(BIC 101,151 and BIC 201) Fundamentals of Biochemistry (2+1)

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Syllabus

THEORY

Chemistry of biomolecules: carbohydrates – classification, structure, physical and chemical properties of monosaccharides, disaccharides, oligosaccharides and polysaccharides. Pectins, seed gums, sea weed and algal polysaccharides.

Amino acids – chemistry, structure and properties . Protein – classification based on solubility and functional properties. Structure of proteins – primary, secondary, tertiary and quarternary. Nutritional quality of proteins – egg, milk and meat.

Lipids – classification, structure and properties. Fatty acids – classification, chemistry and properties. Proximate composition of cereals, pulses, oilseeds, tubers, fruits and vegetables. Dietary fiber.

Enzymes – classification, structure and biological importance; Coenzymes and cofactors. Activators and inhibitors of enzymes; Enzymes kinetics; Factors affecting enzyme action and mechanism of enzyme action; active site. Industrial applications of enzymes. Immobilized enzymes.

Metabolism - glycolysis; TCA cycle, cellular respiration – Lipid metabolism – lipases and phospholipases. Fatty acid metabolism – beta oxidation . Metabolism of proteins – proteolytic enzymes. Metabolic inter-relationship.

PRACTICAL

Qualitative tests for monosaccharides, disaccharides and polysaccharides. Estimation of starch, amylose, proteins, amino acids and vitamins. Assay of an enzyme, Chromatography of amino acids/sugars. Estimation of protein, reducing and non-reducing sugars. Determination of iodine number, acid number and saponification number.

THEORY LECTURE SCHEDULE

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9. Structure of proteins.
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10. Extraction of oil and estimation of oil content.
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12. Determination of acid number.
13. Estimation of protein.
14. Isolation of an enzyme and its assay.
15. Chromatography of amino acids.
16. Assay of lipase.
17. Practical examination.

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What is Biochemistry?

Biochemistry, as the name implies, is the chemistry of living organisms. It bridges the gap between the conventional chemistry and biology. Living organisms have certain extraordinary properties. They can grow, respond to stimuli and replicate themselves with high fidelity. All these activities are ultimately interpretable in chemical terms. The lifeless organic molecules with appropriate complexity and properties make a living thing. The basic phenomena of biochemistry are to understand how the collections of inanimate molecules that constitute living organisms interact with each other to maintain life. The basic life processes or chemistry remains broadly the same whether it is an unicellular microorganism or the higher organisms such as human or plants. Life is nothing but thousands of ordered chemical reactions. In other words, chemistry is the logic of all biological phenomena.

The growth of biochemistry in chronological order is presented in the first chapter. The various biomolecules such as carbohydrates, amino acids, proteins, fatty acids, lipids, enzymes and coenzymes are presented in chapters 2 to 8. The metabolism of these biomolecules is described in chapters 9 to 14. . The interrelationship existing between the metabolisms of these biomolecules is presented in nutshell in the final chapter 15. At the end of each chapter, the summary and simple exercise are given. The exercise includes not only the objective and subjective type review questions but also, simple problems in order to help the students to understand the subject more effectively. This will help the students to take up higher studies in biotechnology or to write competitive examinations.

CHAPTER 1

INTRODUCTION TO BIOCHEMISTRY

Lecture-1

1.1 History of biochemistry

Only during 17^{th} and 18^{th} centuries, important foundations were laid in many fields of biology. The 19^{th} century observed the development of very crucial concepts, which include the cell theory by Schleiden and Schwann, Mendel's study of inheritance and Darwin's theory of evolution. The real push to biochemistry was given in 1828 when total synthesis of urea from lead cyanate and ammonia was successfully achieved by Wohler who thus initiated the synthesis of organic compound from inorganic compound. Louis Pasteur, during 1857, did a great deal of work on fermentations and pointed out categorically the central importance of enzymes in this process. The breakthrough in enzyme research and hence, biochemistry was made in 1897 by Edward Buckner when he extracted enzyme from yeast cells in crude form which could ferment a sugar molecule into alcohol. Neuberg introduced the term biochemistry in 1903.

The early part of 20th century witnessed a sudden outburst of knowledge in chemical analysis, separation methods, electronic instrumentation for biological studies (X-ray diffraction, electron microscope, etc) which ultimately resulted in understanding the structure and function of several key molecules involved in life processes such as proteins, enzymes, DNA and RNA.

In 1926, James Sumner established the protein nature of enzyme. He was responsible for the isolation and crystallization of urease, which provided a breakthrough in studies of the properties of specific enzymes.

The first metabolic pathway elucidated was the glycolytic pathway during the first half of the 20th century by Embden and Meyerhof. Otto Warburg, Cori and Parnas also made very important contributions relating to glycolytic pathway. Krebs established the citric acid and urea cycles during 1930-40. In 1940, Lipmann described the central role of ATP in biological systems.

The biochemistry of nucleic acids entered into a phase of exponential growth after the establishment of the structure of DNA in 1953 by Watson and Crick followed by the discovery of DNA polymerase by Kornberg in 1956. From 1960 onwards, biochemistry plunged into an interdisciplinary phase sharing much in common with biology and molecular genetics.

Frederick Sanger's contributions in the sequencing of protein in 1953 and nucleic acid in 1977 were responsible for further developments in the field of protein and nucleic acid research.

The growth of biochemistry and molecular biology was phenomenal during the past two decades. The development of recombinant DNA research by Snell and coworkers during 1980 allowed for further growth and emergence of a new field, the genetic engineering.

Thus there was progressive evolution of biology to biochemistry and then to molecular biology, genetic engineering and biotechnology. What is next? That lies in the hands of young

students like you. The chronological development of biochemistry and other related fields are shown in table 1.1.

Table 1.1 Important scientists and their contribution to biochemistry and other related fields.

1780-1789	Lavoisier	Recognized that respiration is oxidation and first measured oxygen consumption by human subject
1828	Wohler	Synthesized the first organic compound, urea from inorganic components
1837	Berzelius	Postulated the catalytic nature of fermentation. He also identified lactic acid as a product of muscle activity.
1838	Schleiden and Schwann	Enunciated the cell theory
1854-1864	Louis Pasteur	Proved that fermentation is caused by microorganisms
1866	Mendel	Reported the principles of segregation and independent assortment of genes
1869	Miescher	Discovered DNA
1877	Kuhne	Proposed the term 'Enzyme'
1894	Emil Fischer	Demonstrated the specificity of enzymes and the lock and key relationship between enzyme and substrate
1897	Buckner	Discovered alcoholic fermentation in cell-free yeast extract
1902	Emil Fischer	Demonstrated that proteins are polypeptides
1903	Neuberg	First used the term 'biochemistry'
1905	Harden and Young	Showed the requirement of phosphate in alcoholic fermentation and identified first coenzyme, cozymase, later shown to be NAD
1912	Neuberg	Proposed chemical pathway for fermentation
1913	Michaelis and	Developed kinetic theory of enzyme action

	Menten	
1926	Sumner	First crystallized an enzyme, urease and proved it to be a protein
1933	Embden Meyerhof and Parnas	Demonstrated crucial intermediates in the chemical pathway of glycolysis and fermentation
1937	Krebs	Discovered citric acid cycle
1940	Lipmann	Role of ATP in biological systems
1940	Beadle and Tatum	Deduced one gene-one enzyme relationship
1944	Avery, MacLeod and McCarty	Demonstrated that bacterial transformation was caused by DNA
1948	Calvin and Benson	Discovered that phosphoglyceric acid is an early intermediate in photosynthetic CO_2 fixation
1950	Pauling and Corey	Proposed the α-helix structure for keratins
1950-1953	Chargaff	Discovered the base composition of DNA
1953	Sanger and Thompson	Determined the complete amino acid sequence of insulin
1953	Watson and Crick	Proposed the double-helical model for DNA structure
1954	Arnon and Colleagues	Discovered photosynthetic phosphorylation
1956	Kornberg	Discovered DNA polymerase
1958	Meselson and Stahl	Confirmed the Watson-Crick model of semi conservative replication of DNA
1960	Hamilton and Daniel Nathans	Restriction endonucleases

1961	Jacob & Monod	Proposed the operon hypothesis and postulated the function of messenger RNA
1961	Nirenberg and Matthaei	Reported that polyuridylic acid codes for phenylalanine and this opened the way to identification of genetic code
1961-1965	Nirenberg Khorana and Ochoa	Identified the genetic code words for amino acids
1969	Arber	Restriction endonucleases
1977	Sanger	Determination of DNA sequence
1980	Snell	Development of recombinant DNA research leading to genetic engineering
1984	Kary Mullis	Polymerase chain reaction
1997	Wilmut	Viable offspring derived from fetal and adult mammalian cells.
1999	Ingo potrykus	Golden rice rich in β-carotene

CHAPTER 2

CARBOHYDRATES

Lectures 2-6

2.1 Occurrence and importance

Compounds with empirical formula, $(CH_2O)n$, were called as carbohydrates (hydrates of carbons). With the discoveries of many diverse carbohydrates it was noticed that many, but not all, carbohydrates have the above empirical formula; some also contain nitrogen, phosphorus or sulfur. There are some carbohydrates (derivatives) that do not possess $(CH_2O)n$. On the other hand, there are a few non-carbohydrate compounds like lactic acid with empirical formula

$(CH_2O)n$. Hence carbohydrates are chemically defined as polyhydroxy aldehydes or ketones, their derivatives and their polymers.

The carbohydrates comprise one of the major groups of naturally occurring biomolecules. This is mainly because; the light energy from the sun is converted into chemical energy by plants through primary production and is transferred to sugars and carbohydrate derivatives. The dry substance of plants is composed of 50-80% of carbohydrates. The structural material in plants is mainly cellulose and related hemicelluloses. Starch is the important form of storage polysaccharide in plants. Pectins and sugars such as sucrose and glucose are also plant constituents. Many non-carbohydrate organic molecules are found conjugated with sugars in the form of glycosides. The carbohydrates in animals are mostly found in combination with proteins as glycoproteins, as well as other compounds. The storage form of carbohydrates, glycogen, found in liver and muscles, the blood group substances, mucins, ground substance between cells in the form of mucopolysaccharides are few examples of carbohydrates playing important roles in animals. Chitin found in the exo-skeleton of lower animals, is a polymer of N-acetyl glucosamine. Carbohydrates are also universally found in other polymeric substances. For example, fats are fatty acid esters of a sugar alcohol, glycerol. Ribose and deoxyribose are constituent of nucleic acids. Moreover, in all living forms, the energy needed for mechanical work and chemical reactions are derived from carbohydrates. Adenosine triphosphate and related substances that contain ribose as a constituent are key substances in energy storage and transfer. The carbon skeletons of almost all organic molecules are derived from carbohydrates. Besides, the carbohydrates are the basic raw material of many important industries including sugar and sugar products, starch products, paper and wood pulp, textiles, plastics, food processing and fermentation.

2.2 Classification

Carbohydrates are classified into three major groups: monosaccharides, oligosaccharides and polysaccharides (Table 5.1). Monosaccharides are the simplest form that cannot be

hydrolyzed further into smaller units. Monosaccharides are classified into simple monosaccharides and derived monosaccharides. Simple monosaccharides are further classified based on the type of functional group and the number of carbon atoms they possess. Derived monosaccharides include the derivatives of simple monosaccharides such as oxidation products, reduction products, substitution products and esters (Table 5.2).

Oligosaccharides contain two to ten monosaccharide units joined by glycosidic linkages that can be easily hydrolyzed (Table 5.3). The monosaccharides and oligosaccharides are crystalline compounds that are soluble in water and have sweet taste. These compounds are also referred as sugars.

Polysaccharides are high molecular weight polymers. They are either linear or branched in structure. If the polysaccharides are made from a single kind of monosaccharide, they are called as homopolysaccharides. Heteropolysaccharides are made up of more than one type of monosaccharides (Table 5.4). Polysaccharides are usually tasteless insoluble compounds.

2.2.1 Monosaccharides

Some of the monosaccharides occur free in nature and most of them are found as components of various oligosaccharides, polysaccharides and glycosides. The simplest monosaccharide that possesses a hydroxyl group and a carbonyl group with an asymmetric carbon atom is the aldotriose-glyceraldehyde. (A carbon is said to be asymmetric if four different groups or atoms are attached to it. The carbon is also called as a chiral center). Glyceraldehyde is considered as a reference compound and it exists in two optically active forms, D and L. The two families of monosaccharides, D-and L occur based on the configuration of D and L glyceraldehyde (Figure 2.1). In general, the D-family of sugars occurs in nature.

For monosaccharides with two or more asymmetric carbons, the prefixes D or L refer to the configuration of the penultimate carbon (i.e, the asymmetric carbon farthest from the

carbonyl carbon). If the hydroxyl group on the penultimate carbon is on the right-hand side of the carbon chain when the aldehyde or ketone group is written at the top of the formula it belongs to the D family. The D has nothing to do with optical activity. D sugars may be dextro- or levorotatory. The important monosaccharides containing aldehyde group belonging to the D-family are D-erythrose, the aldotetrose; D-ribose, D-arabinose and D-xylose, the aldopentoses; D-glucose, D-mannose and D-galactose, the aldohexoses (Figure 2.2). The important monosaccharide belonging to the L-family is L-arabinose.

The important ketoses are the ketotriose, dihydroxy acetone (It is optically inactive since there is no asymmetric carbon); the ketotetrose, D-erythrulose; the ketopentoses,D-ribulose and D-xylulose and the ketohexose, D-fructose (Figure 2.3).

2.3 Physical properties

In organic chemistry, isomerism is defined as the existence of more than one compound with the same molecular formula. A close observation of the structure of monosaccharides (hexoses) indicate that they possess same molecular formula ($C_6H_{12}O_6$) but with different physical and chemical properties.

There are different types of isomerism. For example, D-glucose and D-fructose differ in the position of carbonyl group (aldehyde and ketone group). These two compounds are functional isomers. Another type of isomerism exhibited by compounds possessing asymmetric carbon atom like monosaccharides, is stereoisomerism. These stereoisomers differ in the spatial arrangement of atoms or groups. There are two types of stereoisomerisms-geometrical and optical isomerism. Geometrical isomers (*cis*-*trans*) differ in the spatial arrangement of atoms across a double bond. Geometrical isomerism is not noticed among carbohydrates. Optical isomers differ in the arrangement of atoms around an asymmetric carbon atom. The number of possible optical isomers can be calculated using the formula 2^n where n=number of asymmetric carbon atoms. For example, glucose contains four asymmetric carbon atoms and the possible optical isomers of glucose are $2^4 = 16$.

2.3.1 Optical activity

To understand optical activity one must have a knowledge on plane-polarized light. A ray of ordinary light vibrates in all directions at right angles to the direction in which the ray is travelling. When this light is passed through a Nicol prism, the emerged light vibrates in only one direction and such light is called as a 'plane polarized light ' (Figure 2.4).

When a beam of plane polarized light is passed through a sugar solution, that is optically active,the plane-polarized light will be rotated either to the right (clockwise) or to the left (anticlockwise). When the plane polarized light is rotated to the right, the compound is dextrorotatory and is written as (+). If the plane polarized light is rotated to the left, the compound is levorotatory (-)

Optical activity is measured using polarimeter. Optical activity varies with the concentration of the sugar solution and length of the polarimeter tube where sugar solution is placed. Specific rotation (α) of a sugar molecule is calculated by the formula

$$(\alpha)_T^D = \frac{\text{Observed rotation}}{\text{Length of tube (dm) x concentration (g/ml)}}$$

Where T=temperature and D=D line of spectrum.

The specific rotation of some important sugars are given below:

Sugar	Specific rotation
D - glucose (dextrose)	+ 52.2
D - fructose (levulose)	-92.0

D - galactose	+ 80.5
D - mannose	+ 14.6
L - arabinose	+ 104.5
Sucrose	+ 66.5

2.3.2 Epimers, enantiomers and diastereomers

Monosaccharides differing in configuration around a single carbon atom other than the carbonyl carbon are known as epimers. e.g. Mannose and glucose are epimers with respect to carbon 2: galactose and glucose are epimers with respect to carbon 4 Enantiomers are non-superimposable mirror images of each other. They differ in the ability to rotate the plane-polarized light. A solution of one enantiomer rotates the plane of such light to the right, and a solution of the other to the left. D-glucose and L-glucose are examples of enantiomers. Diastereomers are stereoisomers that are not mirror images of each other. D-glucose, D-mannose, D-galactose and other members of aldohexose are diastereoisomers.

2.4 Structure of monosaccharides

The monosaccharides exist either in cyclic or acyclic. There are many evidences to show that the pentose and hexose monosaccharides are present in cyclic form. The evidences are 1. Glucose and other aldoses fail to give the Schiff 's test for aldehydes. 2. Solid glucose is quite inert to oxygen whereas aldehydes are easily auto-oxidizable. 3. Glucose and other aldoses do not form bisulfite or aldehyde ammonia compound. 4.Glucose pentaacetate does not react with hydroxylamine. 5. Presence of two forms of glucose with different physical and chemical properties. 6. X-ray analysis definitely proves the existence of the ring structure and also the size of the ring. 7. Mutarotation.

When an aldehyde or a ketone group is present in a molecule that also possesses hydroxyl groups, an intramolecular arrangement may occur to form a hemiacetal or a hemiketal, respectively. This intramolecular hemiacetal or hemiketal is the basis for the cyclic structure of

the sugars. Hence, Haworth (an English chemist) proposed a cyclic hemiacetal structure that accounts completely for its chemical properties (Figure 2.5).

Two types of ring structures are possible, the five-membered furanose and the six-membered pyranose ring if the carbonyl group interact with the third or fourth hydroxyl group. These names are derived from the parent compounds 'furan' and 'pyran' (Figure 2.5). The most common ring structure for aldohexoses is the pyranose ring structure that involves the first carbonyl carbon and the hydroxyl group attached to the fifth carbon. The furanose ring structure is formed by interaction of carbonyl carbon with the hydroxyl group attached to the fourth carbon.This furanose form is less stable than the pyranose strucure and is not very common among aldohexoses. Very seldom is a seven-membered ring formed.

Fructose exists in solution and in compounds as a furanose; however, in the crystalline state only the pyranose ring is believed to exist. Ribose occurs as the furanose structure in many important biological compounds. While writing the cyclic form (Haworth) of monosaccharides it is sometimes difficult to judge whether an OH group should be above or below the plane of the ring. A few rules can be followed for writing Haworth's structure for carbohydrates.

Write the oxygen at the upper right hand corner of the ring structure (pyranose) and the carbons clockwise around the ring. At the fifth carbon it is necessary to rotate the bond to 90^{o} to make the ring closure. For the D-family of sugars, it is customary to write the terminal CH_2OH above the plane of the ring. If the hydroxyl group or hydrogen atom occurs on the right-hand side of the carbon chain in the linear structure it is placed below the plane of the ring in the cyclic structure. Conversely, if the hydroxyl group or hydrogen atom is on the left-hand side of the carbon chain, it is placed above the plane of the ring in the structure formula (Figure 5.6). Writing the formula with the carbons counter-clockwise around the ring is equivalent to flipping the ring over (Figure 2.6). Furanose ring structure for fructose and ribose are shown in figure 2.7.

2.4.1 Conformational structure

The six-membered pyranose ring is not actually planar, as suggested by Haworth, but assume usually the stable chair conformation. The substituents are represented either axially or equatorially (Figure 2.8).

The axial substituents project almost parallel with the vertical axis through the ring and the equatorial substituents project roughly perpendicular to this axis. Substituents in the equatorial positions are less sterically hindered by neighbouring substituents. Conformations with their bulky substituents in equatorial positions are favoured.

2.4.2 Mutarotation

Mutarotation refers to the change in optical rotation when an aqueous sugar solution is allowed to stand. Sugars having potential free aldehyde or keto group exhibit mutarotation. Many sugars exist in two crystalline forms. For example, when D-glucose is dissolved in water and allowed to crystallize out by evaporation of water, one form of D-glucose is obtained. If D-glucose is crystallized from acetic acid or pyridine, another form of D-glucose is obtained. These two forms exhibit different physical and chemical properties.

