base pairs such substitution, addition or deletion
- **Substitution mutations include:**
  - **Transitions**:- purine for purine or pyrimidine for pyrimidine substitutions
  - **Transversions**:- purine for pyrimidine or vice versa substitutions.
- **Addition mutations** involve the insertion of a single base pair.
- **Deletion mutations** involve the removal of a single base pair.

## Mutation

### • Substitution mutations



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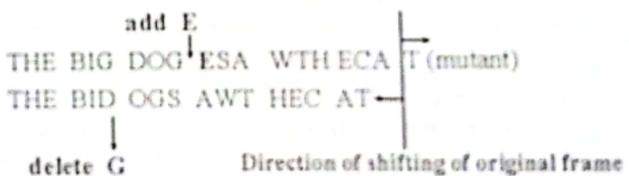
## Mutation

- **Frameshift mutations:**
  - Result from addition or deletion of single base pairs.
  - They alter the reading frame of all base-pair triplets that specify codons in mRNA and amino acids in a polypeptide gene product.

## Mutation

### Substitution and Frame-shift Mutations

- Substitution mutation (e.g. H for a G)  
THE BIG DOG SAW THE CAT (original)  
THE BIG DOH SAW THE CAT (mutant)
- Frame-shift mutation (insertion of an E and deletion of a G)



Source: Adapted from Ouellette, R.J. (1997)

716

## Mutation

- **Sickle cell disease**
  - a mutation that results from the replacement of glutamic acid (an acidic amino acid) of hemoglobin with valine (a hydrophobic amino acid).
- **Osteoarthritis**
  - a mutation that results from the replacement of cysteine of collagen with arginine.
  - cysteine is important for S-S bond formation in proteins.

717

## Mutation

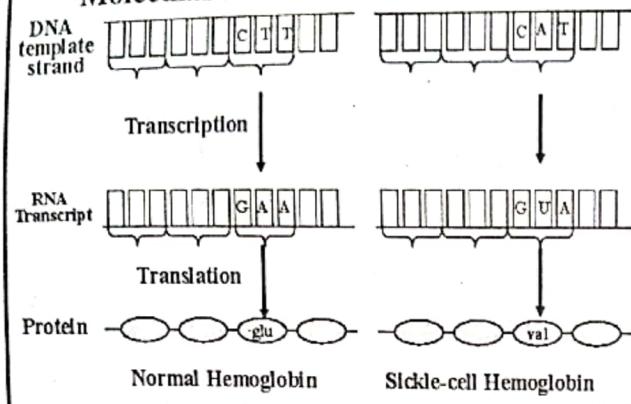
### Molecular basis of sickle-cell disease

- Conformational differences between hemoglobin A (normal) and hemoglobin S (in sickle cell) arise from a single base-pair substitution in the hemoglobin gene.
- This involves a single amino acid change from a polar charged glutamic acid to valine, a hydrophobic amino acid.
- The change introduces a hydrophobic area on the surface of the molecule which leads to the formation of insoluble strands that result in the sickle shape of the red blood cell.

718

## Mutation

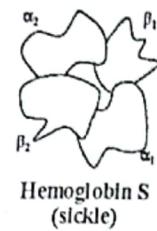
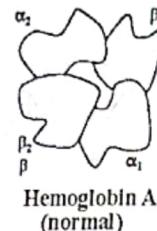
### Molecular Basis of Sickle-cell Disease



721

## Mutation

### Normal and Sickle-cell Hemoglobin



Conformational differences between hemoglobin A and hemoglobin S.

Source: Redrawn from Nelson, D. L. and Cox, M. M. (2000) Lehninger, Principles of Biochemistry;

722

## Mutation

### Mutagens and carcinogens

- Mutagens are agents that cause changes in DNA.
- Mutagens are most often carcinogens (cancer-causing chemicals)
- Mutagens that cause changes in DNA include:
  - Chemical agents,
  - Radiation
  - Chemical agents include:
    - base analogs (e.g. 5-Bromouracil, 2-Aminopurine)
    - acridine dye (e.g. proflavin:- causes intercalation)
    - nitrous acid:- causes oxidative deamination of adenine to hypoxanthine).
    - alkylating agents (e.g. 7-Ethylguanine:- causes depurination)

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## Mutation

### Radiation (ionizing and non-ionizing)

- Ionizing radiation ( e.g.  $\gamma$ -ray; X-ray, cosmic rays). They are of high energy and penetrate tissues colliding with atoms in their path and cause release of electrons, leaving a trail of positively charged ions. Used in medical diagnosis (X-rays)
- Non-ionizing radiation (e.g. Ultraviolet (UV) light). Lower energy, penetrate surface of cells. There is a direct correlation between UV absorption and mutagenicity.