A cyclic (ring) structure is formed as a result of internal rearrangement of atoms between the carbonyl carbon and hydroxyl group to form a hemiacetal or hemiketal. A new asymmetric carbon is introduced in the molecule due to this rearrangement. As a result of this new asymmetric centre, two isomers are formed for glucose or fructose. When the newly formed hydroxyl group is written below the planar structure, it is called as α-D-glucopyranose. When the hydroxyl group is above the planar structure it is β-D-glucopyranose. Isomeric forms of monosaccharides that differ only in their configuration about the hemiacetal or hemiketal carbon atom are called anomers. A freshly prepared aqueous solution of α-D-glucose has a specific rotation of $+113^{o}$. If the solution of α- D-glucose is allowed to stand, the specific rotation changes to $+52.2^{o}$. Similarly, a fresh solution of β- D-glucose has a specific rotation of $+19^{o}$ which changes to $+52.2^{o}$ on standing. This change in optical rotation is called mutarotation. On standing in solution, the hemiacetal ring opens and reformed to give a mixture

of α- and β-D-glucose having a specific rotation of +52.2^{o}. The anomeric and epimeric structures of glucose are shown in figure 2.9.

2.5.Derived monosaccharides

The important functional groups present in monosaccharides are hydroxyl and carbonyl groups. The hydroxyl group forms esters, usually with phosphoric acid or is replaced by a hydrogen or amino group. The carbonyl group undergoes reduction or oxidation to produce number of derived monosaccharides.

2.5.1 Deoxysugars

In sugars, the hydroxyl group is replaced by a hydrogen to produce deoxy sugars (devoid of oxygen). The important deoxy sugar is 2-deoxy ribose that occurs in deoxy ribonucleic acid. (Figure 5.10a). Other important deoxy sugars are L-fucose and L. rhamnose (Figure 2.10 a).

The substitution of the hydroxyl group at C-6 of L. galactose or L.mannose with hydrogen produces fucose or rhamnose respectively. L-fucose occurs in the cell wall polysaccharides namely hemicelluloses and L-rhamnose occurs in pectic polysaccharides namely rhamnogalacturonan. These deoxy sugars are also found in the complex oligosaccharide components of glycoproteins and glycolipids.

2.5.2 Amino sugars

The hydroxyl group, usually at C-2, is replaced by an amino group to produce amino sugars such as glucosamine, galactosamine and mannosamine. The amino group may be condensed with acetic acid to produce N-acetyl amino sugars, for example, N-acetyl glucosamine (Figure 2.10b). This glucosamine derivative is important constituent of many structural polymers (chitin, bacterial cell wall polysaccharides etc.).

2.5.3 Polyols (alditols)

Both aldoses and ketoses are reduced to polyhydric alcohols (polyols) when treated with enzymes, sodium amalgam, and hydrogen under high pressure with catalyst or sodium borohydride. Each aldose yields the corresponding alcohol upon reduction while a ketose forms two alcohols because of the appearance of a new asymmetric carbon atom in the process. By this reduction process, the following sugars give rise to their respective alcohols under specified conditions.

Glucose	----------→	Sorbitol
Fructose	----------→	Sorbitol and mannitol
Mannose	----------→	Mannitol
Glyceraldehyde	----------→	Glycerol
Erythrose	----------→	Erythritol
Ribose	----------→	Ribitol
Galactose	----------→	Dulcitol

Polyols occur in many plant products. Sorbitol was first isolated from the berries of mountain ash *(Sorbus aucuparia).* Commercially sorbitol is manufactured by the hydrogenation of glucose. Mannitol occurs in many terrestrial and marine plants. Potential food applications of polyols include confectionery products, bakery products, deserts, jams and marmalade. Sorbitol is an excellent moisture conditioner and is used in pharmaceutical preparations such as elixirs and syrups. Sorbitol, as a humectant in creams and lotions helps to stabilize the water content, providing better moisture control. The use of sorbitol or xylitol in toothpaste and mouthwashes is highly desirable.

2.5.4 Oxidation products

When aldoses are oxidized under proper conditions with different types of oxidizing agents, three types of acids are produced, namely aldonic acids, uronic acids and aldaric acids or saccharic acids.

Aldonic acids

Oxidation of an aldose with bromine water at neutral pH converts the aldehyde group to a carboxyl group. Hydrobromous acid formed by the reaction of water with bromine acts as an oxidizing agent (Figure 2.11). Ketoses are not readily oxidized by bromine water. Aldoses are not only oxidized by bromine water but also by the alkaline iodine solution.

$$I_2 + 2\ NaOH \longrightarrow NaOI + Nal + H_2O$$

$$Glucose + NaOI \longrightarrow gluconic\ acid$$

Uronic acids

When aldoses are oxidised with hydrogen peroxide (H_2O_2) uronic acids are formed. In this reaction only primary alcohol group is oxidized to carboxyl group, whereas the aldehyde group remains unchanged (Fugure 2.11). Uronic acids are constituents of pectic polysaccharides.

Aldaric or saccharic acid

When aldoses are oxidised with nitric acid, saccharic acids are formed. Both aldehyde and primary alcohol groups are oxidised to carboxyl groups (Figure 2.11). Glucose on oxidation with nitric acid produces glucaric or glucosaccharic acid. The aldaric acid produced from galactose is called as mucic acid.

2.6 Oligosaccharides

The oligosaccharides commonly encountered in nature belong to disaccharides. The physiologically important disaccharides are maltose, lactose, trehalose and sucrose. Disaccharides consist of two monosaccharides joined covalently by an O-glycosidic bond. The hydroxyl group formed as a result of hemiacetal formation is highly reactive when compared to other hydroxyl groups. This hydroxyl group present in one monosaccharide reacts with any one of the hydroxyl groups attached to C-1, C-2, C-3, C-4, or C-6 of another monosaccharide to

produce 1→1, 1→2, 1→3, 1→4, and 1→6 linked disaccharides. When only one anomeric carbon is involved in glycosidic bond formation, reducing disaccharides are formed. If both anomeric carbon atoms of monosaccharides are involved in glycosidic bond formation that results in the formation of a non-reducing disaccharides such as trehalose (aldosyl-aldosyl disaccharide) or sucrose (aldosyl-ketosyl disaccharide)'. In the case of reducing disaccharides, one end of the molecule having free anomeric carbon is called reducing end and the other end, where the anomeric carbon is involved in glycosidic bond, is called as non-reducing end (Figure 2.12).

2.6.1 Reducing disaccharides

Maltose

Maltose is a diasaccharide made up of two glucose residue joined by a glycosidic linkage between C-1 of one glucose residue and C-4 of the other. The configuration of the anomeric carbon of glucose involved in the linkage is α and hence the glycosidic linkage is α (1→4). The anomeric carbon atom of the second glucose is free and therefore maltose is a reducing sugar. The second glucose residue is capable of existing in α or β configuration (Figure 5.12.). Maltose has been recorded occasionally in plants. It is usually obtained as a product of the enzyme hydrolysis of starch during germination or malting process.

Lactose

Lactose is a reducing disaccharide found only in milk. It is made up of galactose at the non-reducing end and glucose at the reducing end. They are connected by a β (1 →4) linkage (Figure 2.12).

2.6.2 Non-reducing disaccharides

Trehalose

Trehalose, a non-reducing disaccharide., occurs as a major constituent of the circulating fluid (hemolymph) of insects and serves as an energy storage compound. It is also present to a limited extent in the fat body of a variety of insects. It gives twice the amount of energy as that

of glucose and at the same time maintains the osmotic balance. It has been described as an important adaptation of insects engaged in flight. The anomeric carbons of both glucose moieties are involved in the formation of glycosidic bond (Figure2.13).

Sucrose

Sucrose, a sugar of commercial importance, is widely distributed in higher plants. Sugarcane and sugar beet are the sole commercial sources. It is made up of glucose and fructose. The anomeric carbon atom of glucose (C-1) and fructose (C-2) are involved in linkage and is therefore a non-reducing disaccharide (Figure 5.13) Surcose is a major intermediate product of photosynthesis and it is the principal form in which sugar is transported from the leaves to other portions of plants via their vascular systems.

Invert sugar

The hydrolysis of sucrose when followed polarimetrically the optical rotation changes from positive (dextro-) to negative (levo-). The dextrorotatory sucrose on hydrolysis yield levorotatory mixture of glucose and fructose. The levorotaion is due to the presence of fructose which is by itself more levorotatory (-92°) than dextrorototary glucose (+52.2). This phenomenon is called inversion and the mixture of glucose and fructose is called invert sugar. This reaction is catalysed by the enzyme invertase. Invert sugar is more sweeter than sucrose. Honey contains plenty of invert sugar and therefore is very sweet.

2.6.3 Sucrosyl oligosaccharides

The degree of polymerization (DP) of sucrosyl oligosaccharides normally ranges from 3 to 9. Though sucrose is found at higher concentration in all plants, members of the sucrosyl oligosaccharides occur at least in traces in each plant family. The main accumulation of surosyl oligosaccharides is found in storage organs such as roots, rhizomes and seeds. The important members of sucrosyl oligosaccharides are raffinose (DP-3), stachyose (DP-4), verbascose (DP-5) and ajugose (DP-6). All sucrosyl oligosaccharides are non-reducing in nature (Figure 2.14).

Raffinose occupies the second position next to surcose in abundance in the plant kingdom. Raffinose occurs only at low concentration in the leaves of leguminous plants, but

accumulates in the storage organs such as seeds and roots. Most of the leguminous seeds contain these oligosaccharides in large amounts. Bengal gram has higher amounts of raffinose. Red gam and green gram have significantly high amounts of verbascose and stachyose than bengal gram and black gram. These sucrosyl oligosaccharides are responsible for flatulence following the consumption of these legumes. The funtion of sucrosyl oligosaccharides as reserve material is evident, and their contribution to frost resistance is also very well documented.

2.7 Polysaccharides

The polysaccharides found in nature either serve a structural function (structural polysaccharides) or play a role as a stored form of energy (storage polysaccharides).

2.7.1 Storage polysaccharides

Starch, galactomanans and inulin are important storage polysaccharides in plants.

Starch

The principal food-reserve polysaccharide in the plant kingdom is starch. It forms the major source of carbohydrate in the human diet. Starch has been found in some protozoa, bacteria and algae. But the major source is plants where it occurs in the seeds, fruits, leaves, tubers and bulbs in varying amount from a few percent to over 74%. Starch is an alpha-glucan that has structurally distinct components called amylose and amylopectin. A third component referred as the intermediate fraction has also been identified in some starches. Starch molecules are organized into quasicrystalline macromolecular aggregates called granules. The shape of the granules are characteristics of the source of the starch. The two components, amylose and amylopectin, vary in amount among the different sources from less than 2% of amylose in waxy rice or waxy maize to about 80% amylose in amylomaize. The majority of starches contain 15 to 35% of amylose. The ratio of amylose and amylopectin is a function of the enzymes, granulosis bound starch synthase (GBSS) and soluble starch synthase (SSS). GBSS is able to synthesise amylose in a form that is not a substrate for branching enzyme to form amylopectin. Waxy mutants containing only amylopectin lack the GBSS but still contain soluble starch synthase.

Amylose

Amylose is made up of α- D-glucose units linked mostly in a linear way by 1 → 4 linkages (Figure 2.15). It has a molecular weight of 150,000 to 1,000,000 depending on its biological origin. It has been shown that amylose has some elements of nonlinearity. Amylose consists of a mixture of linear molecules with limited, long-chain branching involving α 1 → 6 linkages. The branches contain several hundred glucose residues. Amylose gives a characteristic blue color with iodine due to the ability of the iodine to occupy a position in the interior of a helical coil of glucose units. Pure amylose binds 20% of iodine at 20°C

Amylopectin

Amylopectin is a branched, water-insoluble polymer comprised of thousands of D-glucose residues. The main chain of amylopectin consists of D-glucose residues joined by α (1 →4) glycosidic bonds. Side chains of glucose residues are attached to the main chain by α (1→ 6) glycosidic bonds. Each chain contains 15-25 glucose residues joined by α (1→4) bonds. It contains 94-96% α 1→4 and 4-6% α- 1→-6 linkages. The molecular weight of amylopectin is in the order of 10^7 - 10^8. Robin and co-workers have proposed a model for amylopectin (Figure 5.16). In this model, A and B chains are linear and have degree of polymerization as 15 and 45 respectively. The B chain form the backbone of the amylopectin molecule and extend over two or more clusters. Each cluster of A chain are primarily responsible for the crystalline regions within the granule. The intercrystalline regions occur at regular intervals (60 - 70 °A) containing the majority of α- 1 - 6 linkages. The amylopectin molecule is 100 - 150 A in diameter and 1200-4000 A long. Within the granule, amylose may be located between amylopectin molecules and associated with the linear regions of the amylopectin molecule. Amylopectin produces a purple to red color with iodine.

Inulin

Inulin is a non-digestible fructosyl oligosaccharide found naturally in more than 36000 types of plants. It is a storage polysaccharide found in onion,garlic,chicory,artichoke,asparagus,banana,wheat and rye.It consists of mainly,if not exclusively, of β- 2→1 fructosyl-fructose links.A starting glucose moiety can be present,but is

not necessary.Inulin is asoluble fibre that helps maintain normal bowel function,decreases constipation,lowers cholesrerol and triglycerides.It is used for fat replacement and fibre enrichment in processed foods.

2.7.2 Structural polysaccharides

Cellulose

Cellulose is the most abundant organic substance found in nature. It is the principal constituent of cell walls in higher plants. It occurs in almost pure form (98%) in cotton fibres and to a lessor extent in flax (80%), jute (60-70%), wood (40-50%) and cereal straws (30-43%).

It is linear, unbranched homoglycan of 10,000 to 15,000 D-glucose units joined by β-1→ 4 linkages (Figure 5.17a). The structure of cellulose can be represented as a series of glucopyranose rings in the chair conformation. The most stable conformation for the polymer is the chair turned 180° relative to the adjacent glucose residues yielding a straight extended chain. Celluose molecules within the plant cell walls are organized into biological units of structure known as microfibrils. A microfibril consists of a bundle of cellulose molecules arranged with its long axis parallel to that of the others. This arrangement permits the formation of intramolecular hydrogen bonding between the hydroxyl group of C-3 of one glucose residue and the pyranose ring oxygen atom of the next glucose residue. This hydrogen bond impart a double bond character to the glycosidic bond and impedes the rotation of adjacent glucose residues around the glycosidic bond. Within the microfibril, the adjacent cellulose molecules are linked by intermolecular hydrogen bond between C-6 hydroxyl group of one molecule and the glycosidic bond oxygen atom of adjacent cellulose molecule (Figure 2.17 a).

The cross section of the microfibril consists of a central crystalline core of about 5–30 nm short diameters. The central crystalline core contains around 50-100 cellulose molecules which are arranged in perfect three dimensional array and exhibits a crystalline structure. Surrounding this crystalline core is a region of paracrystalline matrix which contains about 100 polysaccharide molecules of cellulose and hemicellulose (Figure 2.17 b). This region does not

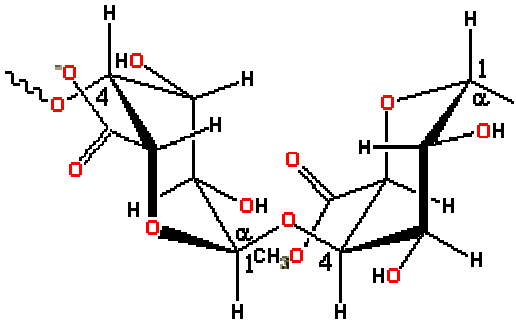
have perfect three-dimensional order and water molecules are able to penetrate the paracrystalline region but not the crystalline core. Algal Polysaccharides

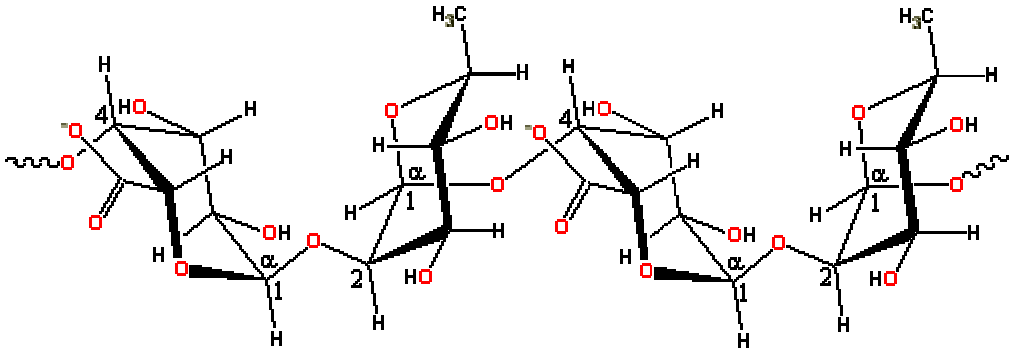
2.8.Pectin

Pectin is a structural polysaccharide, found in fruit and vegetables and mainly prepared from citrus peel.

Structural unit

The majority of the structure consists of partially methylated poly- -(1 4)-D-galacturonic acid residues ('smooth', see right) but there are substantial 'hairy' non-gelling areas (see below) of alternating - (1 2)-L-rhamnosyl- -(1 4)-D-galacturonosyl sections containing branch-points with mostly neutral side chains (1 - 20 residues) of mainly L-arabinose and D-galactose but may also contain other residues such as D-xylose, L-fucose, D-glucuronic acid, D-apiose, 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) and 3-deoxy-D-*lyxo*-2-heptulosonic acid (Dha).





Molecular structure

Generally, pectins do not possess exact structures .D-galacturonic acid residues form most of the molecules, in blocks of 'smooth' and 'hairy' regions. The molecule does not adopt a straight conformation in solution, but is extended and curved ('worm like') with a large amount of flexibility. The `hairy' regions of pectins are even more flexible and may have pendant arabinogalactans. The carboxylate groups tend to expand the structure of pectins as a result of their charge, unless they interact through divalent cationic bridging (their pK_a of about 2.9 ensuring considerable negative charge under most circumstances). Methylation of these carboxylic acid groups forms their methyl esters, which take up a similar space but are much more hydrophobic and consequently have a different effect on the structuring of the surrounding

water. The properties of pectins depend on the degree of esterification, which is normally about 70%. Low methoxyl-pectins (< 40% esterified) gel by calcium di-cation bridging between adjacent two-fold helical chains forming so-called 'egg-box' junction zone structures so long as a minimum of 14-20 residues can cooperate [326]. It may well be that the two carboxylate groups have to cooperate together in prizing the bound water away from the calcium ions to form the salt links that make up these junction zones. The gelling ability of the di-cations is similar to that found with the alginates (Mg^{2+} << Ca^{2+}, Sr^{2+} < Ba^{2+}) with Na^{+} and K^{+} not gelling. If the methoxyl esterified content is greater than about 50%, calcium ions show some interaction but do not gel. The similarity to the behavior of the alginates is that poly- -(1 4)-D-galacturonic acid is almost the mirror image of poly- -(1 4)-L-guluronic acid, the only difference being that the 3-hydroxyl group is axial in the latter. The controlled removal of methoxyl groups, converting high methoxyl pectins to low-methoxyl pectins, is possible using pectin methylesterases but the reverse process is not easily achieved.

High methoxyl-pectins (> 43% esterified) gel by the formation of hydrogen-bonding and hydrophobic interactions in the presence of acids and sugars.

Functionality

Pectins are mainly used as gelling agents, but can also act as thickener, water binder and stabilizer. Low methoxyl pectins (< 50% esterified) form thermoreversible gels in the presence of calcium ions and at low pH (3 - 4.5) whereas high methoxyl pectins rapidly form thermally irreversible gels in the presence of sufficient (e.g. 65% by weight) sugars such as sucrose and at low pH (< 3.5); the lower the methoxyl content, the slower the set. The degree of esterification can be (incompletely) reduced using commercial pectin methylesterase, leading to a higher viscosity and firmer gelling in the presence of Ca^{2+} ions. Highly acetylated pectin from sugar beet is reported to have considerable emulsification ability due to its more hydrophobic nature, but this may be due to protein impurities [309].