• UV light causes dimerization (covalent linking) of pyrimidine bases on the same DNA strand (pyrimidine dimers). This prevents the formation of hydrogen bonding between the pyrimidine base and the complementary base on the opposite strand. UV light can be used to kill bacteria and hence is used as a germicidal agent.

## Mutation

### Ames test for carcinogens

- Compounds which cause mutation (mutagens) may also cause cancer (carcinogens). Using animal models to test for cancer is expensive and takes several years.
- There is a correlation between exposure to mutagens and cancer.
- A simple, sensitive and rapid test developed by Bruce Ames (Ames Test) is used to test for mutagens.
- The test which takes between 48-72 hours to perform uses mutants of the bacterium *Salmonella typhimurium* that cannot grow in the absence of histidine.

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## Mutation

### Ames test for carcinogens

- The bacterium is exposed to a chemical to see whether it can cause a reversal of the original mutation to produce revertant mutant cells that can synthesize their own histidine and hence grow.
- The degree of reversion is an indication of the potency of a mutagen.
- Compounds found to be mutagenic by Ames Test can then be confirmed using animal models.

726

# 10 Viruses

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## Characteristics of Viruses

- A virus is an infectious parasitic particle, usually smaller than bacteria, that consists of either DNA or RNA encapsulated by a protein coat.
- Viruses are genetic entities that lie somewhere between living and non-living states.
- **Living characteristics of viruses**
  - rapid reproduction (but only in living host cell)
  - ability to mutate.
- **Nonliving characteristics of viruses**
  - are acellular (contain no cytoplasm or cellular organelles).
  - Do not carry out metabolism on their own. Must replicate using the host cell's metabolic machinery.

728

## Characteristics of Viruses

### Composition of viruses

- A viral particle consist of :
  - only one kind of nucleic acid (DNA or RNA) as their genome. Never both.
  - the nucleic acid is encased in a protein shell or coat called **capsid** which is surrounded by a lipid containing membrane.
  - the capsid together with the enclosed nucleic acid is called a **nucleocapsid**.

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## Classification of Viruses

- Viruses are classified by;
  - the type of nucleic acid that makes up their genome (RNA or DNA, single-stranded or double stranded)
  - the shape of their capsid (helical or polyhedral)
  - whether they are enveloped or naked (lack an envelope). The envelope is a lipid-containing membrane that surrounds some virus particles.

730

## Classification of Viruses

- Viruses can store their genetic information in 6 different types of nucleic acids, which are named based on how that nucleic acid eventually forms the viral mRNA
  - **(+/-) double-stranded DNA.** The (-) DNA strand is directly transcribed into viral mRNA. Include most bacteriophages, *Papovaviruses*, *Adenoviruses*, *Herpesviruses*.
  - **(+) DNA or (-) DNA.** Once inside the host cell, the single-stranded DNA is converted into a double-stranded DNA. The (-) DNA strand is transcribed into viral mRNA. e.g. *Phage M13*, *Parvoviruses*.

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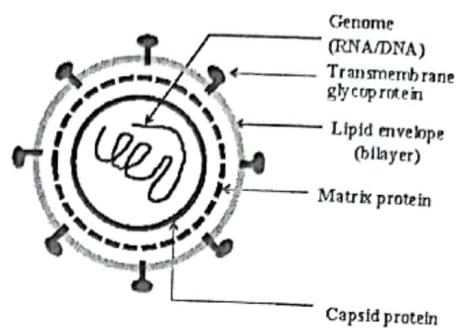
## Classification of Viruses

- **(+)RNA.** A (+) RNA is copied into (-) RNA that is transcribed into viral mRNA. e.g. *Picornaviruses*, *Togaviruses*, *Coronaviruses*.
- **(-) RNA.** The (-) RNA is copied into a (+) RNA which functions as viral mRNA. e.g. *Orthomyxoviruses*, *Paramyxoviruses*, *Rhabdoviruses*.
- **(+/-) double-stranded RNA.** The (+) of the (+/-) RNA functions as the viral mRNA. e.g. *Reoviruses*.
- **(+) RNA.** The (+) RNA is reverse transcribed into (-) DNA that makes a complementary copy to become (+/-) DNA. The (-) DNA is transcribed into viral mRNA. e.g. *Retroviruses*