As with other viscous polyanions such as carrageenan, pectin may be protective towards milk casein colloids, enhancing the properties (foam stability, solubility, gelation and emulsification) of whey proteins whilst utilizing them as a source of calcium.

General information about pectin substances.

Pectin substances are natural components of plants and their fruits. They occur in plants in connection with cellulose and such substances are called **protopectin**. **Protopectin** is the binder of cell walls. Especially large amounts of **pectin** substances are present in fruit such as: currant, gooseberry, citrus fruits and apples. **Pectin** is a preparation obtained in industrial conditions, containing pectin substances isolated from plant material and soluble in water. Those preparations are used as food and medicine additives and they have the ability to make gels in proper conditions. Raw material for our pectin is dried apple pomace, containing 8-12 % **pectin** substances, and dried lemon peel, containing 18-25 % **pectin** substances, from where they are extracted by diluted acid solution and subsequently precipitated by alcohol, purified, dried and

crumbled. Being the substance of plant origin, it is the best gelling agent for jams and fruit jellies production. Being the naturally compound of fruit, it makes products manufactured with its addition retain fully organoleptic characteristics

Pectin classification

Depending on the applied raw material the following pectins can be distinguished:

- Apple pectins
- Citrus-apple pectins
- Citrus pectins

Depending on degree of esterification (DE) they are divided into :

- High esterificated pectins (DE above 50 %)
- Low esterificated pectins (DE below 50 %)

2.9Agar

Agar is a heteropolysaccharide obtained from red algae.It is composed of

agarose –neutral gelling fraction

Agaropectin – sulfated non-gelling fraction

It is the most effective gelling agents known andis soluble in hot water.It melts in the temperature range of 60 –90^{o}C and sets between 32 and 39^{o}C to form gel.

Uses As Solidifying agent, emulsifier

Pharmaceuticals, cosmetics and food

Laxative

Sizing material in tentile industry

Emulsifierin dairy products

Microbial lab.

2.10.Seaweed Polysaccharides

Structurally they are highly branched and composed of many different monosaccharides.

Alginic acid is obtained from seawood principally from brown algae. It is composed of β1 →4 linked D-mannuronic acid and α → 4 linked L-guluronic acid – random or alternating sequence

Uses: Sauces, frozen deserts, fruit pies

Tabilize emulsions

Soft drinks (gum Arabic)

Beer making to stabilize foam (gum arabic0

Cosmetics and lotions, paints, ink

Adhesive, laxative, foods paper industry (karaya)

Food industry-ice creams, salad dressings, pie fillings (locust gum)

Ice cream, paper industry, fire hoses, medicines (guar)

Salad dressing, cheese (improves sprcading) lower cholesterol, helps diabetics to control sugar.

2.11.Gums

Gums are hydrocolloids High affinity for H_2O

More complex in structure than starch or pectin

Primary building block is galactose (1000s)

They are plant extractives, not digested but are soluble fibres –Low calorie foods

Sources are

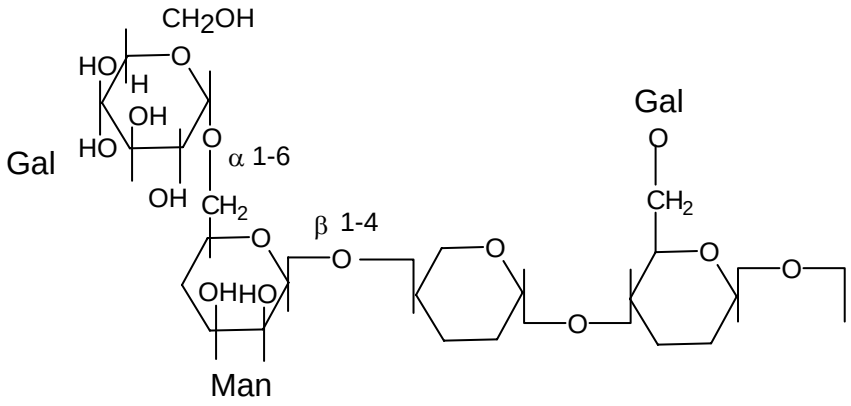
Guar gum	-	cyamopsis tetragonolobus
Taragacanth	-	Astragalaus gummifer
Locust bean gum (Carob)	-	Ceratonia siliqua
Gum Karaya	-	Sterulia urens
Xanthan gum (bacterial)		
Gum Arabic	-	Acacia Senegal

Guar gum

Galactomannan

Mannose β 1 → 4

Galactose α 1 → 6 (every other mannose)



Locust bean gum

Galactomannan

Galactose for every 4th mannose

Xanthan gum

Glucose 2.8 moles

Mannose 3.0 moles

Glucuronic 2.0 moles

All β 1→4 linkage

Backbone similar to cellulose

Trisaccharide side chain at 3 position alternate glucose.

Fenugreek gum (Trigonella foenum –grae cum)

Monnose and galactose 1:1

Prosopsis juliflora gum man: Gal 5:4

2.12 Chemical properties of carbohydrates

Monosaccharides

Reactions of monosaccharides are due to the presence of hydroxyl (-OH) and the potentially free aldehyde (-CHO) or keto (>C=O) groups.

2.12.1 Reaction with alkali

Dilute alkali

Sugars in weak alkaline solutions undergo isomerization to form 1,2-enediol followed by the formation of a mixture of sugars. (Figure 2.18).

Strong alkali

Under strong alkaline conditions sugar undergo caramelization reactions.

2.12.2 Reducing property of sugars

Sugars are classified as either reducing or non-reducing depending upon the presence of potentially free aldehyde or keto groups. The reducing property is mainly due to the ability of these sugars to reduce metal ions such as copper or silver to form insoluble cuprous oxide, under alkaline condition. The aldehyde group of aldoses is oxidized to carboxylic acid. This reducing property is the basis for qualitative (Fehling's, Benedict's, Barfoed's and Nylander's tests) and quantitative reactions. All monosaccharides are reducing. In the case of oligosaccharides, if the molecule possesses a free aldehyde or ketone group it belongs to reducing sugar (maltose and lactose). If the reducing groups are involved in the formation of glycosodic linkage., the sugar belongs to the non- reducing group (trehalose, sucrose, raffinose and stachyose).

2.12.3 Reaction with phenylhydrazine

When reducing sugars are heated with phenylhydrazine at pH 4.7 a yellow precipitate is obtained. The precipitated compound is called as osazone. One molecule of reducing sugar reacts with three molecules of phenylhydrazine. The reaction of phenylhydrazine with glucose is shown in figure 2.19.

D-mannose and D-fructose form same type of osazone as that of D-glucose since the configuration of C-3, C-4, C-5 and C-6 is same for all the three sugars. The osazone of D-galactose is different. Different sugars form osazone at different rates. For example, D-fructose forms osazone more readily than D-glucose. The osazones are crystalline solids with characteristic shapes, decomposition points and specific optical rotations. The time of formation and crystalline shape of osazone is utilized for identification of sugars. If methyl

phenylhydrazine is used instead of phenylhydrazine in the preparation of osazone, only ketoses react. This reaction serves to distinguish between aldose and ketose sugars.

2.12.4 Reaction due to hydroxyl group

Glycoside formation

The hydroxyl group formed as a result of hemiacetal formation in monosaccharides react with methanol and HCl to form methyl α- and β-glycosides (Figure 5.20). The derivaties of each sugar are named according to the name of the sugar, that is, the derivaties of glucose as glucosides, of galactose as galactosides and of arabinose as arabinosides etc. Glycosides are acid-labile but are relatively stable at alkaline pH. Since the formation of glycosides convert the aldehydic group to an acetal group, the glucosides are not a reducing sugars.

Glycosides are also formed with a non-sugar component, the aglycone. The sugars which are connected to the non-sugar moiety are pentoses, hexoses, branched sugars or deoxy or dideoxy sugars. The chain length varies from one to five monosaccharide sugar residues per glycosides. Apart from O-glycosides, three other classes of glycosides are found in higher plants namely S-glycosides, N-glycosides and C-glycosides.

2.12.5 Reaction with acids

Monosaccharides are generally stable to hot dilute mineral acids though ketoses are appreciably decomposed by prolonged action.

Heating a solution of hexoses in a strong non-oxidising acidic conditions, hydroxy methyl furfural is formed. The hydroxymethyl furfural from hexose is usually oxidized further to other products When phenolic compounds such as resorcinol, α-naphthol or anthrone are added, mixture of coloured compounds are formed (Figure 2.21)

The molisch test used for detecting carbohydrate in solution is based on this principle. When conc. H_2SO_4 is added slowly to a carbohydrate solution containing α-naphthol, a pink color is produced at the juncture. The heat generated during the reaction hydrolyse and

dehydrate it to produce furfural or hydroxymethyl furfural which then react with α-naphthol to produce the pink color.

2.12.6 Formation of esters:

When sugars are treated with appropriate acid anhydride or acid chloride under proper conditions, the hydroxyl groups get esterified and form sugar esters (Figure 2.22).

Summary

Carbohydrates are chemically defined as polyhydroxy aldehydes or ketones and their polymers. They are classified into monosaccharides, oligosaccharides and polysaccharides. Monosaccharides, the simplest form of carbohydrates, are classified based on the functional groups as aldoses and ketoses or based on the carbon atoms they possess as triose, tetrose, pentose and hexose. Oligosaccarides contain two to ten monosaccharide units joined by glycosidic linkages.Monosaccharides and oligosaccharides are crystalline compounds and soluble in water having sweet taste. These compounds are also referred as sugars. Most naturally occurring monosaccharides belong to D-family which is determined by the hydroxyl group attached on th right-hand side of the penultimate carbon. Optical isomers differ in the configuration around the asymmetric carbon atoms. Mutarotation refers to the change in optical rotation when an aqueous sugar solution is allowed to stand. Sugars having potential free aldehyde or keto group exhibit mutarotation.

Monosaccharides exist either in cyclic or acyclic forms. The intramolecular hemiacetal or hemiketal formation is the cyclic structure of the monosaccharides. Cyclic form may possess a pyranose or a furanose ring structure. Sugars differing in the configuration of the hydroxyl and hydrogen attached to the carbonyl carbon are called anomers.Epimers differ in the configuration around a carbon atom other than the carbonyl carbon.Glucose and galactose are epimers. Derived monosaccharides are formed when the hydroxyl group forms esters or is replaced by hydrogen (deoxysugars) or amino group (amino sugars). The carbonyl group undergoes reduction to form polyols or oxidation to yield aldonic, uronic or aldaric acids.

The hydroxyl group formed as a result of hemiacetal is highly reactive and reacts with any one of the hydroxyl groups attached to a carbon atom of another monosaccharide through glycosidic bond to form disaccharides, oligosaccharides and polysaccharides. Non-reducing sugars are formed when both anomeric hydroxyl groups are involved in glycosididc bond formation. Thus maltose and lactose are (1-4) linked reducing disaccharides. Sucrose and trehalose are non-reducing disaccharides. Sucrose is called as invert sugar because, the dextrorotatory sucrose is converted into a levorotatory sugar solution after hydrolysis.

Starch and cellulose are important polysaccharides. Starch, the storage homo polysaccharide is composed of the linear amylose with α (1$\rightarrow$4) linked glucose units and branched amylopectin molecules with α(1$\rightarrow$4) and α (1$\rightarrow$6) linked glucoses. Cellulose, the structural homopolysaccharide is a linear molecule with β(1$\rightarrow$4) linkages.

Review Questions

A. Multiple choice questions

1. Identify the non reducing sugar from the following
   a. Maltose b. Lactose c. Sucrose d. Glucose
2. Which one of the following is levorotatory
   a. Glucose b. Mannose c. Sucrose d. Fructose
3. The epimer of glucose is
   a. Galactose b. Fructose c. Arabinose d Ribose
4. Identify the aldopentose from the following
   a. Xylulose b. Ribulose c Ribose d. Erythrose
5. An example of heteropolysaccharide is
   a. Amylose b. Hemicellulose c. Cellulose d. Amylopectin

B. Fill up the blanks

6. Number asymmetric carbons present in α-D-glucopyranose is ----------.

7. Dihydroxyacetone is optically ----------.
8. Starch and glycogen are polymers of ----------.
9. The repeating unit in chitin is ----------.
10. Acid catalysed dehydration of pentose yields ----------.

C. Write short answers for the following

11. Define the following
    a. Anomer b. Epimer c. Enantiomer d Diastereomer e. Reducing sugar
    f. Non-reducing sugar g. Mutarotation h. Optical activity
12. Explain mutarotation with an example.
13. What are glycosides?
14. What are the evidences for the ring structure of glucose?
15. What is inversion sucrose? Which is the invert sugar ? Explain.

D. Write in detail on the following.

16. How are carbohydrates classified? Give example for each group.
17. Compare the structural differences between amylose, amylopectin and cellulose.
18. Explain the reactions of carbohydrates with conc H_2SO_4, HNO_3 and dilute alkali.
19. Describe chemistry and functions of starch.
20. Give the structure and functions of cellulose.

E. Solve the following problems.

21. Draw the structure of β-D-glucopyranose and answer the following
    a. Draw the anomeric form of this sigar.
    b. Name the enantiomer
    c. How many asymmetric carbon atoms it possess?
    d. How many steroisomers of this sugar are possible?
    e. Can you draw three epimeric structures and name them?
22. Compound X is a non reducing sugar with a optical rotation + 66.50. On hydrolysis

the optical rotation changes to –39.80 and yield equimolar concentration of glucose and fructose. Idetify X and draw its structure. Why there is a change in optical rotation upon hydrolysis?

23. The specific rotation of D-mannose is + 14.60. If a solution of D-mannose rotates the incident light by 1.50 in an optical path length of 1 dm, calculate the concentration of D-mannose in the solution.
24. One molar solution of sucrose is hydrolysed using hydrochloric acid. Calculate the molarity of glucose and fructose.
25. Why can't human beings digest cellulose which is made up of glucose only?
26. Can sucrose mutarotate? Justify your answer.

F. Gain additional knowledge by further reading

27. Industrial uses of starch
28. Cell wall structure
29. Pharmaceutical uses of sorbitol
30. Flatulence

Table 2.1 Classification of carbohydrates

Carbohydrates		
Monosaccharides (Simple sugars)	**Oligosaccharides**	**Polysaccharides (Glycans)**

Low molecular weight carbohydrates and cannot be hydrolysed further	Contain 2-10 monosaccharides joined by glycosidic bonds. Low molecular weight carbohydrates which can be hydrolysed by enzymes or acids to yield monosaccharides	Contain many monosaccharides joined by glycosidic bonds. They can be hydrolysed by enzymes or acids.
Crystalline, soluble in water, and sweet in taste.	Powdery or crystalline, soluble in water and sweet in taste	Insoluble in water, tasteless, linear or branched
Classified into triose, tetrose, pentose, hexose and heptose depending upon the number of carbon atoms. They may be either aldoses or ketoses depending upon whether they contain a (potential) free aldehyde or ketone group, respectively	Classified into disaccharide, trisaccharide, tetrasaccharide and pentasaccharide depending upon the number of monosaccharides they contain.	Classified into homoglycans and heteroglycans depending upon the kind of monosaccharides present. Depending upon the function, they are classified as storage and structural polysaccharides.
All monosaccharides are reducing in nature	Some of them are reducing and some of them are non reducing in nature	Non reducing in nature and give deep blue (amylose) or red colour (amylopectin) with iodine.

Table 2.2 Classification of monosaccharides

Monosaccharides	**No. of carbon atoms**	Aldose	**Ketose**	**Occurrence**

Simple				
Triose	3	D-Glycerose	Dilhydroxy acetone	Intermediary metabolities in glucose metabolism
Tetrose	4	D-Erythrose	D-Erythrulose	
Pentose	5	D-Ribose	D-Ribulose	Ribose is a constituent of nucleic acid
		L-Arabinose	-	Occurs in olysaccharides
		D-Xylose	D-Xylulose	Gum arabic, cherry gums, wood gums, proteoglycans
Hexose	6	D-Glucose	D-Fructose	Fruit juices and cane sugar
		D-Galactose	-	Lactose, constituent of lipids
		D-Mannose	-	Plant mannosans and glycoproteins
Heptose	7	-	D-Sedoheptulose	Intermediate in arbohydrate metabolism

Derived

Deoxysugar	5	2-Deoxy ribose	-	DNA
	6	L-Rhamnose	-	Component of cell wall
Aminosugar	6	D-Glucosamine	-	A major component of polysaccharide found in insects and crustaceans (chitin)
Polyol	6	Sorbitol	-	Berries
	6	Mannitol	-	Commercially prepared from mannose and fructose
Aldonic acid	6	Gluconic acid	-	-
Uronic acid	6	Glucuronic acid	-	Constituent of chondroitin sulfate
	6	Galacturonic acid	-	Constituent of pectin
Aldaric acid (Saccharic acid)	6	Glucaric acid	-	Oxidation product of glucose
	6	Mucic acid	-	Oxidation product of galactose

Table 2.3 Composition, sources and properties of common disccharides

Disaccharides	Constituent monosaccharides	Linkage	Source	Properties
Reducing disaccharides				
Maltose	α-D-glucose + α-D-glucose	α(1→4)	Germinating cereal and malt	Forms osazone with phenylhydrazine. Fermentable by enzyme maltase present in yeast. Hydrolysed to two molecules of D-glucose. Undergoes mutarotation.
Lactose	β-D-galactose + α-D-glucose	β(1 →4)	Milk. In trace amounts it can be seen in urine during pregnancy	It shows reactions of reducing sugars including mutarotation. Decomposed by alkali. Not fermentable by yeast. Hydrolysed to one molecule of galactose and one molecule of glucose by acids and the enzyme lactase
Non reducing disaccharides				
Sucrose	α-D-glucose + β-D-fructose	α,β(1→ 2)	Sugar beet, sugar cane, sorghum and carrot roots	Fermentable. Hydrolysed by dilute acids or enzyme invertase (sucrase) to one molecule of glucose and one molecule of fructose. Relatively stable to reaction with alkali

Trehalose	α-D-glucose + α-D-glucose	α,α(1→1)	Fungi and yeast. It is stored as a reserve food supply in insect's hemolymph	It is hydrolysable by acids to glucose with difficulty. Not hydrolysed by enzymes.

Table 2.4 Classification of polysacharides

Polysaccharides

Structural	**Storage**
Important constituents of plant cell wall. Include both homo and heteropolysaccharides	Occurs in the storage organs such as seeds and tubers. Include both homo and heteropolysaccharides
Cellulose is a homopolysaccharide made up of glucose	Starch and inulin are homopolysaccharides made from glucose and fructose, respectively
Hemicelluloses are heterpolysaccharides containing pentoses, hexoses and monosaccharide derivatives	Arabinogalactan is an example for storage heteropolysaccharide
The configuration of major linkages are β-type	The configuration of major linkages are α-type

Chapter 3

Lectures 7-10

AMINO ACIDS AND PROTEINS

3.1 Occurrence and importance

The word "Protein" was coined by J.J. Berzelius in 1838 and derived from the Greek word "Proteios" meaning the ‘first rank’. Proteins are macromolecular polymers composed of amino acids as the basic unit. These biopolymers contain carbon, hydrogen, oxygen, nitrogen and sulphur. The elementary composition of most proteins is very similar; approximate percentages are C=50-55, H=6-8, O=20-23, N=15-18 and S=Traces - 4. Proteins are found in all living cells. They form essential constituent of protoplasm, cell membrane and nuclear material. They may be present as simple proteins or complexes with lipids or nucleic acids. . Proteins from different tissues such as muscle, bone, brain, blood and other biological fluids differ in composition and properties. In cereal and leguminous plants, seeds contain comparatively higher amounts of protein than stem, leaves and flowers. Tuber crops usually contain less amounts of protein in all parts. Enzymes are specialized proteins with catalytic activities and are present in all living organisms.. Proteins serve as regulators of metabolic reactions, directly as components of enzymes and indirectly in the form of chemical messengers known as hormones as well as receptors for hormones. They regulate and integrate the numerous physiological and metabolic processes in the body. Proteins are the center of action in many biological processes.

The structure and properties of amino acids are described first since they are the fundamental structural units of all proteins.

3.2 Amino acids

All proteins are formed from 20 different amino acids. All the amino acids have trivial or common names based on the source from which they were first isolated or based on their properties. Asparagine was named so, as it was isolated from asparagus and glycine was so named because of its sweet taste (Greek:'glykos' meaning sweet).

All the 20 amino acids, except proline, found in proteins have an amino group and a carboxyl group attached to the same carbon atom, namely the α-carbon. They differ only in the side chains (R groups). The 20 amino acids found in proteins are referred as the standard or normal or protein amino acids. There are many other amino acids found in nature but do not occur in proteins.They are referred as non-protein amino acids.

3.2.1 Classification of protein amino acids

The protein amino acids are classified according to the chemical nature of their R groups as aliphatic, aromatic, heterocyclic and sulphur containing amino acids. More meaningful classification of amino acids is based on the polarity of the R groups. The polarity of the R groups varies widely from totally non-polar to highly polar. The 20 amino acids are classified into four main classes whose structures, three-letter and one-letter symbols are shown in figure 3.1.

a. Amino acids with non-polar or hydrophobic, aliphatic R groups

This group of amino acids includes glycine, alanine, valine, leucine, isoleucine and proline (Figure 3.1). The hydrocarbon R groups are non-polar and hydrophobic. The side chains of alanine, valine, leucine and isoleucine are important in promoting hydrophobic interactions within protein structures. The minimal steric hindrance of the glycine side chain (hydrogen) allows more flexibility than other amino acids. On the other hand, the imino group of proline is held in a rigid conformation and reduces the structural flexibility of the protein.

b) Amino acids with non-polar aromatic R groups

This group includes phenylalanine, tyrosine and tryptophan (Figure 3.1.). All these amino acids participate in hydrophobic interactions, which is stronger than aliphatic R groups because of stacking one another. Tyrosine and tryptophan are more polar than phenylalanine due to the presence of hydroxyl group in tyrosine and nitrogen in the indole ring of tryptophan. The absorption of ultraviolet (UV) light at 280 nm by tyrosine, tryptophan and to a lesser extent by phenylalanine is responsible for the characteristic

strong absorbance of light by proteins. This property is exploited in the characterization and quantification of proteins.

c) Amino acids with polar, uncharged R groups

This group of amino acids includes serine, threonine, cysteine, methionine, asparagine and glutamine (Figure 3.1). The hydroxyl group of serine and threonine, the sulphur atom of cysteine and methionine and the amide group of asparagine and glutamine, contribute to the polarity. The R groups of these amino acids are more hydrophilic than the non-polar amino acids.

d) Amino acids with charged R groups

i) Acidic: The two amino acids with acidic R groups are aspartic and glutamic acids (Figure 3.1). These amino acids have a net negative charge at pH 7.0.

ii) Basic: This group includes lysine, arginine and histidine (Figure 3.1). The R groups have a net positive charge at pH 7.0. The lysine has a second ε-amino group; arginine has a positively charged guanidino group; and histidine has an imidazole group.

3.3 Properties of amino acids

3.3.1 Physical

Amino acids are white crystalline substances. Most of them are soluble in water and insoluble in non-polar organic solvents (e.g., chloroform and ether). Aliphatic and aromatic amino acids particularly those having several carbon atoms have limited solubility in water but readily soluble in polar organic solvents. They have high melting points varying from 200-300^{o}C or even more. They are tasteless, sweet or bitter. Some are having good flavour. Sodium glutamate is a valuable flavouring agent and is used in the preparation of certain dishes and sauces.

Amphoteric nature of amino acids

Amino acids are amphoteric compounds, as they contain both acidic (COOH) and basic (NH_2) groups. They can react with both alkalies and acids to form salts. In acid solution amino acids carry positive charges and hence they move towards cathode in an electric field. In alkaline solution, on the other hand, the amino acids carry negative charges and therefore move towards anode. But when an amino acid is dissolved in water, it exists as inner salt carrying both positive and negative charges. This occurs as a result of dissociation of carboxyl group to release the H^+ ion, which passes from the carboxyl to the amino group as shown in the figure 3.2. The amino acids possessing both positive and negative charges are called zwitterions.

Figure 3.2

The zwitterion reacts as an acid with a base by liberating a proton (H^+) from the NH_3^+ group and as a result possesses a net negative charge. On the other hand, zwitterion reacts with an acid as base, combining with the proton (H^+) of the acid resulting in the formation of a compound having a net positive charge. These reactions are reversible. The pH at which the amino acid has no tendency to move either towards positive or negative electrode is called isoelectric pH or isoelectric point. At this pH, the amino acid molecule bears a net charge of zero.

Isomerism

All amino acids except proline, found in protein are α-amino acids because NH_2 group is attached to the α-carbon atom, which is next to the COOH group. Examination of the structure of amino acids reveals that except glycine, all other amino acids possess asymmetric carbon atom at the alpha position. Because of the presence of asymmetric carbon atom, amino acids exist in optically active forms. For example, in the steric configuration for serine, the carboxyl group is written on the top, while the amino group is written to the left in the case of L-serine and to the right in the case of D-serine (Figure 3.3). This distinction will hold good for all the amino acids having asymmetric carbon atoms. 'D' and 'L' do not refer to the optical rotation, but to the steric configuration of amino group to the right and left side of the carboxyl group. The direction of optical rotation of amino acid is indicated by the symbol + or -, which follows the designation 'D' or 'L'. The steric configuration and optical rotation of an amino acid may be simultaneously expressed as D (+) or D (-) and L (+) or L (-). L-forms are more common than D-forms and most of the naturally occurring amino acids are L-amino acids.

3.3.2 Chemical properties

3.3.2.1 Reactions due to amino group

Reaction with formaldehyde (Formal titration)

Amino acid exists as zwitterion in aqueous medium. If an amino acid solution is treated with excess of neutralized formaldehyde solution, the amino group combines with formaldehyde forming dimethylol amino acid which is an amino acid formaldehyde complex (Figure 3.4). Hence the amino group is protected and the proton released is titrated against alkali. This method is used to find out the amount of total free amino acids in plant samples.

Reaction with nitrous acid

Nitrous acid reacts with the amino group of amino acids to form the corresponding hydroxyacids and liberate nitrogen gas (Figure 3.5).

Reaction with ninhydrin

Ninhydrin is a strong oxidizing agent. When a solution of amino acid is boiled with ninhydrin, the amino acid is oxidatively deaminated to produce ammonia and a ketoacid. The keto acid is decarboxylated to produce an aldehyde with one carbon atom less than the parent amino acid. The net reaction is that ninhydrin oxidatively deaminates and decarboxylates α-amino acids to CO_2, NH_3 and an aldehyde. The reduced ninhydrin then reacts with the liberated ammonia and another molecule of intact ninhydrin to produce a purple coloured compound known as Ruhemann's purple (Figure 3.6).

This ninhydrin reaction is employed in the quantitative determination of amino acids. Proteins and peptides that have free amino group(s) (in the side chain) will also react and give colour with ninhydrin.

3.3.2.2 Reactions due to carboxyl group

Decarboxylation

The carboxyl group of amino acids is decarboxylated to yield the corresponding amines. Thus, the vasoconstrictor agent, histamine is produced from histidine (Figure 3.7). Histamine stimulates the flow of gastric juice into the stomach and the dilation and constriction of specific blood vessels. Excess reaction to histamine causes the symptoms of asthma and various allergic reactions.

3.4 Essential amino acids

Most of the prokaryotic and many eukaryotic organisms (plants) are capable of synthesizing all the amino acids present in the protein. But higher animals including man possess this ability only for certain amino acids. The other amino acids, which are needed for normal functioning of the body but cannot be synthesized from metabolic intermediates, are called essential amino acids. These must be obtained from the diet and a deficiency in any one of the amino acids prevents growth and may even cause death. Methionine, Arginine, Threonine, Tryptophan, Valine, Isoleucine, Leucine, Phenylalanine, Histidine, and Lysine are the essential amino acids (Remember MATTVILPHLy).

3.5 Peptide

Amino acids are linked together by formation of covalent bonds. The covalent bond is formed between the α-carboxyl group of one amino acid and the α-amino group of the next amino acid. The bond so formed between the carboxyl and the amino groups, after elimination of a water molecule is called as a peptide bond and the compound formed is a peptide (Figure 3.8).

The peptide formed between two amino acids is a dipeptide; three amino acids is a tripeptide; few amino acids are an oligopeptide and many amino acids is a polypeptide. In writing the peptide structure, the amino terminal (N-terminal) amino acid is written first and carboxyl terminal (C-terminal) amino acid written last.

3.5.1 Peptides of physiological interest

Glutathione is a commonly occurring tripeptide (γ-glutamyl cysteinyl glycine) in many living organisms (Figure 3.9). It has a role in detoxification of toxic compounds in physiological system.

The nanapeptides (nine amino acids), oxytocin and vasopressin are important animal peptide hormones. Oxytocin induces labor in pregnant women and controls contraction of uterine muscle. Vasopressin plays a role in control of blood pressure by regulating the contraction of smooth muscles.

A dipeptide L-aspartyl-L-phenylalanine, is of commercial importance. This dipeptide is about 200 times sweeter than cane sugar. The methyl ester of this dipeptide is called as aspartame and marketed as an artificial sweetener for diabetics.

3.6 Proteins

3.6.1 Classification of protein

Proteins are classified based on their
(a) Solubility and composition
(b) Function
(c) Shape & size

(a) Classification based on solubility and composition

According to this classification, proteins are divided into three main groups as simple, conjugated and derived proteins.

(i) Simple proteins

Simple proteins yield on hydrolysis, only amino acids. These proteins are further classified based on their solubility in different solvents as well as their heat coagulability.

Albumins

Albumins are readily soluble in water, dilute acids and alkalies and coagulated by heat. Seed proteins contain albumin in lesser quantities. Albumins may be precipitated out from solution using high salt concentration, a process called 'salting out'. They are deficient in glycine. Serum albumin and ovalbumin (egg white) are examples.

Globulins

Globulins are insoluble or sparingly soluble in water, but their solubility is greatly increased by the addition of neutral salts such as sodium chloride. These proteins are coagulated by heat. They are deficient in methionine. Serum globulin, fibrinogen, myosin of muscle and globulins of pulses are examples.

Prolamins

Prolamins are insoluble in water but soluble in 70-80% aqueous alcohol. Upon hydrolysis they yield much proline and amide nitrogen, hence the name prolamin. They are deficient in lysine. Gliadin of wheat and zein of corn are examples of prolamins.

Glutelins

Glutelins are insoluble in water and absolute alcohol but soluble in dilute alkalies and acids. They are plant proteins e.g., glutenin of wheat.

Histones

Histones are small and stable basic proteins and contain fairly large amounts of basic amino acid, histidine. They are soluble in water, but insoluble in ammonium hydroxide. They are not readily coagulated by heat. They occur in globin of hemoglobin and nucleoproteins.

Protamines

Protamines are the simplest of the proteins. They are soluble in water and are not coagulated by heat. They are basic in nature due to the presence of large quantities of arginine. Protamines are found in association with nucleic acid in the sperm cells of certain fish. Tyrosine and tryptophan are usually absent in protamines.

Albuminoids

These are characterized by great stability and insolubility in water and salt solutions. These are called albuminoids because they are essentially similar to albumin and globulins. They are highly resistant to proteolytic enzymes. They are fibrous in nature and form most of the supporting structures of animals. They occur as chief constituent of exoskeleton structure such as hair, horn and nails.

ii. Conjugated or compound proteins

These are simple proteins combined with some non-protein substances known as prosthetic groups. The nature of the non-protein or prosthetic groups is the basis for the sub classification of conjugated proteins.

Nucleoproteins

Nucleoproteins are simple basic proteins (protamines or histones) in salt combination with nucleic acids as the prosthetic group. They are the important constituents of nuclei and chromatin.

Mucoproteins

These proteins are composed of simple proteins in combination with carbohydrates like mucopolysaccharides, which include hyaluronic acid and chondroitin sulphates. On hydrolysis, mucopolysaccharides yield more than 4% of amino-sugars, hexosamine and uronic acid e.g., ovomucoid from egg white. Soluble mucoproteins are neither readily

denatured by heat nor easily precipitated by common protein precipitants like trichloroacetic acid or picric acid. The term glycoproteins is restricted to those proteins that contain small amounts of carbohydrate usually less than 4% hexosamine.

Chromoproteins

These are proteins containing coloured prosthetic groups e.g., haemoglobin, flavoprotein and cytochrome.

Lipoproteins

These are proteins conjugated with lipids such as neutral fat, phospholipids and cholesterol (Refer chapter 4).

Metalloproteins

These are metal-binding proteins. A β-globulin, termed transferrin is capable of combining with iron, copper and zinc. This protein constitutes approximately 3% of the total plasma protein. Another example is ceruloplasmin, which contains copper.

Phosphoproteins

These are proteins containing phosphoric acid. Phosphoric acid is linked to the hydroxyl group of certain amino acids like serine in the protein e.g., casein of milk.

iii. Derived proteins

These are proteins derived by partial to complete hydrolysis from the simple or conjugated proteins by the action of acids, alkalies or enzymes. They include two types of derivatives, primary-derived proteins and secondary-derived proteins.

Primary-derived proteins

These protein derivatives are formed by processes causing only slight changes in the protein molecule and its properties. There is little or no hydrolytic cleavage of peptide bonds.

Proteans

Proteans are insoluble products formed by the action of water, dilute acids and enzymes. These are particularly formed from globulins but are insoluble in dilute salt solutions e.g., myosan from myosin, fibrin from fibrinogen.

Metaproteins

These are formed by the action of acids and alkalies upon protein. They are insoluble in neutral solvents.

Coagulated proteins

Coagulated proteins are insoluble products formed by the action of heat or alcohol on natural proteins e.g., cooked meat and cooked albumin.

Secondary-derived proteins

These proteins are formed in the progressive hydrolytic cleavage of the peptide bonds of protein molecule. They are roughly grouped into proteoses, peptones and peptides according to average molecular weight. Proteoses are hydrolytic products of proteins, which are soluble in water and are not coagulated by heat. Peptones are hydrolytic products, which have simpler structure than proteoses. They are soluble in water and are not coagulated by heat. Peptides are composed of relatively few amino acids. They are water-soluble and not coagulated by heat. The complete hydrolytic decomposition of the natural protein molecule into amino acids generally progresses through successive stages as follows:

Protein ------→ Protean --------→ Metaprotein ------→
Proteoses ------→ Peptones --------→ Peptides ------→ amino acids

b. Classification of proteins based on function

Proteins are classified based on their functions as:

Catalytic proteins - Enzymes

The most striking characteristic feature of these proteins is their ability to function within the living cells as biocatalysts. These biocatalysts are called as enzymes. Enzymes represent the largest class. Nearly 2000 different kinds of enzymes are known, each catalyzing a different kind of reaction. They enhance the reaction rates a million fold. (Refer chapter 6 for more detail).

Regulatory proteins - Hormones

These are polypeptides and small proteins found in relatively lower concentrations in animal kingdom but play highly important regulatory role in maintaining order in complex metabolic reactions e.g., growth hormone, insulin etc.

Protective proteins - Antibodies

Some proteins have protective defense function. These proteins combine with foreign protein and other substances and fight against certain diseases. e.g., immunoglobulin. These proteins are produced in the spleen and lymphatic cells in response to foreign substances called antigen. The newly formed protein is called antibody which specifically combines with the antigen which triggered its synthesis thereby prevents the development of diseases. Fibrin present in the blood is also a protective protein.

Storage proteins

A major class of proteins which has the function of storing amino acids as nutrients and as building blocks for the growing embryo. Storage proteins are source of essential amino acids, which cannot be synthesized by human beings. The major storage protein in pulses is globulins and prolamins in cereals. But in rice the major storage protein is glutelins. Albumin of egg and casein of milk are also storage proteins.

Transport proteins

Some proteins are capable of binding and transporting specific types of molecules through blood. Haemoglobin is a conjugated protein composed of colourless basic protein, the globin and ferroprotoporphyrin or haem. It has the capacity to bind with oxygen and transport through blood to various tissues. Myoglobin, a related protein, transports oxygen in muscle. Lipids bind to serum proteins, principally, albumin and transported as lipoproteins in the blood.

Toxic proteins

Some of the proteins are toxic in nature. Ricin present in castor bean is extremely toxic to higher animals in very small amounts. Enzyme inhibitors such as trypsin inhibitor bind to digestive enzyme and prevent the availability of the protein. Lectin, a toxic protein present commonly in legumes, agglutinates red blood cells. A bacterial toxin causes cholera, which is a protein. Snake venom is protein in nature.

Structural proteins

Some proteins serve as structural materials or as important components of extra cellular fluid. Examples of structural proteins are myosin of muscles, keratin of skin and hair and collagen of connective tissue. Carbohydrates, fats, minerals and other cellular components are organized around such structural proteins that form the molecular framework of living material.

Contractile proteins

Proteins like actin and myosin function as essential elements in contractile system of skeletal muscle.

Secretary proteins

Fibroin is a protein secreted by spiders and silkworms to form webs and cocoons.

Exotic proteins

Antarctic fishes live in -1.9^{o}C waters, well below the temperature at which their blood is expected to freeze. These fishes are prevented from freezing by antifreeze glycoproteins present in their body.

C. Classification based on size and shape

Based on size and shape, the proteins are also subdivided into globular and fibrous proteins. Globular proteins are mostly water-soluble and fragile in nature e.g., enzymes, hormones and antibodies. Fibrous proteins are tough and water-insoluble. They are used to build a variety of materials that support and protect specific tissues, e.g., skin, hair, fingernails and keratin

3.7 Conformation of proteins

Conformation of a protein refers to the three-dimensional structure in its native state. There are many different possible conformations for a molecule as large as a protein. A protein can perform its function only when it is in its native condition. Due to the complexity of three-dimensional structures, the structure of protein is discussed at different levels of its organization.

Four levels of structural organization can be distinguished in proteins:

1. Primary
2. Secondary
3. Tertiary
4. Quaternary

3.7.1 Primary structure

Primary structure of protein refers to the number of amino acids and the order in which they are covalently linked together. It also refers to the location of disulfide bridges, if there are any, in a polypeptide chain.

The peptide bond is covalent in nature, quiet stable and referred as backbone of the protein. They can be disrupted by chemical or enzymatic hydrolysis but are not directly influenced by salt concentration, change in pH or solvent.

Frederick Sanger in 1953 determined the complete amino acid sequence of insulin for the first time.The important steps involved in determining the primary structure of protein are

a. Determination of number of (chemically different) polypeptide chains or subunits in the protein.
b. Separation of polypeptide chains if more than one are present in a protein.
c. Determination of the amino acid sequence of the subunits.
d. Elucidation of the position of the disulfide bonds, if any, between and within the subunits.

Determination of number of polypeptides or subunits

Determination of the number of C-terminal or N-terminal amino acids will indicate the number of polypeptides in a protein.

H_2N ----------$\rightarrow$ COOH

N-terminal Polypeptide C-terminal

N-terminal identification

Fluoro dinitro benzene (FDNB), known as Sanger's reagent, was used to identify the N-terminal amino acid. This reagent was replaced by dansyl chloride and Edman's reagent (phenyl isothiocyanate, PITC).

Edman's reagent is also used to determine the amino acid sequence of a polypeptide chain from the N-terminal by subjecting the polypeptide to repeated cycles of Edman degradation. After every cycle, the newly liberated phenylthiohydantoin (PTH) amino acid was identified (Figure 3.10). The sequence of peptides containing 30-40 amino acids can be determined using a sequencer by adopting the Edman's degradation method.