## Classification of Viruses

Virus	Type of Nucleic Acid	Shape of capsid	Enveloped or Naked	Disease caused
Parvoviruses	ssDNA	polyhedral	naked	gastroenteritis
Human papilloma viruses	ds circular DNA	polyhedral	naked	Genital warts and cancers
Herpes simplex viruses (HSV), Hepatitis B virus	ds DNA	polyhedral	enveloped	Oral and genital herpes, Hepatitis B
Chikungunya virus, Hepatitis A virus (HAV)	(+) ss RNA	polyhedral	naked	Common cold, Hepatitis A
Hepatitis C virus	(+) ss RNA	polyhedral	enveloped	Hepatitis C
Ebola virus	(-) ss RNA	pleomorphic	enveloped	Hemorrhagic fevers
Influenza virus	(-) multiple strands of RNA		enveloped	Influenza
HIV	(+) ssRNA	Bullet shaped or polyhedral	enveloped	HIV infection, T-cell leukemia
HTLV				
Reoviruses	ds RNA	polyhedral	enveloped	Tick fever viruses

## Structures of Viruses



Cross-section of an enveloped virus

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## Viral Replication

- A virus may have a broad or limited host range.
- They are known to infect unicellular organisms such as bacteria, mycoplasmas, and algae and all higher plants and animals.
- Viruses insert their genetic material into a susceptible host, and literally take over the host's function.
- An infected cell produces more viral protein and genetic material instead of its own products.
- Viruses penetrate, replicate and exit a cell.
- Virus replication can be divided arbitrarily into three phases: **initiation, replication, and release**.
- Each of these phases consist of two or more steps.

## Viral Replication

- Initiation involves;
  - attachment:**
    - specific binding of viral attachment protein (VAP) to a cellular receptor.
    - type of receptor expressed by a cell type determines which virus can infect.
  - penetration:**
    - viral penetration is an energy-dependent process, i.e. the host cell must be metabolically active for this to occur.
  - uncoating:**
    - the capsid is removed and the virus genome exposed usually as a nucleoprotein complex.

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## Viral Replication

- The **replication** step involves;
  - genome synthesis, RNA production and protein synthesis.
- The replication strategy of a virus depends on the nature of its genome.
- Majority of RNA viruses replicate in the cytoplasm while a majority of DNA viruses replicate in the nucleus.
- Genomic size, composition and organization of viruses show tremendous diversity.
- Viral DNA integrates into host DNA to produce a **provirus**.

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## Viral Replication

- All viruses make proteins which function to:
  - ensure replication of the genome.
  - package the genome into virus particles.
  - alter the metabolism of the infected cell so that viruses are produced.
- Protein synthesis from genetic materials varies for different classes of viruses.

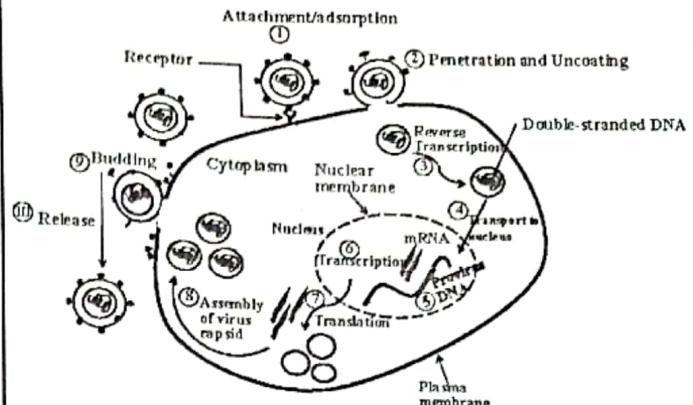
737

## Viral Replication

- The release phase involves:
  - assembly
  - maturation
  - exit from host cell
- Viral proteins are responsible for exit from host cell.
- The proteins are assembled and mature into infectious viral particles that are released from the cell to infect new cells.