C-terminal identification

C-terminal amino acid can be determined by methods similar to those used for the N-terminal acid. Hydrazine is used to find out the C-terminal amino acid. It reacts with the carbonyl group of each peptide bond except C-terminal amino acid. The bond is cleaved and each amino acid derivative is released as the hydrazide derivative

(hydrazinolysis). Since the carboxyl group of C-terminal amino acid is not involved in a peptide bond, it remains in the mixture as the only unmodified amino acid (Figure 3.11). After chromatographic separation and comparison with the standards, the C-terminal amino acid can be identified. Carboxypeptidases are used for enzymic determination of the C-terminal amio acid.

Separation and purification of polypeptide chains

Determination of C-terminal and/or N-terminal amino acids reveals the presence of one or more polypeptide chains in a protein. If the protein contains more than one polypeptide chain, separation of polypeptide chain is essential. If the polypeptide chains are connected by disulfide bond, they are cleaved to separate the individual peptide chains. The polypeptide is treated with 2-mercaptoethanol ($HS\text{-}CH_2\text{-}CH_2OH$) so that reductive cleavage occurs and the polypeptide chains are separated. The resulting free-SH groups are usually alkylated by treatment with iodoacetic acid (Figure 3.12).

After cleaving the disulfide links using mercaptoethanol, subunits are dissociated using denaturing agents such as urea or guinidinum ion or detergents such as sodium dodecyl sulphate (SDS). The dissociated subunits are then separated using ion exchange or gel filtration chromatographic method.

Amino acid sequencing of polypeptides

The amino acid sequence in polypeptides with 30-40 amino acids can be determined by Edman reaction. For polypeptides containing more than 40 amino acids, both enzymatic and chemical methods are employed to break polypeptide chains into smaller peptides. The enzyme, trypsin hydrolyses the peptide bond on the carboxyl side of the basic amino acid residues of lysine or arginine. The chemical reagent, cyanogen bromide cleaves peptide bond on the carboxyl side of methionine residues. The hydrolyzed peptides are separated and the amino acid sequence is determined by Edman reaction. The hydrolysis of the original polypeptide by two different methods separately gives overlapping regions, from which the sequence is derived (Figure 3.13).

3.7.2 Secondary structure

Secondary structure refers to the steric relationship of amino acids that are close to one another in the linear sequence. The folding of a linear polypeptide chain occurs to form a specific coiled structure. Such coiling or folding is maintained by hydrogen bonds and hydrogen bond is the only bond responsible for secondary structure. X-ray studies of several polypeptides by Linus Pauling and Robert Corey revealed that the peptide group has a rigid, planar structure which is a consequence of resonance interactions that give the peptide bond a 40% double bond character. Peptide groups mostly assume the trans-conformation in which successive C2 atoms are on opposite sides of peptide bond joining them. The cis configuration creates steric interference. If a polypeptide chain is twisted by the same amount each of its C atoms, it assumes a helical conformation (Figure 3.14).

Helix structure

The α-helix is the most stable arrangement of polypeptides (Figure 3.15).The helix structure of proteins is stabilized by intramolecular hydrogen bonding.

In this structure, hydrogen bonds are formed between the C=O group of one peptide bond and the N-H group of another after 3 amino acid units. The polypeptide chain constituted by L-amino acids form a right-handed helix, whereas the polypeptide chains made up of D-amino acids form a left-handed helix. In the α-helical conformation, all the side chains lie outside the helix whereas C, N, O and H of the peptide bond lie in the same plane.

Certain amino acids tend to disrupt the α-helix. Among these are proline (the N-atoms is part of the rigid ring and no rotation of the N-C bond can occur) and amino acid with charged or bulk R groups that either electrostatically or physically interferes with helix formation.

The β-pleated sheet structure

Pauling and Corey also proposed a second ordered structure, the β-pleated sheet for polypeptide. This structure is a result of intermolecular hydrogen bonding between the polypeptide chains to form a sheet like arrangement (Figure 3.16).

There are two ways in which proteins chains can form the pleated sheet structure. One is with the chains running in the same direction i.e. the -COOH or NH_2 ends of the polypeptide chains lying all at the top or all at the bottom of the sheet. This is called

parallel pleated-sheet structure. In another type, known as antiparallel β-pleated sheet structure, the polypeptide chains alternate in such a way that the -COOH end of the one polypeptide is next to the $-NH_2$ end of the other i.e. polypeptide chains run in opposite directions.

The random coil

Regions of proteins that are not identifiably organized as helices or pleated sheets are said to be present in random coil conformation. Considerable portion of the protein may be present in this conformation. The term 'random' is unfortunate which imply less biological significance than more highly repeating regions. But in terms of biological function, the regions of random coil are of equal importance to those of helix and pleated sheet.

3.7.3 Tertiary structure

Tertiary structure refers to the steric relationship of amino acid residues that are far apart in the linear sequence. This leads to the twisting of polypeptide chains into specific loops and bends which are maintained chiefly by five kinds of bonds.

Hydrogen bonds

Hydrogen bonds are formed between the side chain (R group) of amino acids having a hydrogen donor group and an acceptor group (Figure 3.17).
Salt-linkages (electrostatic forces; ionic bonds)

Salt linkages are due to the interaction between amino groups of basic amino acids and the carboxyl group of acidic amino acids present in the R group (Figure 3.18).

Disulfide bonds (S-S linkages)

The S-S linkages are formed by the oxidation of sulfhydryl (-SH) group of two cysteine side chains (Figure 3.19).

Hydrophobic bonds

Hydrophobic bonds are formed as a result of interaction between non-polar side chains (Figure 3.20).

Dipole-dipole interaction

This interaction occurs between polar unionized side chains (Figure 3.21).

The folding of a polypeptide chain due to different covalent and non-covalent interactions is shown in figure 3.22.

Out of the above bonds, the disulfide bond (covalent bond) is the strongest and cannot be affected by solvent, pH, temperature and salts whereas the above conditions. The disulfide bond can be split and reformed by oxidation/reduction respectively. The tertiary structure gains special importance in the case of enzymes.

Domain

Domains are structurally independent units that have the characteristics of a small globular protein. Domains often have a specific function such as the binding of a small molecule.

A long peptide strand of a protein will often fold into multiple, compact semi-independent folded regions or domains. Each domain having a characteristic spherical geometry with a hydrophobic core and polar surface very much like the tertiary structure of a whole globular protein. The domains of a multidomain protein are often interconnected by a segment of polypeptide chain lacking regular secondary structure. In enzymes with more than one substrate or allosteric effector sites the different binding sites are often located in different domains. In multifunctional proteins, the different domains perform different tasks.

3.7.4 Quaternary structure

Proteins that have more than one subunit or polypeptide chains will exhibit quaternary structure. Quaternary structure refers to a functional protein aggregate

(organization) formed by interpolypeptide linkage of subunits or polypeptide chains. These subunits are held together by noncovalent surface interaction between the polar side chains. Proteins formed like above are termed oligomers and the individual polypeptide chains are variously termed protomers, monomers or subunits. The most common oligomeric proteins contain two or four protomers and are termed dimers or tetramers, respectively.

Myoglobin has no quaternary structure since, it is composed of a single polypeptide chain. Hemoglobin molecule, which consists of four separate polypeptide chains, exhibits quaternary structure.

Quaternary structure may influence the activity of enzymes. Some enzymes are active only in their quaternary state and become inactive when split into smaller units. Other enzymes are inactive in the quaternary state and are activated only when they are dissociated to form monomeric state.

3.8 Physical and chemical properties of proteins

3.8.1 Physical

Pure proteins are generally tasteless, though the predominant taste of protein hydrolysates is bitter. Pure proteins are odourless. Because of the large size of the molecules, proteins exhibit many properties that are colloidal in nature. Proteins, like amino acids, are amphoteric and contain both acidic and basic groups. They possess electrically charged groups and hence migrate in an electric field. Many proteins are labile and readily modified by alterations in pH, UV radiation, heat and by many organic solvents.

The absorption spectrum of protein is maximum at 280 nm due to the presence of tyrosine and tryptophan, which are the strongest chromophores in that region. Hence the absorbance of the protein at this wavelength is adapted for its determination.

Denaturation of protein

The comparatively weak forces responsible for maintaining secondary, tertiary and quaternary structure of proteins are readily disrupted with resulting loss of biological activity. This disruption of native structure is termed denaturation. Physically, denaturation is viewed as randomizing the conformation of a polypeptide chain without affecting its primary structure (Figure 3.23).

Physical and chemical factors are involved in the denaturation of protein

a) Heat and UV radiation supply kinetic energy to protein molecules causing their atoms to vibrate rapidly, thus disrupting the relatively weak hydrogen bonds and salt linkages. This results in denaturation of protein leading to coagulation. Enzymes easily digest denatured or coagulated proteins.

b) Organic solvents such as ethyl alcohol and acetone are capable of forming intermolecular hydrogen bonds with protein disrupting the intramolecular hydrogen bonding. This causes precipitation of protein.

c) Acidic and basic reagents cause changes in pH, which alter the charges present on the side chain of protein disrupting the salt linkages.

d) Salts of heavy metal ions (Hg^{2+}, Pb^{2+}) form very strong bonds with carboxylate anions of aspartate and glutamate thus disturbing the salt linkages. This property makes some of the heavy metal salts suitable for use as antiseptics.

Renaturation

Renaturation refers to the attainment of an original, regular three-dimensional functional protein after its denaturation.

When active pancreatic ribonuclease A is treated with 8M urea or β-mercaptoethanol, it is converted to an inactive, denatured molecule. When urea or mercaptoethanol is removed, it attains its native (active) conformation.

3.8.2 Chemical

Colour reactions of proteins

The colour reactions of proteins are of importance in the qualitative detection and quantitative estimation of proteins and their constituent amino acids. Biuret test is extensively used as a test to detect proteins in biological materials.

Biuret reaction

A compound, which is having more than one peptide bond when treated with Biuret reagent, produces a violet colour. This is due to the formation of coordination complex between four nitrogen atoms of two polypeptide chains and one copper atom (Figure 3.24).

Xanthoproteic reaction

Addition of concentrated nitric acid to protein produces yellow colour on heating, the colour changes to orange when the solution is made alkaline. The yellow stains upon the skin caused by nitric acid are the result of this xanthoproteic reaction. This is due to the nitration of the phenyl rings of aromatic amino acids.

Hopkins-Cole reaction

Indole ring of tryptophan reacts with glacial acetic acid in the presence of concentrated sulphuric acid and forms a purple coloured product. Glacial acetic acid reacts with concentrated sulphuric acid and forms glyoxalic acid, which in turn reacts with indole ring of tryptophan in the presence of sulphuric acid forming a purple coloured product.

3.9.Nutroitional quality of proteins

In judging the adequacy of dietary proteins to meet the human needs, not only the quantity, but the nutritional quality of the dietary proteins also matters. Proteins present in different foods vary in their nutritional quality because of differences in their amino acid composition. Amino acids are the building blocks of proteins. There are 19 of them in proteins, nine of them are designated as “essential amino acids”, since they cannot be synthesized in the body the rest of the amino acids are called “non-essential” as they can be formed in the body by interconversion of other essential amino acids or synthesized from simple compounds derived from the break down of carbohydrate or fat in the body.The body drives from the dietary protein the amino acids, particularly the essential amino acids which it cannot synthesise and makes its

own tissue proteins. The quality of dietary protein depends on the pattern of essential amino acids it supplies. The best quality protein is the onw whichprovides essential amino acid pattern very close to the pattern of the tissue proteins. Egg. proteins, human milk protein, satisfy these criteria and are classified as high quality proteins and serve as reference protein for defining the quality of other proteins. Apart fro these proteins, the minimum amount of essential amino acids required by infants are also taken as a reference pattern for defining the quality of proteins. The quality of dietary proteins are computed on the basis of the extent to which its essential amino acid pattern deviates from that of standard reference pattern as found in egg or breast milk. This mode of chemical assessment (chemical score) does not take into account the digestibility of dietary proteins. Hencebiological methods based on growth or N retention are used to determine the overall quality of a protein. Such biological measures of quality are 'net protein utilization' (NPU) which takes both absorption and retentin and 'biological value' (BV) takes only retention into consideration.

The proteins of animal foods like milk, meat, fish etc. generally compare well with egg in their essential amino acid composition and are categorized as good quality proteins. They are also highly digestible. Plant proteins on the other hand are of poorer quality since EAA composition is not well balanced and a few EAA deviate much from the optimal level present in egg. For instance that in comparison with egg protein cereal proteins are poor in amino acid lysine. Pulses and oilseed proteins are rich in lysine but they are poor in sulphur containing amino acids. Such proteins individually are therefore incomplete proteins. However, relative insufficiency of a particular amino acid of any vegetable food can be overcome by judicious combination with other vegetable foods which may have adequate level of that limiting amino acid. The amino acid composition of these proteins complement each other and the resulting mixture will have an amino acid pattern better than either of the constituent proteins of the mixture. This is the procedure normally used to improve quality of vegetable proteins. Thus a protein of cereals, deficient in lysine and pulses with adequate lysine content have a mutually supplementary effect, a deficiency of an amino acid in one can be made good by an adequate level in another, if aboth are consumed together. A combination of cereal and pulse in the ratio

5:1 has been found to give an optimum combination. Thus the habitual diets of vegetarians in India based on cereal and pulse has indeed a rational basis.

Another factor to be considered in assessing the value of the proteins of a food stuff is their digestibility. In general, proteins of uncooked vegetable foods (particularly pulses) are less digestible than those of animal foods. Often the low digestibility of plant proteins is due to the presence of trypsin inhibitors, which are destroyed on cooking. Soya bean has a powerful trypsin inhibitor which is destroyed only on autoclaving. Hence cooking/heat treatment improves the digestibility of proteins in several foods. Excessive heat treatment particularly dry heat treatment should be avoided since it affects the quality of vegetable proteins by making some of the essential amino acids like lysine and methionine unavailable. On excessive heating the lysine in proteins reacts with reducing sugars in foods and renders part of lysine unavailable.

Summary

Proteins are polymers of varying size that are constructed from 20 different amino acids. They are structurally and functionally diverse molecules and play important roles in human and other organisms than any other class of compounds. The basic unit of proteins, amino acids, share a general structure composed of a carbon centre surrounded by a hydrogen, a carboxyl group,an amino group and side chain R that differs for each amino acid. All 20 amino acids are white crystalline, high melting solids, soluble in water and insoluble in non-polar organic solvents. At physiological pH values, amino acids exist as dipolar ionic species called zwitterions. All amino acids contain acidic and basic functional groups that can be dissociated; therefore their structures change depending on the pH of their environment. They also exhibit isomerism. All the amino acids found in proteins belong to L-amino acids. Except glycine, all other amino acids possess asymmetric carbon atoms.

The 20 protein amino acids are classified according to the chemical nature of their R groups as aliphatic, aromatic, heterocyclic and sulphur containing amino acids. They are also classified into four groups depending on the chemical reactivity of their side chain as i) those

with nonpolar side chains ii) those with polar uncharged side chains iii) those with charged acidic side chains or R groups (acidic) and iv) those with charged basic side chains or R groups (basic). They undergo chemical reactions due to amino and carboxyl groups.

Peptides and proteins are formed by linking the amino acids through peptide bonds. All polypeptide chains have an amino terminus and a carboxyl terminus.

Proteins are involved in different functions. Based on function they are classified as enzymes, storage proteins, toxic and structural compounds, secretary and exotic proteins and as antibodies apart from being involved in the activities of transport, muscle contraction and metabolic regulation in biological systems.

They are also classified based on their solubility and composition as simple, compound and derived proteins. Simple proteins include albumins globulins, prolamins, glutelins histones, protamins, and albuminoids. Compound or conjugate proteins constitute nucleoproteins, mucoproteins, chomoproteins, lipoproteins metalloproteins and phosphoprteins. Derived proteins include primary and secondary derived proteins.

Conformation of a protein refers to the three-dimensional structure in its native state. The molecular architecture of proteins can be organized into four levels; i) primary structure-the order in which amino acids are arranged in a polypeptide chain ii) secondary structure-the steric relationship of amino acids that are close to one another on linear sequence iii) the tertiary structure defines the complete three-dimensional arrangement in space (native conformation) iv) quaternary structure-the specific manner in which separate polypeptide chains are packed together to form a functional protein aggregate.

Most pure proteins are generally tasteless and odourless. They are colloidal and amphoteric in nature. Most proteins are labile and readily modified by pH, UV radiation, heat and organic solvents. They undergo chemical reactions due to peptide bond and side chain R groups.

Review questions

A. Multiple choice questions

1. Which one of the following amino acids is optically inactive?
   a. Alanine b. Glycine c. Leucine d. Aspartic acid

2. The reagent that is used for quantitative determination of amino acids is
   a. Sanger's b. Molisch's c. Benedict's d. Ninhydrin

3. The protein with quaternary structure is
   a. Insulin b. Myoglobin c. Hemoglobin d. Keratin

4. The interaction, which is involved in the secondary structure of protein, is
   a. Hydrogen bond b. Hydrophobic c. van der Waals force d. Ionic bond.

5. The bond, which is not disturbed during denaturation of protein, is
   a. Covalent bond b. Hydrogen bond c. Ionic bond d. van der Waals force.

B. Fill up the blanks

6. The decarboxylated product of histidine is ---------- .
7. Below isoelectric point proteins possess ---------- charge.
8. In denaturation of protein, ---------- bond is not broken.
9. The phosphoprotein present in egg yolk is----------.
10. All the amino acids found in the protein are of L-configuration except ----------.

C. Write short answers for the following

11. Define the following
    a. Peptide b. Zwitterions c. Ruhemann's purple d. Essential amino acids
    e. Simple proteins f. Conjugated proteins g. Denaturation
    h. Renaturation i. Conformation of protein j. Prosthetic group
12. Amphoteric nature of amino acids.
13. Reaction of amino acids with ninhydrin.
14. Essential amino acids.
15. Hydrophobic interaction.

D. Write in detail on the following.

16. How are amino acids classified? Give example for each group.
17. How are proteins classified based on solubility and functions?
18. Explain the usefulness of the following reagents in the characterization of

proteins
a. Sanger's reagent b. Cyanogen bromide c. Phenyl isothiocyanate
d. 6 N HCl

19. Discuss the structural organization of proteins in different levels.
20. What are peptides? Give an account of the peptides of physiological interest.

E. Solve the following problems.

21. From the list of amino acids given below, select those with side chains capable of forming hydrogen bond or hydrophobic interaction
    a. Alanine b. Phenylalanine c. Serine d. Threonine e. Tyrosine
    f. Asparagine g. Glutamic acid h. Valine.

22. Estimate the possible number of amino acids in each protein given below. Molecular weight is given in the bracket.
    a. Insulin (5.733kDa) b. Immunoglobulin G. (14.5kDa) c. Catalase (250 kDa)

23. Draw the structures of glycine and histidine as it would exist pH 1, pH 5, and pH 11.

24. Draw the structure of the dipeptide Asp-Phe and answer the following questions.
    a. Which amino acid is present at the N terminus? b. Circle all the ionizable groups in the dipeptide c. Which amino acid is present at the C-terminus?

25. Write the possible dipeptide structures that could form from valine and serine under peptide forming conditions.

F. Gain additional knowledge by further reading

26. Artificial peptide and protein sweeteners.
27. PR proteins.
28. Proteinaceous enzyme inhibitors.
29. Lectins.
30. Seed storage proteins.

CHAPTER 4

LIPIDS

Lectures 11-13

4.1.Occurrence and importance

The word lipids is derived from the Greek word 'lipos' meaning fat. Lipids are chemically heterogenous group of compounds that are insoluble in water but soluble in non-polar solvents such as chloroform. Lipids occur in plants and animals as storage and structural components.Structural lipids present in animals and plants are in the form of meat and vegetables respectively. Storage fats occur in milk and adipose tissue of farm animals and in seed oils. Fats supply over twice as much energy per unit weight as proteins or carbohydrates. Lipids are anhydrous due to non-polar nature and represent more energy than carbohydrates which are heavily hydrated due to polar nature. The presence of lipids in diet contributes considerably to palatability. A fat-free diet would be unpleasant to eat. Lipids contribute palatability in two ways. They induce olfactory responses, namely, taste in the mouth and aroma through nose. Secondly, they contribute to the texture of food and is responsible for the mouth-feel. Lipids also supply the essential fatty acids which are not synthesised in human beings but are essential for growth. Lipids are essential for the effective absorption of fat-soluble vitamins A, D, E and K from intestine. Many enzymes require lipid molecules for maximal activity. Examples are microsomal enzyme, glucose 6-phosphatase and mitochondrial enzyme, β-hydroxybutyrate dehydrogenase. Adrenal corticosteroids, sex hormones and vitamin D_3 (Cholecalciferol) are synthesized from lipid derivative-cholesterol. Much of the lipid of mammals is located subcutaneously and acts as insulation against excessive heat loss to the environment. The subcutaneous lipid deposits also insulate the important organs against mechanical trauma.

4.2. Classification

Lipids are broadly classified into simple, compound and derived lipids (Table 4.1). Fatty acids are described first since they occur in simple and complex lipids.

4.3. Plant fatty acids

Fatty acids are carboxylic acids with hydrocarbon chains of 2 to 36 carbons. More than 200 fatty acids have been isolated from higher and lower plants. Of these, only a few are present in large quantities in most plant lipids. These are referred as major fatty acids. Fatty acids present in smaller proportions are called as minor fatty acids. Major and minor fatty acids are usually biosynthesised by analogous pathways. Fatty acids that occur only in a few plant species are called as unusual fatty acids.

Major fatty acids

The major fatty acids are saturated or unsaturated with an unbranched carbon chain. The saturated fatty acids, lauric (dodecanoic), myristic (tetradecanoic), palmitic (hexadecanoic) and stearic (octadecanoic) and the unsaturated fatty acids, oleic (9-octadecenoic), linoleic (9,12-octadecadienoic) and α-linolenic (9,12,15-octadecatrienoic) are usually found in the lipids from all parts of plants (Table 4.2).

The structure of fatty acids are written as a symbol of two numbers separated by a colon: the first number denotes the carbon atoms in the chain and the second number denotes the number of unsaturation centres. The positions of double bonds are specified by superscript numbers following (delta). Thus 18:2 ($\Delta^{9,12}$) indicates an eighteen carbon fatty acid with two double bonds between C-9 and C-10, and between C-12 and C-13. The double bonds of all naturally occurring unsaturated fatty acids are in the cis configuration. The non-polar hydrocarbon chain accounts for the poor solubility of fatty acids in water.

Minor fatty acids

The minor fatty acids that are found in foods and body tissues are shown in table 6.3. The fatty acid composition of cow's and goat's milk are characterised by a high content of short and medium chain saturated fatty acids.

Unusual fatty acids

The unusual fatty acids are found only in few individual species or genus or a whole family. Castor bean (*Ricinus communis*) seed oil is rich in ricinoleic acid (90%) which is 12-hydroxy oleic acid $CH_3(CH_2)_5$-CH(OH)-CH_2-CH=CH-$(CH_2)_7$-COOH. Rape seed *(Brassica napus)* is rich in erucic acid (cis-13-docosenoic acid $CH_3(CH_2)_7$-CH=CH-$(CH_2)_{11}$-COOH). Hydnocarpic and chaulmoogric acids are found in chaulmoogra oil which is used in the treatment of leprosy.

Essential fatty acids

Human body is unable to synthesise all fatty acids found in the body. Those fatty acids that are not synthesised in the body but required for normal body growth and maintenance are called as essential fatty acids. These fatty acids are to be supplied through diet. Linoleic and linolenic acids are essential fatty acids (Figure 6.1). The longer chain fatty acids can be synthesised by the body from dietary linoleic and α-linolenic acids. Arachidonic acid is essential but it can be synthesised by the body from linolenic acid. It is also present in the meat. Linoleic acid is grouped under n-6 family because the 6th carbon from methyl end possesses the double bond.

Other fatty acids that are synthesised in the body from linoleic acid such as γ-linolenic and arachidonic acids also belong to n-6 family. α-Linolenic acid belongs to n-3 family and is an essential fatty acid. The third carbon from the methyl end possess the double bond (Figure 6.1). The organs and tissues that perform the more routine and generalized functions such as adipose tissue, liver, muscle, kidney and the reproductive organs tend to have membranes in which n-6 family of polyunsaturated fatty acids

predominate. Nervous tissue and retina of the eye have a larger proportion of the longer chain acids with 5 or 6 double bonds predominantly of the n-3 family. Fish oils and spirulina are rich in fatty acids of n-3 family.

Arachidonic acid serves as precursor for the synthesis of prostaglandins, thrombaxanes and prostacyclins. These fatty acid derivatives are called as 'eicosanoid' meaning 20 C compounds. The main source of these eicosanoids are the membrane phospholipids from which they are released by the action of phospholipase-A. Phosphatidyl inositol which contains a high concentration of arachidonic acid in carbon-2 of glycerol provides a major store of eicosanoid precursors.

4.4. Simple lipids

Lipids containing only fatty acids and glycerol or long chain alcohols (monohydric) are called as simple lipids which include fats, oils and waxes.

6.4.1.Fats and oils

Triacylglycerols are the simplest lipids constructed from fatty acids and glycerol. They are also referred as triglycerides, fats or neutral fats. Triacylglycerols are composed of three fatty acids esterified to the three hydroxyl groups of glycerol (Figure 6.2). When all the 3 fatty acid molecules are of the same kind the triacylglycerol is said to be simple triacylglycerol. Mixed triacylglycerol possesses two or more different fatty acids.

Triacylglycerol that are solid at room temperature are called as fats, while the liquid triacylglycerols are called as oils. Neutral fats or oils are mostly composed of mixed triacyl glycerol. Fats are usually rich in saturated fatty acids and the unsaturated fatty acids predominate in oils. Most oil-producing plants store their lipids in the form of triacylglycerols.

Storage fats or oils

Triacylglycerols are widely distributed in the plant kingdom. They are found both in vegetative as well as reproductive tissues. Triacylglycerols are normally

stored in the endosperm of the seed although some plants store appreciable quantities of fat in the fleshy fruit mesocarp, for example, avocado. Some plants like the oil palm, store oils in both the mesocarp (Palm oil) and the endosperm (Palm kernel oil). The oil present as droplets in the cytoplasm of the seed cells. These droplets are called as oil bodies and are surrounded by a membrane composed of phospholipids and protein. Most of the common edible oils (groundnut, sunflower, gingelly, soybean, safflower, rice bran) contain limited number of the common fatty acids such as palmitic, stearic, oleic, linoleic and linolenic acids. Palm kernel and coconut oils contain higher amount of medium chain saturated fatty acids (Table 6.4). Seed oils contain small amount of phospholipids, carotenoids, tocopherols, tocotrienols and plant sterols depending on the species of plant and degree of processing.

Structural or hidden fats in plants

The leaves of higher plants contain upto 7% of their dry weight as fats; some of which are present as surface lipids, the others as components of leaf cells, especially in the chloroplast membrane. The fatty acid composition of plant membrane lipids is very simple. Six fatty acids- palmitic, palmitoleic, stearic, oleic,linoleic and α-linolenic generally account for over 90% of the total fatty acids.

4.4.2. Waxes

Waxes are esters of long-chain saturated and unsaturated fatty acids with long chain alcohol. The carbon number of fatty acids vary from 14 to 34 and that alcohol from 16 to 30. For example, beeswax is an ester of palmitic acid with a 30 carbon alcohol, triacontanol (Figure 4.3).

Waxes are the chief storage form of metabolic fuel in marine phytoplanktons. Biological waxes find a variety of applications in the pharmaceutical, cosmetic and other industries. Lanolin from lamb's wool, beeswax, carnauba wax, spermaceti oil from whales are widely used in the manufacture of lotions, ointments and polishes. Waxes are not easily hydrolysed like fats or digested by lipases.

Liquid wax - Jojoba oil

About 50% of the seed dry weight of jojoba consists of a liquid wax which is unique in the plant kingdom and is similar to sperm whale oil. The wax is made up of straight chain esters with an average total chain length of 42 carbons (Figure 6.4). Jojoba wax has a wide range of industrial uses including cosmetics, pharmaceuticals, extenders for plastics, printers ink, gear oil additives and various lubricants. The oil is highly stable and can be stored for years without becoming rancid.

Cuticular wax

The outermost surface of the cell walls of epidermal cells are covered with a hydrophobic cuticle which contains wax called cuticular wax. The main components of cuticular waxes are hydrocarbon (odd chain alkanes) and its derivatives, wax esters, free aldehydes, free acids, free alcohols and other components like mono esters of phenolic acids and aliphatic alcohols. The main function of the cuticular wax is to reduce the excessive losses and gains of water by the underlying tissue. It also helps in protecting the tissues from chemical, physical and biological attack.

4.5. Compound lipids

Compound lipids contain certain chemical groups in addition to alcohol and fatty acids. These group of lipids include glycerophospholipids, sphingo phospholipids, glycolipids, sulpholipids and lipoproteins.

4.5.1.Glycerophospholipids

The important structural lipid in biological membrane is glycero phospholipid which contains glycerol, fatty acids phosphoric acid and a nitrogenous base. The general structure of a glycerophospholipid is given in figure 6.5a. Without alcoholic residue (X), it is called as phosphatidic acid (Figure 4.5 b). Depending on the alcoholic residue attached to phosphatidic acid, they are named as

i. Phosphatidyl choline (lecithin)

ii. Phosphatidyl ethanolamine (cephalin)

iii. Phosphatidyl serine

iv. Phosphatidyl inositol

v. Phosphatidyl glycerol (which include monophosphatidyl glycerol and diphosphatidyl glycerol or cardiolipin).

Phosphatidyl choline (lecithin)

Lecithin contains glycerol, fatty acids, phosphoric acid and a nitrogenous base, choline (Figure 4.5.i). Lecithins are widely distributed in the membranes of cells having both metabolic and structural functions. Dipalmityl lecithin is a very effective surface active agent preventing adherence due to surface tension of the inner surfaces of the lungs. Most phospholipids have a saturated fatty acid in the C_1 position but an unsaturated fatty acid in the C_2 position.

Phosphatidyl ethanolamine (cephalin)

The cephalin differs from lecithin only in the nitrogenous group where ethanolamine is present instead of choline (Figure 4.5 ii).

Phosphatidyl serine

The hydroxyl group of the amino acid L-serine is esterified to the phosphatidic acid (Figure 4.5.iii).

Phosphatidyl inositol

Phosphatidyl inositol (Figure 4.5 iv) is an important constituent of cell membrane phospholipids; upon stimulation by a suitable animal hormone it is cleaved into diacylglycerol and inositol phosphate, both of which act as internal signals or second messengers.

Phosphatidyl glycerol and diphosphatidyl glycerol (Cardiolipin)

Cardiolipin (Figure 4.5 v) is a phospholipid that is found in membranes of mitochondria. It is formed from phosphatidylglycerol (Figure 4.5 vi).

4.5.2 Sphingophospholipids

The phosphate and fatty acids are attached to the alcohol sphingosine instead of glycerol in sphingophospholipids. The fatty acids are attached through an amide linkage rather than the ester linkage. The base present is normally choline. The structure of the parent compound sphingosine and phytosphingosine are shown in figure 4.7. C-1, C-2 and C-3 of the sphingosine or phytosphingosine bear functional groups, -OH, $-NH_2$ and -OH respectively, which are structurally homologous with the three hydroxyl groups of glycerol. Carbon 4 to 18 in sphingosine and C-5 to 18 in phytosphinogsine resembles that of a fatty acid. When a fatty acid is attached by an amide linkage to the $-NH_2$, group the resulting compound is a ceramide which is similar to diacyl glycerol.Ceramide is the fundamental structural unit common to all sphingophospholipids (Figure 6.6). Sphingophospholipids are found in the seeds of several plant species.

There is a range of molecular species among the phospholipid sub groups which differ from one another in the fatty acid composition (All the sub groups of phospholipids are found in plant photosynthetic tissue). Animal phospholipids contain mostly fatty acids with chain length between 16 and 20. The predominant fatty acids are palmitic, stearic, oleic, linoleic and arachidonic. Plant leaf phospholipids have a more limited range with very few fatty acids greater than C-18. The approximate proportion of each phospholipid expressed as a percentage of the total phospholipid present is phosphatidyl choline, 45%; phosphatidyl ethanolamine, 10%; Trace amounts of phosphatidyl serine, phosphatidyl inositol, 8%; monophosphatidyl glycerol, 35%, diphosphatidylglycerol, 2%. The diphosphatidyl glycerol is present in the inner mitochondrial membrane. The phospholipids are only minor components of seed lipids in which triacylglycerol predominate. The most abundant mammalian phospholipid is phosphatidyl choline. The phospholipids carry an electrical charge and interact with water. They are called as polar or hydrophilic molecules and also as amphiphilic

molecules. The sphingomyelins, the main sphingophospholipids of animals, are not present in plants.

4.5.3. Glycolipids and sulpholipids

Glycolipids are structurally characterised by the presence of one or more monosaccharide residues and the absence of a phosphate. They are O-glycoside of either sphingosine or glycerol derivative. The monosaccharides commonly attached are D-glucose, D-galactose or N-acetyl D-galactosamine. Monogalactosyl diglycerides and digalactosyl diglycerides have been shown to be present in a wide variety of higher plant tissues (Fig 4.7).

The 3 position of 1, 2-diacylglycerol is linked to 6- sulpho-6-deoxy D-glucose by an α-glycosidic bond in plant sulpholipid (Figure 6.8). The predominant fatty acid present in sulpholipid is linolenic acid. The sulpholipid is mostly present in chloroplasts, predominantly in the membranes of thylakoid. Cerebrosides are composed of a monosaccharide residue glycosidically linked to C-1 of an N-acylated sphingosine derivative. The monosaccharide is D-glucose in plants and D-galactose in animals.

4.5.4. Lipoprotein

Protein molecules associated with triacylglycerol, cholesterol or phospholipids are called lipoproteins. Triacylglycerols derived from intestinal absorption or from the liver are not transported in the free form in circulating blood plasma, but move as chylomicrons, as very low density lipoproteins (VLDL) or as free fatty acids (FFA) - albumin complexes. Besides, two more physiologically important groups of lipoproteins are low density lipoprotein (LDL) and high density lipoprotein (HDL).

The majpr lipid components of chylomicrons and VLDL are triacylglycerol, whereas the predominant lipids in LDL and HDL are cholesterol and phospholipids

respectively. The protein part of lipoprotein is known as apoprotein. Lipoproteins occur in milk, egg-yolk and also as components of cell membranes.

4.5.5. Sterols

The characteristic structure of sterol is their steroid nucleus consisting of four fused rings, three with six carbons (Phenanthrene) and one with five carbons (cyclopentane). This parent structure is known as perhydro cyclopentano phenanthrene.
(Figure 4.8).

The steroid nucleus is almost planar and relatively rigid. Steroids with methyl groups attached to carbons 10 and 13 and 8-10 carbon atoms in the side chain at position 17, an alcoholic group at position 3 and a double bond between carbons 5 and 6 are classified as sterols. Cholesterol (Figure 6.8) is the most abundant sterol in animals. Cholesterol is a major component of animal plasma membranes and occurs in lesser amounts in the membranes of their subcellular organelles. Its polar OH group gives it a weak amphiphilic character, whereas its fused ring system provides it with greater stability than other membrane lipids. Cholesterol is therefore an important determinant of membrane properties. It is also abundant in blood plasma lipoproteins where 70% of it is esterified to long chain fatty acids to form cholesteryl esters. Plants contain little cholesterol. Rather, the most common sterol components of their membranes are stigmasterol and β-sitosterol (Figure 6.8) which differ from cholesterol only in their aliphatic side chains. Yeast and fungi have another sterol named ergosterol which has a double bond between C7 and C8. In animal system, cholesterol functions as a precursor of various physiologically important compounds such as vitamin D, bile acids, female sex hormones and corticosteroids. In plants, cholesterol functions as an intermediate compound in the synthesis of various phytosteroids such as saponins, cardiac glycosides, phytoecdysteroids and brassinosteroids.

Brassinosteroids

In 1979, a novel plant growth regulating steroidal substance called brassinolide was isolated from rape (*Brassica napus*) pollen (Figure 6.8). More than 24 compounds are known (designated as BR_1, BR_2). Pollen is the richest source. Brassinosterols are active at concentration much lower (nM to pM range) than those of other types of hormones. Brassinosterols elicit a pronounced stem elongation response in dwarf pea epicotyls, mung bean epicotyls that are sensitive also to gibberellic acids but not auxins.

Brassinosteroids are thought by some to be a new class of plant hormones. The evidences are

i. They are widely distributed in the plant kingdom.

ii. They have an effect at extremely low concentration.

iii.They have a range of effects which are different from the other classes of plant hormones.

iv. They can be applied to one part of the plant and transported to another where in very low amounts elicit a biological response.

They are widely distributed including dicots,monocots, gymnosperms and algae, and in various plant parts such as pollen, leaves, flowers, seeds, shoots and stems. Among the naturally occurring brassinosteroids, brassinolide and castasterone are considered to be the most important because of their wide distribution as well as their potent physiological activity.

Physiological effects of brassinosteroids

i. Promotion of ethylene biosynthesis by stimulating ACC synthase activity.

ii. Promote elongation of vegetative tissue in a wide variety of plants at very low concentration.

iii. They are powerful inhibitors of root growth and development (via ethylene).

iv. They have been shown to interfere with ecdysteroids at their site of action, and are thus the first true antiecdysteroids.

v. They enhance resistance to chilling, disease, herbicides and salt stress, promote germination and decrease fruit abortion and drop.

Practical application of BR

Large scale field trials in China and Japan over a six year period have shown that 24-epibrassinolide, an alternative to brassinolide, increased the production of agronomic and horticultural crops (wheat, corn, tobacco, watermelon and cucumber). Environmental stresses were also seem to be allievated by treatment with brassinolide.

4.6. Properties of fat

4.6.1. Physical

Fats are greasy to touch and leave an oily impression on paper. They are insoluble in water and soluble in organic solvents. Pure triacylglycerols are tasteless, odourless, colourless and neutral in reaction. They have lesser specific gravity (density) than water and therefore float in water.

Though fats are insoluble in water, they can be broken down into minute droplets and dispersed in water. This is called emulsification. A satisfactory emulsion is one highly stable and contains very minute droplets with diameter less than 0.5 μm. Examples of naturally occurring emulsions are milk and yolk of egg. But they are not mere fat droplets in water. They contain hydrophilic colloidal particles such as proteins, carbohydrates and phospholipids which act as stabilizing agents. Emulsification greatly increases the surface area of the fat and this is an essential requisite for digestion of fat in the intestine.

4.6.2. Chemical

The most important chemical reaction of neutral fat is their hydrolysis to yield three molecules of fatty acid and one molecule of glycerol. The hydrolysis of fat is effected by alkali and enzyme.

Alkali hydrolysis (saponification)

The process of alkali hydrolysis is called 'saponification' (Figure 4.9). The alkali salt of fatty acid resulting from saponification is soap. The soaps we use for washing consists of Na or K salts of fatty acids like palmitic, stearic and oleic acid. The potassium soaps are soft and soluble whereas the sodium soaps are hard and less soluble in water.

Enzyme hydrolysis

Hydrolysis of triacylglycerol may be accomplished enzymatically through the action of lipases. Lipases are widespread in both plants and animals.

Rancidity

Development of disagreeable odour and taste in fat or oil upon storage is called rancidity. Rancidity reactions may be due to hydrolysis of ester bonds (hydrolytic rancidity) or due to oxidation of unsaturated fatty acids (oxidative rancidity).

Hydrolytic rancidity

This involves partial hydrolysis of the triacylglycerol to mono and diacylglycerol. The hydrolysis is hastened by the presence of moisture, warmth and lipases present in fats or air. In fats like butter which contains a high percentage of volatile fatty acids, hydrolytic rancidity produces disagreeable odour and taste due to the liberation of the volatile butyric acid. Butter becomes rancid more easily in summer.

Oxidative rancidity

The unsaturated fatty acids are oxidised at the double bonds to form peroxides, which then decompose to form aldehydes and acids of objectionable odour and taste.