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## Viral Replication



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## Viral Pathogenicity

- Animal viruses cause disease in the infected host by two main mechanisms:
  - Damaging infected host cells by,**
    - inhibiting normal host cell DNA, RNA, or protein synthesis.
    - causing nicks or breaks in the host cell's chromosomes.
    - depleting the host cell of cellular materials essential for life or normal function
    - stimulating body cells to release inflammatory cytokines and chemokines

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## Viral pathogenicity

- inducing adjacent host cells to fuse together forming giant multinucleated cells.
- causing cytolysis of the infected host cell etc.
- Evading host immune defenses**
  - antigenic shift and drift as seen in influenza virus.
  - mutant strains that differ from the original and cannot be identified by antibodies e.g HIV and HCV
  - blocking the formation of major histocompatibility complex 1(MHC-1) molecules that are needed in the immune response e.g Epstein-Barr virus etc
- Antibiotics are ineffective against viruses; but immunization and viricidal agents are.

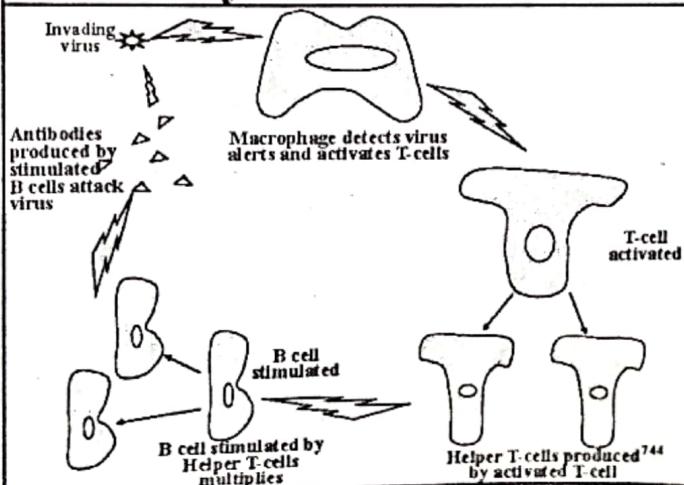
742

## Latent (silent) Viral Infections

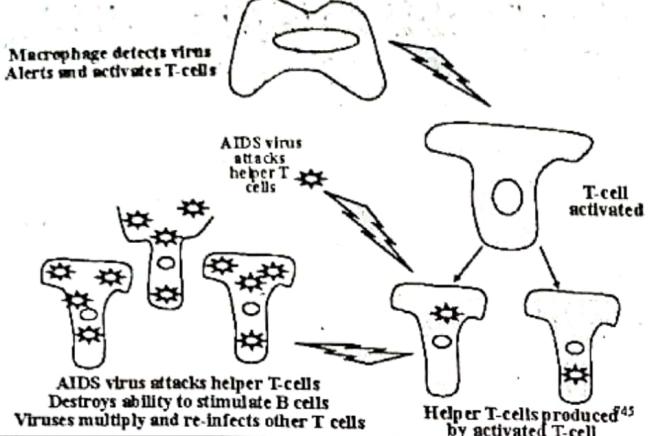
- Some viruses may remain dormant inside host cells for long periods, causing no obvious change in their host cells (a stage known as the **lysogenic phase**).
- When a dormant virus is stimulated, it enters the **lytic phase**: new viruses are formed, self-assembled, and burst out of the host cell, killing the cell and going on to infect other cells.

743

## Immune System and Viral Infection



## Immune System and AIDS virus



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## Antibiotics

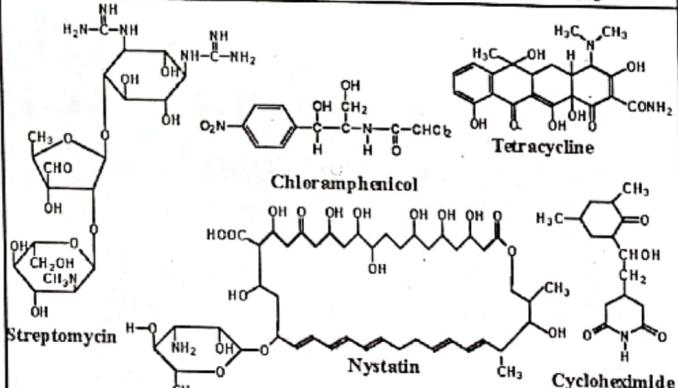
746

### Structures of Antibiotics

- Many antibiotics act by interfering with bacterial protein synthesis.
- They inhibit either transcription or translation.
- Puromycin for example inhibits translation because it structurally resembles a tRNA-amino acid complex.
- During translation, puromycin binds to mRNA and thus halts protein synthesis.

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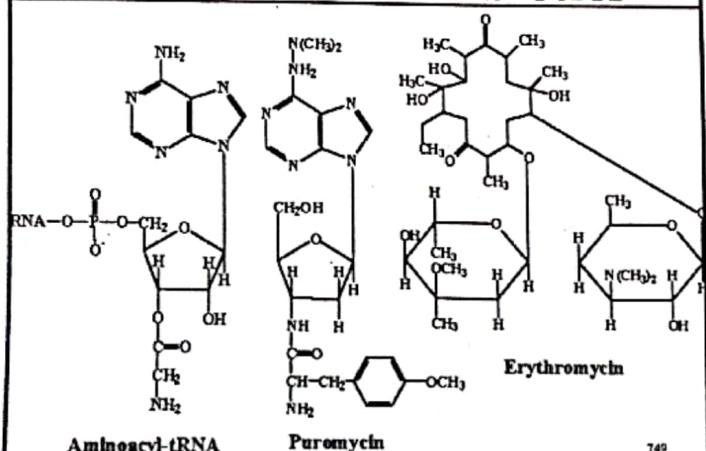
### Structures of Antibiotics



Nystatin and its structural analogue amphotericin B are antifungal agents

748

### Structures of Antibiotics



749

### Mode of Action of Antibiotics

Antibiotic	Eukaryote		Mode of Action
	Cyto	Mito	
Chloramphenicol	+	-	Binds to 50S; blocks chain elongation
Cycloheximide	-	+	Binds 60S; blocks chain elongation
Puromycin	+	+	Binds to A site of larger subunit, causes premature chain termination
Streptomycin	+	+	Binds to smaller ribosomal subunit, blocks chain initiation and elongation.
Erythromycin	+	-	Binds 50S, prevents translocation
Tetracycline	+	-	Binds to 30S ribosomal subunit, blocks A site
Sparsomycin	+	+	Blocks peptidyl transferase

Source: Adapted from Wood, W. B. et al (1974) Biochemistry, A Problems Approach

# 12 Porphyrin Compounds

751

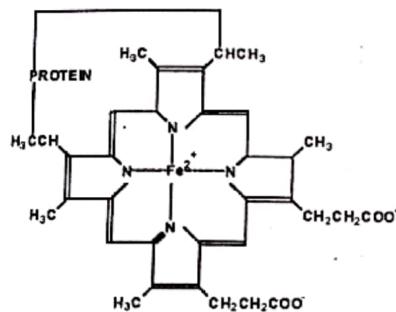
## Porphyrins

- Consist of four pyrrole rings linked through methene groups (-CH<sub>2</sub>-).
- The porphyrin ring system is present in:
  - Heme proteins (e.g. hemoglobin, myoglobin, cytochromes, peroxidase, catalase)
  - Chlorophyll of green plants
- Porphyrins are classified according to the side chain attached to individual pyrrole rings
- The conjugated double bonds in the pyrrole ring system impart certain characteristics to porphyrin-containing compounds e.g.
  - absorption of light in the visible range
  - fluorescence
- The porphyrin molecule is very heat stable.

752

## Porphyrin Compounds

### Heme containing compounds

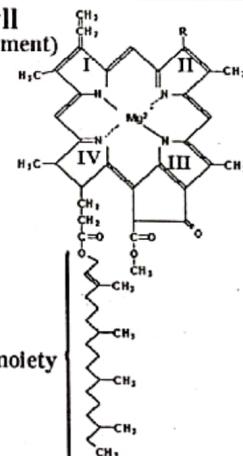


Heme pyrrole ring system is found in heme proteins such as cytochrome C, hemoglobin and myoglobin.

753

## Porphyrin Compounds

### Chlorophyll (green plant pigment)



The structure of chlorophylls *a* and *b*.  
in *a*, R = CH<sub>3</sub>;  
in *b*, R = -CHO

The pyrrole system of chlorophylls resembles that of the heme except that, Mg<sup>2+</sup> is coordinately bound in the center of the tetrapyrrole ring instead of Fe<sup>2+</sup>.