Hydrogenation

The degree of unsaturation of the fatty acids present in triacylglycerol determines whether a fat is liquid or solid at room temperature. The presence of more unsaturated fatty acids lower the melting point. The presence of highly unsaturated fatty acids makes the oil more susceptible to oxidative deterioration. The objective of

hydrogenation is to reduce the degree of unsaturation and to increase the melting point of the oil. The oil can be selectively hydrogenated by careful choice of catalyst and temperature. Hydrogenation of unsaturated fats in the presence of a catalyst is known as hardening. Normally the process of hydrogenation is partial so as to get desired characteristics and to avoid products with high melting points. Hydrogenation is carried out in a closed container in the presence of finely powdered catalyst (0.05 - 0.2% of nickel) at temperature as high as 180^{o}C. The catalyst is usually removed by filtration. During hydrogenation process a proportion of the cis double bonds are isomerized to trans double bonds and there is also migration of double bonds. The hydrogenation process has made it possible to extend the food uses of a number of vegetable oils and marine oils whose melting points are too low.

4.6.3. Constants of fats and oils

Since fats and oils form essential nutrient of human diet, it is necessary to identify a pure fat or to determine the proportion of different types of fat or oil mixed as adulterant in edible oils and fats like butter and ghee. With an adequate knowledge of the characteristic composition of fats or oils, it is possible to identify the fat or oil under investigation. The chemical constants also give an idea about the nature of fatty acids present in fats or oils. Eventhough gas chromatographic method is available to identify and quantify the fatty acids present in fat or oil, the physical and chemical constants are still used in routine public health laboratories where such sophisticated facilities are lacking.

Physical constants

i. Specific gravity

Since different oils have different specific gravity, any variation from normal value shows mixture of oils.

ii. Refractive index

Fats have definite angles of refraction. Variation from the normal value indicates adulteration of fats or oils.

iii. Solidification point or setting point

Solidification point is the temperature at which the fat after being melted, sets back to solid or just solidifies. Each fat has a specific solidification point.

Chemical constants

i. Saponification number

It is defined as milligrams of KOH required to saponify 1 gm of fat or oil. Saponification number is high for fat or oil containing low molecular weight or short chain fatty acids and vice versa. It gives a clue about the molecular weight and size of the fatty acid in the fat or oil.

ii.Iodine Number

It is defined as the number of grams of iodine taken up by 100 grams of fat or oil. Iodine number is a measure of the degree of unsaturation of the fatty acid. Since the quantity of the iodine absorbed by the fat or oil can be measured accurately, it is possible to calculate the relative unsaturation of fats or oil.

iii.Reichert-Meisel number(R.M.number)

This is a measure of the volatile soluble fatty acids. It is confined to butter and coconut oil. It is defined as the number of millilitres of 0.1 N alkali required to neutralise the soluble volatile fatty aicds contained in 5 gm of fat. The determination of Reichert-Meisel number is important to the food chemist because it helps to detect the adulteration in butter and ghee. Reichert-Meisel value is reduced when animal fat is used as adulterant in butter or ghee.

iv. Polanski number

Ghee may be adulterated by the addition of insoluble, non-volatile fatty acids (by addition of animal fat). This can be tested by finding out the Polanski number. It is defined as the number of millilitres of 0.1 N potassium hydroxide solution required to

neutralise the insoluble fatty acids (not volatile with steam distillation) obtained from 5 gm of fat.

v.Acetyl number

It is defined as the amount in millilitres of potassium hydroxide solution required to neutralise the acetic acid obtained by saponification of 1 gm of fat or oil after acetylation. Some fatty acids contain hydroxyl groups. In order to determine the proportion of these, they are acetylated by means of acetic anhydride. This results in the introduction of acetyl groups in the place of free hydroxyl groups. The acetic acid in combination with fat can be determined by titration of the liberated acetic acid from acetylated fat or oil with standard alkali. Acetyl number is thus a measure of the number of hydroxyl groups present in fat or oil.

vi.Acid number

It is defined as the milligram of potassium hydroxide required to neutralise the free fatty acids present in one gram of fat or oil. Acid number indicates the amount of free fatty acids present in fat or oil. The free fatty acid content increases with age of the fat or oil.

Molecular aggregation of phospholipids

Glycerophospholipids are virtually insoluble in water. Depending on the precise conditions and the nature of lipids used, three types of lipid aggregates can form when amphipathic lipids are mixed with water.

Micelles

Free fatty acids, lysophospholipids and sodium dodecyl sulphate (SDS) form micelle. Micelles are relatively small spherical structures involving a few dozen to few thousand molecules arranged so that their hydrophobic regions aggregate in the interior excluding water and their hydrophilic head groups are at the surface in contact with water. This molecular arrangement eliminates unfavourable contacts between water and the hydrophobic tails (Figure 4.10a).

Bilayer

A second type of lipid aggregate in water is the bilayer in which two lipid monolayers combine to form a two dimensional sheet. The hydrophobic portions in each monolayer interact excluding water. The hydrophilic head groups interct with water at the two surfaces of the bilayer lipid bilayers form the structural basis of biological membranes (Figure 4.10b).

Liposomes

The third type of lipid aggregate is formed when a lipid bilayer folds back on itself to form a hollow sphere called a liposome or vesicle. These bilayer vesicles enclose water creating a separate aqueous compartment (Figure 4.10c).

Biological membranes

Proteins and polar lipids account for mass of biological membranes. The relative proportions of protein and lipid differ in different membranes, reflecting the diversity of biological roles. Amphipathic molecules form a lipid bilayer with the non polar region of lipids facing outward. In this lipid bilayer, globular proteins are embedded at regular intervals held by hydrophobic interactions. Some proteins protrude from one or other face of the membrane (peripheral proteins); some span its entire width (integral proteins). The individual lipid and protein subunits in a membrane form a fluid mosaic (Figure 4.10d). The membrane is fluid because the interactions among lipids, between lipids and proteins are non covalent, leaving individual lipid and protein molecules free to move laterally.

One of the key functions of a membrane is to control the passage of substances across it. They are said to be selectively permeable. The different membranes of the cell have different selective permeabilities.

Summary

Lipids are chemically heterogenous group of compounds that are insoluble in water but soluble in non-polar solvents. Lipids occur in plants and animals as storage and structural forms. They are broadly classified into simple, compound and derived lipids. Fatty acids which occur in simple and compound lipids are carboxylic acids with hydrocarbon chains of 2 to 36 carbons. Major fatty acids found in lipids contain 12 to 18 carbons. Fatty acids containing two or more double bonds are not synthesised in humans and are therefore essential fatty acids. There are minor as well as unusual fatty acids.

Lipids containing only fatty acid and glycerol or long chain alcohols are simple lipids, which include fats, oils and waxes. Physical and chemical constants are used to know appoximately about the fatty acid composition of the oils. Fats and oils are hydrolysed by alkalies (saponification) and enzymes. Development of disagreeable odour and taste in fat or oil is due to rancidity that occurs during storage. It may be due to hydrolysis of ester bonds or oxidation of unsaturated fatty acids. The oil can be selectively hydrogenated with the help of catalyst under high temperature and pressure. Saponification number is used to know about the chain length of fatty acids present in oil or fat. Iodine number is useful to find out the degree of unsaturation in fatty acids present in fat or oil. Acid number indicates the degree of rancidity.

Compound lipids contain certain chemical groups in addition to alcohol and fatty acids. They are classified into glycerophospholipids, sphingophospholipids, glycolipids, sulpholipids and lipoproteins. The important structural lipid in all biological membranes is glycerophospholipids. The important glycerophospholipids are phosphatidyl choline(leithin), phosphatidyl ethanolamine(cephalin) phosphatidyl serine, phosphatidylglycerol and phosphatidyl inositol. Sphingophospholipids are derived from sphingosine or phytosphingosine instead of glycerol and are found in the seeds of several plant species. Glycolipids are characterised by the presence of

one or more monosaccharide residues and absence of a phosphate. Lipoproteins contain triacylglycerol, cholesterol or phospholipids in association with protein molecules.

Compounds possessing perhydrocyclopentanophenanthrene rings with an hydroxyl group at position 3 and a double bond between carbons 5 and 6 are classified as sterols. Cholesterol is the most abundant sterol in animals. The most important sterols in plants ae stigmasterol and β-sitosterol. Cholesterol functions as an intermediate compound in the synthesis of various phytosteroids such as saponins, ecdysteroids and brassinosteroids. Brassinosteroids are considered as a novel plant growth regulating steroidal substance.

Review Questions

A. Multiple choice questions

1. Identify the polyunsaturated fatty acid from the following
   a. Palmitoleic b. Oleic c. Linoleic d. Stearic
2. Which one of the folllowing is not a component of glycerophospholipid
   a. Fatty acid b. Choline c. Glycerol d. Sphingosine
3. The lipid that can serve as cellular energy source is
   a. Triacylglycerol b. Waxes c. Bile acids d. Sterols
4. The fatty acid present in butter that contributes to the off-flavour during storage is
   a. Lauric b. Stearic c. Butyric d. Myristic
5. Erucic acid is present in
   a. Palm oil b. Mustard oil c. Rice bran oil d. Soybean oil

B. Fill up the blanks

6. Fatty acid with 18 carbons and three double bonds is ----------
7. The hydroxy fatty acid present in castor oil is ----------.
8. An example of liquid wax is ----------
9. Cerebrosides are composed of ----------
10. The process of alkali hydrolysis of oil is called ----------.

C. Write short answers for the following

11. Define the following
    a. Waxes b. Essential fatty acid c. Rancidity d. Hardening of oil
    e. Saponification number f. Iodine number g. RM number h. Acid number
    i. PUFA
12. What are the different types of waxes? Give example for each.
13. What are essential fatty acids? Name any two of them.
14. Explain rancidity reactions in oil
15. What is hardening of oil?
16. Differentiate fats and oils.
17. Differentiate lecithin and cephalin.

D. Write in detail on the following.

18. How are lipids classified ? Explain with examples.
19. Explain the significance of physical and chemical constants in the identification of fats and oils.
20. What are steroids?Describe the important steroids of biological importance.
21. What are glycerophospholipids? Describe the classification, structure and function of phospholipids.
22. Describe the composition and model of biological membranes
23. What are brassinosteroids and their important functions?

E. Solve the following problems.

24.. What are the products of linoleic acid with

a. Bromine b. H_2 (in presence of catalyst and under pressure)

c. Aqueous KOH solution

25. The hydrolysis of a 1 mole of optically active triacylglycerol yields, 1 mole each of glycerol, stearic acid and 2 moles of oleic acid. Write the structure of the triacylglycerol. If it is optically inactive what will be its structure?

26. The chemical constant of two edible oils are given. Answer the following questions based on the data provided.

Constants	Oil 1	Oil 2
Saponification value	175	100
Iodine value	60	130
Acid number	15	5

a. Which oil contains more of unsaturated fatty acids?

b. Which oil contains more of longer chain fatty acids?

c. Which oil is fresh without much rancidity?

d. Can you get any information on the composition of oleic acid?

e. Which oil solidifies first when the temperature is reduced?

27. Crude oil and essential oils are soluble in fat solvents, yet they are not classified as lipids. Why?

28. The hydrolysis of one mole of phospholipid yielded 1 mole each of palmitic, oleic, glycerol, phosphoric acid and choline.

a. Write the possible structure for the phospholipid

b. Can you write more than one structure for it? If so, is any one of them possess chiral centre?

29. Five gram oil is extracted from sunflower seeds and stored for three months. It required 30ml of O.1N NaOH for complete neutralization. Calculate the acid number.

F. Gain additional knowledge by further reading

30. Atherosclerosis
31. Phosphatidyl inositol and signal transduction
32. Refining of edible oil
33. Fatty acid analysis by GLC
34. VLDL, LDL, HDL, triglyceride and their clinical importance.

Table 4.1 Classification of lipids

Lipids

Simple lipids	Compound lipids	Derived lipids
Esters of fatty acids with glycerol and monohydric alcohols	Esters containing chemical groups in addition to alcohol and fatty acids	Substances derived form simple and compound lipids by hydrolysis. Alcohols, fatty acids, aldehydes, ketones, sterols and hydrocarbons
Depending upon the constituent alcohols they are further subdivided into fats or oils and waxes	Depending upon the chemical groups they are further subdivided into phospholipids, glycolipids, sulpholipids and lipoproteins	
Fats, also termed as triacylglycerols are esters of fatty acids with glycerol e.g. Plants–vegetable oils; Animals–ghee and butter	Phospholipids contain phosphate group. Phopholipids are further grouped as glycerophospholipids e.g., Lecithin if the constituting alcohol is glycerol or as sphingophospholipids if the alcohol is sphingosine e.g., sphingomyelin	
Waxes are esters of fatty acids and alcohols other than glycerol e.g., Plant wax-carnauba wax; **Insect wax–beeswax;** Animal wax -lanolin	Glycolipids contain hexose units preferably galactose alongwith fatty acids and alcohol eg. cerebrosides Plant sulpholipids contain sulfated hexose with fatty acids and alcohol Lipoproteins contain protein subunits along with lipids.	

	Depending upon density and lipid compound they are further classified as VLDL. LDL and HDL.	

Table 4.2 Some major fatty acids

Common name	Carbon skeleton	Systematic name	Structure
Lauric acid	12:0	Dodecanoic	$CH_3(CH_2)_{10}$ COOH
Myristic acid	14:0	Tetradecanoic	$CH_3(CH2)_{12}$ COOH
Palmitic acid	16:0	Hexadecanoic	$CH_3(CH_2)_{14}$ COOH
Stearic acid	18:0	Octadecanoic	$CH_3(CH2)_{16}$ COOH
Oleic	18:1	Octadecenoic	$CH_3(CH_2)_7$ CH=CH-$(CH_2)_7$-COOH
Linoleic	18:2	Octa decadienoic	$CH_3(CH_2)_4CH=CH-CH_2CH=CH(CH_2)_7COOH$
α-Linolenic	18:3	Octa-decatrienoic	CH_3-CH_2 -CH=CH-CH_2-CH=CH-CH_2-CH=CH-$(CH_2)_7COOH$

Table 4.3 Some minor fatty acids

Common name	Carbon skeleton	Systematic name
Butyric	4:0	Butanoic
Caproic	6:0	Hexanoic
Caprylic	8:0	Octanoic
Capric	**10:0**	**Decanoic**

Table 4.4 Fatty acid composition of some common edible oils (g/100 g total fatty acids)

Oil	Caprylic (8:0)	Capric (10:0)	Lauric (12:0)	Myristic (14:0)	Palmitic (16:0)	Stearic (18:0)	Oleic (18:0)	Linoleic (18:2)	(18
Coconut	8	7	48	16	9	2	7		
2	0	1							
Gingelly	0	0	0	0	10	3	41		
45	0	1							
Groundnut	0	0	tr	1	11	3	49		
29	1	6							
Mustard	0	0	0	0	2	2	13		
13	15	55*							
Palm kernel	4	4	45	18	9	3			
15	2	0	-						
Palm	0	0	tr	1	42	4	43		
8	tr	2							
Rice bran	0	0	0	1	15	3	45		
35	1	-							
Safflower	0	0	0	0	4	3	16		
76	-	1							
Soybean	0	0	tr	tr	10	4	25		
52	7	2							
Sunflower	0	0	tr	tr	6	6	33		
52	tr	3							

Lecture 14 &15

Proximate composition of foods

Cereals and millets

The major cereals and millets consumed in India are rice, wheat (cereals), jower, bajra, ragi (millets). In recent years the productin and consumption of maize has lso increased. These grains are the main source of energy in Indian diets contributing as they do 70-80 % of daily energy intake of majority of Indians. Since cereals /millets are the cheapest, widely available source of energy, their contribution to energy intake is the highest among the poor income. Even with the highest income groups, cereals account for at least 50-60% of the energy intake. In view of the large intake, cereals are also an important source of several other nutrients in Indian diets: protein, calcium iron and B-complex vitamins. Cereals contain 6-12% protein which is generally deficient in lysine intake. Among cereals rice protein is of better quality than the others. However, cereaks when eaten with pulses, as is the common practice in India, the protein quality improves due to mutual supplementation between cereal and pulse proteins, the former being deficient while the latter being rich in lysine.

Cereals are also source of some nutrients like Ca & Fe. Although they are not rich in these minerals, they contribute significantly due to fairly large amounts of cereals consumed daily. However rice among the cereals is poorer in these two minerals, the content depending upon the extant of polishing. Hence diets based predominantly on rice have lower Ca & Fe content. Ragi is rich in minerals, especially calcium. Millets including ragi are rich in minerals and fibre. Inclusion of some amount of millets in a diet will help in making up in providing bulk (fibre) to diet, especially the rice based ones. It must be pointed out that these millets are also rich in phytate and tannin and hence interfere withmineral availability. A balance has to be struck between the positive and negative aspects of nutritional quality of millets.

Cereals, particularly the whole grains are an important source of B-vitamins in our diet. Since most of these vitamins reside in outer bran or polishing refining these grains (removal of bran) or polishing rice reduces B-vitamin content to different degrees depending upon the extent

of refining and polishing. Highly polished rice has therefore very low level of B-vitamins. Parboiling which includes soaking in water and steaming of paddy results in seeping of vitamins present in outer layer into the grain. Hence milled and polished parboiled rice retains much os the vitamins. Sake considerations apply to high extraction wheat flour (viz., maida) and pearled millets.

Cereals do not contain either vitamin A or vitamin C except that yellow maize and some varieties of sorghum contain small amounts of β-carotene.

Cereals are generally considered to have low fat content as determined by ether extraction, which represents only the free fat. Recent studies however have shown that cereals contain much more fat, if the bound fat is also taken into account. The total fat including bound and free fat can be determined by hydrolysis with HC1 followed by chloroform methanol extraction. The total fat content thus determined in cereals may vary from 2 to 5 per 100g. Considering the amount of cereals consumed it is estimated that fat present in cereals in our diests can meet more than 50% of our essentially fatty acid requirement. Cereals together with pulses can nearly meet the EFA requirement of an adult.

Pulses

Pulses (or legumes as they are also called) are rich source of protein in our diets. In a vegetarian diet or a diet containing low amounts of animal foods, they are an important source of protein. The major pulses which find an important place in our dietaries are tur (Arhar), benngalgram, balackgram, greengram, lentil. Some of then (bengalgram, greengram) are used as whole gram also. Others rajma. In amounts used, pulses and legumes do not contribute much to the total mineral intake. However being rich in B-vitamins they can contribute significantly to B-vitamin A or Vitamin C but germinated legumes contain some vitamin C.

The protein of pulses/legumes are of low quality since they are deficient in methionine and red gram is deficient in tryptophan also. However, they are rich in lysine. Hence they can supplement proteins of cereals and the quality of the protein from a mixture of cereals and pulses is superior to that of the either one. The most effective combination to achieve maximum supplementaryeffect is 4 parts of cereal protein +1 part of pulse protein. In terms of the grains it will be 8 parts of cereals and 1 part of pulses.

Nut and oilseeds

Like pulses, oilseeds and nuts are rich in protein, and in addition they contain a high level of fat. They are thus source of protein and concentrated source of energy . They do not contain an appreciable amount of carbohydrate but contain high levels of B-vitamins. Groundnuts are particularly rich in thiamine and nicotinic acid. However in amounts they are consumed they may not ocntribute much to the intake of vitamin and minerals. Oilseeds produced in the country are mostly used for oil extraction. The meal obtained after extraction of the oil from the seed is richer in protein than the seed itself. Oilseed cake was mostly used for cattlefeed and in the past the quality of cake produced was not good enough for human consumption. Further Ghani pressed cake had high level of residual oil leading to rancidity of the cake ons torage. In recent years however improved method of extraction and careful handling of cake have helped production of edible grade deoiled meal. This protein-rich mel is being used for the development of various products, for use in feeding programmes. Protein isolates are also being produced from these deoiled meal.

Oilseed proteins, as other legume proteins are of inferior quality; deficient in amino acid; methionine but rich in lysine. Sesame seed proteins are however richer in methionine. Because of their high lysine content oilseed meals /proteins are used along with cereals for product development for child feeding and as weaning foods.