754

# 13 Secondary Plant Metabolites

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## Alkaloids

- The name alkaloids means “like an alkali”.
- They are heterocyclic nitrogen compounds found in plants.
- Are basic and can be extracted by dilute acid and regenerated by subsequent treatment with aqueous base.
- They have profound physiological activity.
- They are used as:
  - analgesics
  - anesthetics
  - anti-depressants
  - stimulants
- They are habit forming (addictive)

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## Alkaloids

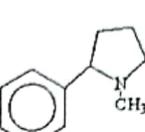
### Nicotine

- Is toxic. Nicotine sulfate is used as an insecticide.
- Acts by stimulating nervous system in small doses. Continuous doses can depress the nervous system. It is responsible for the addiction of smoking.

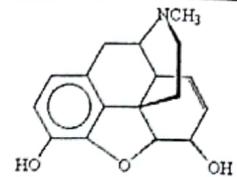
### Morphine

- Obtained from the gum and seeds of opium poppy.
- Codeine is a naturally occurring methoxy derivative of morphine.
- Heroin, is a synthetic diacetyl derivative of morphine. A much more addictive form of morphine.

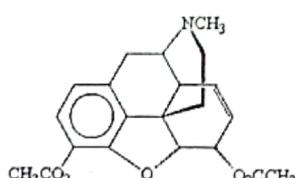
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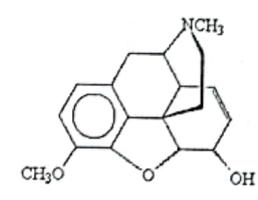
nicotine



morphine



heroin



codeine

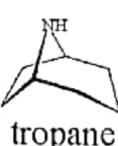
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## Alkaloids

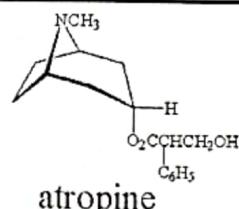
### Tropane alkaloids

- They contain the tropane ring system
  - Atropine
    - used in eye drops to dilate pupils
    - used as anesthetic for eye examination.
    - used to accelerate slow heart rates.
  - Cocaine
    - a habituating stimulant and pain reliever.

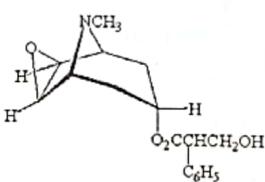
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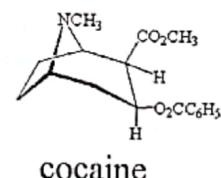
tropane



atropine



scopolamine



cocaine

760

## Saponins

- Plants contain a high percent of glycosides called saponins (Latin *sapo*, soap)
- Have detergent properties and produce frothing in aqueous solution.
- Are toxic and have hemolytic properties.
- Differ in the nature of the sugar(s) present or the aglycone (sapogenin) structure. The sugar moieties are attached at carbon-3
- Two types of saponins are known depending on the nature of the aglycone
  - tetracyclic triterpenoids (steroidal saponins)
  - pentacyclic triterpenoids

761

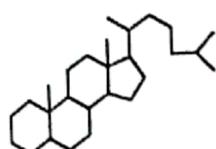
## Saponins

- Steroidal saponins are pharmaceutically important because of their relationship to other steroid compounds e.g. sex hormones.
- Saponins in powder form have been used as foaming and emulsifying agents in the manufacture of toothpastes, foam fire extinguishers, foam in beverages, shampoo, liquid soaps and cosmetics.

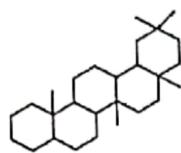
762

## Saponins

### General Structure



Skeleton of tetracyclic triterpenoid saponins



Skeleton of pentacyclic triterpenoid saponins

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## Saponins

Steroidal Saponin	Constituent Sugars
Gitonin	1 glucose, 2 galactose, 1 xylose
Digitonin	2 glucose, 2 galactose, 1 xylose
Dioscin	1 glucose, 2 rhamnose

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## Flavonoids

- Flavonoids (from Latin *flavus* meaning yellow)
- They are plant metabolites with various functions.
- Constitute the largest group of naturally occurring phenolic compounds (more than 2000 known).
- Occur in the free state and as glycosides
- Function as medicinal agents and are common constituents of herbal remedies. They are known for:
  - anti-inflammatory, antithrombotic, vasoprotective, anti-allergic properties.
- They are believed to inhibit cell proliferation.
- Activity thought to be due to effects of flavonoids on arachidonic acid metabolism.

765

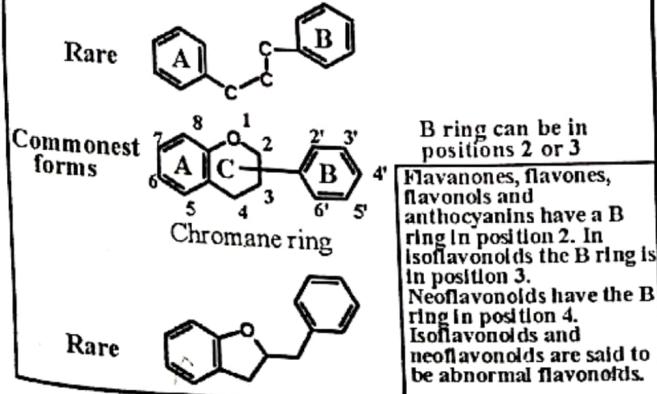
## Flavonoids

- Some flavonoids are also known for antifungal, antibacterial and antitumour properties.
- Intensity of yellow color increases with pH and the number of hydroxyl groups.

766

## Flavonoids

### Flavonoid Skeletons



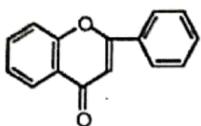
## Flavonoids

### Subgroups of flavonoids

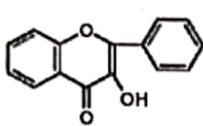
- Anthocyanins
- Chalcones
- Flavone
- Flavonol
- Flavanone
- Isoflavonoids (e.g. rotenone is an insecticide, fish poison and mitochondrial electron transport inhibitor)
- Neoflavonoids
- Anthoxanthins

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## Flavonoids



Flavone



Flavonol

Cocoa and chocolate products have a high flavonol content, which makes them good at protecting the body against cardiovascular diseases.

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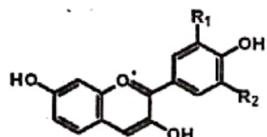
## Anthocyanins

- From the Greek *antho-*, flower, and *kyanos*, blue
- They are flavonoid glycosides of great economic importance
- Occur in practically all parts of most higher plants.
- Most red and blue flowers owe their colors to anthocyanins.
- They serve as,
  - attractants in pollination.
  - natural antioxidants, anti-inflammatory agents
  - promoters of healthy vision, skin and brain function.
  - preventers of premature aging
  - colorants for fruit juices, wine and some beverages.

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## Anthocyanins

### General Structure of Anthocyanidin



the aglycone core of anthocyanins

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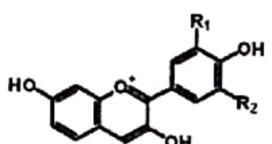
## Anthocyanins

- Anthocyanins vary in different types of fruits.
- Differences in R<sub>1</sub> and R<sub>2</sub> substituents on the B ring distinguishes anthocyanins into three main families of compounds, namely; the **delphinidins**, the **cyanins** and the **pelargonins**.
- R<sub>1</sub> and R<sub>2</sub> can be one of the following H, OH, or OCH<sub>3</sub>
- Anthocyanins usually have the sugar moiety attached in a glycosidic linkage to positions 3,5 or 7.
- Anthocyanidins** are anthocyanins without sugar attachments and are referred to as **delphinidin**, **cyanidin**, and **pelargonidin**. <sup>772</sup>

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## Anthocyanins

### General Structure of Anthocyanidin



the aglycone core of anthocyanins

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## Anthocyanins

- The number and types of sugars attached to the aglycone core affect the polarity and electrochemical properties of anthocyanins.
- The modified properties cause the varieties of reds and blues in flowers and fruits.
- The actual color created by an anthocyanin depends on pH (red in acid, violet in neutral and blue in a basic environment).
- Anthocyanins with sugars attached to position 7 are not common.
- Those with a sugar attached only to position 3 are called monoglycosides, whereas those with sugar moieties attached at both positions 3 and 5 are called diglycosides.

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## Anthocyanins

- Sugar(s) can be removed chemically by acid hydrolysis to give the aglycone, anthocyanidin.
- It has been postulated that anthocyanins when injected by the body are converted into anthocyanidins at the site of use.
- When many anthocyanidins are linked, the polymer is called a proanthocyanidin or more technically, oligomeric proanthocyanidins.

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## Further Reading

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