It has been shown inrecent years that oilseeds particularly the groundnut get affected with fungi if they are not dried and handled at the post harvest stage. The fungus affects many foods besides groundnut. This fungus elaborates some toxins which are deleterious to health and some of them are carcinogenic. Aflatoxin produced by *Asparigillus flavus* which affects groundnut has been shown to cause liver damage. Chronic consumption of the foods contaminated with aflatoxin can lead to liver carcinogenesis in experimental animals, man and monkeys. It is necessary that only clean groundnuts should be used as food and for oil extraction, the seeds should be properly dried, handled properly during storage, transporation, to avoid fungal attack

so that neither the oil nor the cake has aflatoxin efforts should be made to keep the level of aflatoxin contamination within safe limits.

Fruits and vegetables

Fruits: Fruits are generally good sources of vitamin C. Amla an dguava are the rich sources of this vitamin. Yellow fruits like mango and papaya in addition contain β -carotene, aprecursoe of Vitamin A. The commonly used banana is a good source fo carbohydrates an dhence energy. Dried fruits are good source of iron. Fruits also contain pectins which provide bulk to the diet and helps bowel movement. Seasonal fruits must be included in the diet to supply vitamin C and β -carotene.

Vegetables

Green leafy vegetables : Many types of greens are consumed all over our country. The commonly consumed greens are : palak, amarnath, fenugreek, drumstick, mint, etc. The green leafy vegetables are rich source of calcium, iron and β-carotene and vitamin C, riboflavin and folic acid. These greens are inexpensive and it is advisable to include ateast 50 g of GLV daily in one's diet. They contain all important nutrients required for growth and maintenance of health. Hence GLV must be consumed by childrem, pregnant and nursing women to obtain much needed β -carotene, calcium and iron. This is particularly so on a predominantly cereal based diets of the poor who suffer from the dietary deficiency of these nutrients. Hence steps must be taken to encourage cultivation fo GLV in kitchen gardens and school gardens so that they are available all through the year. Use of green leaves from trees like drumstick, agathi, etc, helps to obtain them regularly without much effort if a tree is planted in the backyard.

Roots and tubers: Some of the important root vegetable whicha restriction endonucleases commonly consumed in our country are tapioca, potato, sweet potato, carrots, yam, colocacia. They are all rich in carbohydrate and can form an important source of energy in our diets. Carrots and yellow varieties of yam are rich in carotene and potato is a significant source of

vitamin C. Tapioca and yams are rich in clcium. Tapioca consumed in Kerala as energy source helps to meet a short supply in cerals during drought conditions.

Other vegetables: Vegetables, which do not come under green leavy vegetables and roots and tubers, are classified under this head. This food grouop includes, several commonly used vegetables like brinjal, ladies fingers, French beans, guar beans, various gourds, tomatoes, etc. They not only add variety to the diet but also provide vitamin C and some minerals. These vegetables are also a source of dietary fibre in the diet and provide bulk to the diet.

Lecture 16

DIETARY FIBER

Dietary fiber is found only in plant foods. It is composed of a group of structurally related substances: cellulose, hemicelluloses, lignin, gums, and pectins. Good sources of dietary fiber include whole grains, vegetables, fruit, nuts and seeds. Meat, fish, poultry, eggs, dairy products, fats, oils and sugar contain no dietary fiber.

WHY SHOULD WE INCLUDE DIETARY FIBER IN OUR DIETS?
Some of the benefits of dietary fiber are to increase fecal bulk, soften stools, stimulate the healthy movement of foodstuffs through the digestive tract, and assist the muscles of the digestive tract. Consuming high fiber foods thus may help prevent and treat **constipation**. In addition, researchers have shown that dietary fiber can play an important role in the prevention or treatment of various diseases and disorders. These include: obesity, diabetes, cardiovascular disease, colon cancer, diverticular disease, irritable bowel syndrome, as well as **constipation**.

WHAT ARE SOLUBLE AND INSOLUBLE FIBERS?
Dietary fiber is frequently classified as *soluble* or *insoluble*. Soluble fibers dissolve in water and consist of pectins, gums, and some hemicelluloses. Insoluble fibers do not dissolve in water and consist of cellulose, lignin, and some hemicelluloses. Soluble fiber has been shown to be effective in reducing the risk of cardiovascular disease and diabetes by reducing total blood cholesterol and regulating blood sugar levels. Insoluble fiber has been shown to be effective in reducing the risk of colon cancer, preventing diverticular disease, and treating constipation.

WHAT ARE SOME SOURCES OF SOLUBLE AND INSOLUBLE FIBERS?
Ideally, we should incorporate both soluble and insoluble fibers. Good sources of soluble fiber include oats, barley, legumes (dried beans and peas), and some vegetables and fruits. Good sources of insoluble fiber include whole grain products (breads, cereals, rice, pasta), nuts, seeds, and some vegetables.

HOW MUCH DIETARY FIBER SHOULD WE CONSUME EACH DAY? Experts are now recommending a dietary fiber intake in the range of 20-35 grams/day for the healthy adult. The average American consumes between 10-20 grams/day; well below the current recommendations.

CAN PROBLEMS ARISE FROM EATING TOO MUCH FIBER? Yes. Dietary fiber, if suddenly increased, can cause gas, diarrhea and bloating. It should be added gradually, allowing time for your body to adjust to the extra fiber. For example, begin by increasing your fiber intake to 20 grams/day; allow a few days for adjustment, and then increase it to 25 grams/day. As you add fiber to your diet, it is very important to drink plenty of fluids, since fiber draws water into the intestines. Health care providers recommend at least 8 glasses of water each day. Although excessive intakes of dietary fiber may interfere with the absorption of some nutrients, moderate intakes (i.e., 20 - 35 grams/day) do not appear to pose a problem for the healthy adult

CHAPTER 5

ENZYMES

Lectures 18-23

5.1. Introduction

One of the unique characteristics of a living cell is its ability to permit complex reactions to proceed rapidly at the temperature of the surrounding environment. The principal agents which participate in the remarkable transformations in the cell belong to a group of proteins named enzymes. In the absence of enzymes in the cell, these reactions would proceed too slowly. Enzymes are proteins specialised to catalyse biological reactions with the following characteristics.

Characteristics of enzymes

Enzymes being proteins exhibit all properties of proteins. They have their specific isoelectric points at which they are least soluble. Like proteins, they can be denatured by changes in pH and temperature. The enzyme-catalysed reactions occur below $100^{o}C$, at atmospheric pressure and nearby neutral pH.

Enzymes undergo physical changes during the reaction but revert to their original form at the end of the reaction. Enzymes exhibit enormous catalytic power. The rates of enzymatically catalysed reactions are 10^{6} - 10^{12} times greater than those of the corresponding uncatalysed reactions and several times greater than those of the corresponding chemically catalysed reactions. For example the carbonic anhydrase enzyme catalyses the conversion of carbondioxide to carbonic acid.

$$CO_2 + H_2O \rightarrow H_2CO_3$$

In this reaction, each enzyme molecule can hydrate 10^5 molecules of carbondioxide per second.

Enzyme activity is regulated in a variety of ways, ranging from controls over the amount of enzyme protein synthesised by the cell or modulation of activity through reversible interaction with metabolic inhibitors and activators or through isoenzymes.

Specificity of the enzymes

One of the characteristic feature which distinguishes enzymes from catalysts is their specificity. Enzymes are specific in the reaction catalysed and in their choice of substrates. It usually catalyses a single chemical reaction or a set of closely related reactions. Three kinds of specificities are observed.

i. Absolute specificity

When enzymes catalyse only one particular reaction they are said to exhibit absolute specificity. e.g. Urease acts only on urea.

ii. Group specificity

Enzymes acting on a group of substances that possess a particular type of linkage common to that group of substances are said to exhibit group specificity. Amylase hydrolyses the group of substances like starch, dextrin and glycogen, which have the same type of glycosidic linkages (α 1,4).

iii. Optical specificity

Almost all enzymes show a high degree of optical specificity. Thus there are certain enzymes which catalyse the hydrolysis of same group of substances possessing same optical activity i.e. D-amino acid oxidase acts on D-amino acid and L-amino acid oxidase acts on L-amino acid. Maltase catalyses the hydrolysis of α-but not β- glycosides.

5.2. Classification of enzymes

In olden days enzymes have been named by adding the suffix -ase to the name of the substrate (the molecule on which the enzyme acts).

Ex. Urease (Substrate urea)

Arginase (Substrate arginine)

Recent studies on the mechanism of enzyme catalysed reactions have led to a more rational classification of enzymes. The International Union of Biochemistry (IUB) established a commission on enzyme nomenclature to adopt a systematic classification and nomenclature of all the existing and yet to be discovered enzymes. This system is based on the substrate and reaction specificity. Although, this International Union of Biochemistry system is complex, it is precise, descriptive and informative.

IUB system classifies enzymes into six major classes (should be written in specific order only) (Table 5.1).

1. Oxidoreductases
2. Transferases
3. Hydrolases
4. Lyases
5. Isomerases
6. Ligases

Again each class is divided into subclasses according to the type of reaction catalysed. Each enzyme is assigned a recommended name usually a short for everyday use, a systematic name which identify the reaction it catalyses and a classification number which is used where accurate and unambiguous identification of an enzyme is required.

Oxidoreductases

Enzymes catalysing oxido-reductions between two substrates, S and S'.

S reduced + S' oxidised $\rightarrow$ S oxidsed + S' reduced

Example:

CH_3-CH_2-OH+NAD^+ --------→ CH3-CHO + NADH + H^+

(reduced) (oxidised) (oxidised) (reduced)

Enzyme: Recommended name Alcohol dehydrogenase

Systematic name Alcohol:NAD^+ oxido-reductase

Enzyme Commission number E.C.1.1.1.1

First digit 1 indicates oxido-reductase (Major class)

Second digit 1 indicates enzymes acting on CH-OH group of donors (Sub-class)

Third digit 1 indicates NAD^+ as the electron acceptor (Sub-sub class)

Fourth digit 1 indicates the specific enzyme

Transferases

Enzymes catalysing the transfer of a functional group (G) other than hydrogen between substrates.

$$S - G + S' \rightarrow S' - G + S$$

Example: Phosphorylation of glucose by hexokinase (Figure 5.1)

Enzyme : Recommended name: Hexokinase

Systematic name: ATP:D-hexose, 6- phosphotransferase

Enzyme commission No:2.7.1.1

2. Transferase group (major class)
7. Transfer of phosphate group (sub-class)
1. Alcohol group as acceptor of phosphate group (Sub-sub-class)
1. Hexokinase

Hydrolases

Enzymes catalysing hydrolysis of ester, peptide or glycosidic bonds.

Example

Acetyl choline + H_2O ----------→ Acetic acid + Choline

Enzyme: Acetyl choline esterase

Choline:acetyl hydrolase

E.C : 3.1.1.8

Lyases

Enzymes catalysing the removal of groups from substrates by mechanism other than hydrolysis leaving a double bond in one of the products. Example: convertion of malic acid to fumaric acid by fumarase (Figure 5.2) .

Enzyme : Fumarase (Fumarate hydratase)

L. Malate hydrolyase

E.C.No.4.2.1.2

Isomerases

Enzymes catalysing interconversion of optical, geometrical or positional isomers. Example

All-*trans* retinal $\rightarrow$11 *cis*-retinal

Enzyme Retinene isomerase

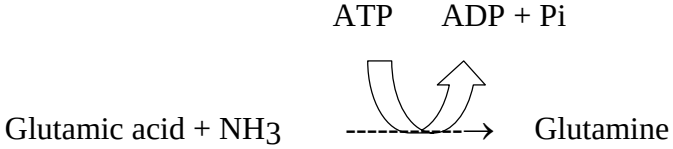
All-*trans* retinene:11-*cis* isomerase

E.C.No. 5.2.1.3

Ligases

Enzymes catalysing the joining together of two compounds with the hydrolysis of a high energy compound.

Example



Enzyme: Glutamine synthetase

L.Glutamate: Ammonia ligase

E.C.6.3.1.2

Major subclasses in different classes of enzymes are presented in table 7.1.

5.3.Apoenzymes, coenzymes and cofactors

A large number of enzymes require an additional non-protein component to carry out its catalytic functions. Generally these non-protein components are called as cofactors. The cofactors may be either one or more inorganic ions such as Fe^{2+}, Mg^{2+}, Mn^{2+} and Zn^{2+} or a complex organic molecules called coenzymes. Some enzymes require both coenzyme and one or more metal ions for their activity. A coenzyme or metal ion that is covalently bound to the enzyme protein is called prosthetic group. A complete, catalytically active enzyme together with its coenzyme and/or metal ions is called holoenzyme. The protein part of such an enzyme is called apoenzyme or apoprotein. Coenzymes function as transient carriers of specific functional groups

Cofactors

Metals are required as cofactors in approximately two thirds of all enzymes. Metalloenzymes contain a definite quantity of functional metal ion that is retained throughout. Metal-activated enzymes bind metals less tightly but require added metals. The distinction between metalloenzymes and metal activated enzymes thus rests on the affinity of a particular enzyme for its metal ion. The mechanisms whereby metal ions perform their function appear to be similar both in metallo enzymes and metal activated enzymes. Metals participate through their ability to act as Lewis acids and through chelate formation. Example of a metal functioning as a Lewis acid is the zinc in carbonic anhydrase. The metal can also promote catalysis by binding substrate at the site of bond cleavage. In carboxypeptidase, the carbonyl oxygen is chelated to the zinc.

The iron-sulfur enzymes are unique class of metalloenzymes in which the active centre consists of one or more clusters of sulfur-bridged iron chelates. These are of greater importance in plant systems. Enzymes requiring inorganic elements as cofactors are shown in table 5.2

5.4. Mechanism of enzyme action

A chemical reaction such as A $\rightarrow$ P takes place because a certain fraction of the substrate possesses enough energy to attain an activated condition called the transition state. This transition state is at the top of the energy barrier separating the reactants and products. The rate of a given chemical reaction is proportional to the concentration of this transition state species. The energy of activation is the amount of energy required to bring all the molecules in 1 mole of a substance at a given temperature to the transition state. Enzymes combine transiently with the substrate to produce a transition state intermediate having a lower energy of activation than the uncatalysed reaction. Thus they accelerate chemical reactions by lowering the energy of activation (Figure 5.3).

Example

H_2O_2 ----------$\rightarrow$ H_2O +(O)

Catalase

Reaction condition	Activation energy (KCal mol^{-1})
Uncatalysed	18
Catalysed by colloidal Pt	13
Catalysed by catalase	7

It is generally believed that the catalytic reactions occur in at least two steps.

Step 1: A molecule of enzyme(E) and a molecule of substrate(S) collide and react to form an intermediate called the enzyme-substrate complex (ES).

Step 2: The decomposition of ES complex to give product(s) and the active enzyme

[S] + [E] ----------$\rightarrow$ [ES] ----------$\rightarrow$ P+[E]

The formation of an ES complex affords a lower activation energy.

Active site

The functional groups that are essential for the formation of ES complex occur at a specific location on the surface of the enzyme molecule. This section of enzyme where substrate binding and transformation of substrate to product occurs is called as active site. Many attempts have been made to implicate specific amino acid residues (side chain or R groups) as being part of the active site of various enzymes. Some of the amino acids occurring at the active site of enzymes are hydroxyl group of serine, sulfhydryl group of cysteine, imidazole group of histidine and carboxyl group of aspartic acid. Two theories were proposed to explain the mechanism of enzyme action.

Fischer's lock and key theory (Rigid template model)

According to this theory proposed by Emil Fischer during 1890s, the active site possesses a unique conformation which is complementary to the structure of the substrate thus enabling the two molecules to fit together in much the same way as a key fits into a lock (Figure 5.4). An unfortunate feature of this model is the implied rigidity of the catalytic site.

Koshland's induced-fit theory

Koshland had advocated a theory to account for the specificity of enzymes. He postulated that the essential functional groups on the active site of the free enzyme are not in their optimal positions for promoting catalysis. When the substrate molecule is bound by the enzyme the catalytic groups assume of favourable geometrical position to form the transition state. The enzyme molecule is unstable in this active conformation and tends to revert to its free form in the absence of substrate. In the induced fit model, the substrate induces a conformational change in the enzyme which aligns the amino acid residues or other groups for substrate binding, catalysis or both. (Figure 5.5).

5.5. Factors affecting enzymatic reaction

The factors that mainly influence any enzyme-catalysed reaction are

1. Substrate concentration
2. Enzyme concentration
3. Temperature
4. pH
5. Inhibitors

Other factors such as state of enzyme (oxidation), time and activators also affect enzyme-catalysed reaction to certain extent.

Substrate concentration

Keeping the factors such as pH, temperature and enzyme concentration at optimum levels, if the substrate concentration is increased, the velocity of the reaction recorded a rectangular hyperbola. (Figure 7.6). At very low substrate concentration the initial reaction velocity (v) is nearly proportional to the substrate concentration (first order kinetics). However, if the substrate concentration is increased the rate of increase slows down (mixed order kinetics). With a further increase in the substrate concentration the reaction rate approaches a constant (zero order-reaction where velocity is independent of substrate concentration).

At initial point, eventhough the substrate molecules are present in excess than enzyme on molar basis, not all the enzyme molecules present combine with the substrate. Hence, increasing the substrate concentration will increase the amount of enzyme associated with substrate as ES and thus v will depend on [S]. At Vmax, all the enzyme molecules are saturated with substrate molecules so that further increase in [S] cannot result in increased reaction rate. Michaelis-Menten derived an equation to explain this type of behaviour.

$$v = \frac{V_{max}[S]}{K_m + [S]}$$ Where K_m = Michaelis-Menten constant

$$v = \frac{Vmax\,[S]}{Km + [S]}$$

[S] = Substrate concentration

Vmax = Maximum velocity

v = Velocity of the reaction

At half maximal velocity $[S] = Km$

i.e $\frac{Vmax}{2} = \frac{Vmax\,[S]}{Km+[S]}$

$\frac{Km + (S)}{2} = \frac{Vmax\,(S)}{Vmax}$

$Km + (S) = 2\,(S)$

$Km = 2\,(S) - (S) = (S)$

Hence, Michaelis - Menten constant, Km, is defined as the substrate concentration at half maximal velocity and is expressed as mole per litre.

The Michaelis-Menten equation can be algebraically transformed into more useful way to plot the experimental data. Lineweaver and Burk have taken the reciprocal of both [S] and v of the Michaelis-Menten equation to give

$$\frac{1}{v} = \frac{Km}{Vmax}\cdot\frac{1}{[S]} + \frac{1}{Vmax}$$

A plot of 1/v versus 1/ [S] (the double reciprocal) yields a straight line. This line intercept X-axis at -1/Km and Y-axis at 1/Vmax. The slope of the line is Km/Vmax. The Lineweaver-Burk plot has the great advantage of allowing more accurate determination of Vmax and Km (Figure 5.7).

Significance of Km

i. Km value may vary with substrate.

ii. An enzyme whose Km is very low will have a high degree of affinity for its substrate (Table 5.3).

Enzyme concentration

When compared to substrate concentration, the concentration of enzyme is always very very low on molar basis. Hence, increasing the enzyme concentration will always increase the reaction rate (Figure 5.8).

Temperature

Over a limited range of temperature, the velocity of enzyme-catalysed reactions roughly doubles with a $10^{O}C$ rise in temperature. Enzymes, being proteins, are denatured by heat and become inactive as the temperature increases beyond a certain point. Most of the enzymes are inactivated at temperatures above $60^{O}C$. The temperature at which the reaction rate is maximum is known as optimum temperature (Figure 5.9).

pH

Most enzymes have a characteristic pH at which their activity is maximum; above or below this pH, the activity declines (Figure 5.10). The pH affects the ionic state of the enzyme and frequently that of the substrate also. If a negatively charged enzyme (E^{-}) reacts with a positively charged substrate (SH^{+}), the ESH is formed. At low pH

values, E^- will be protonated and ESH is not formed. Similarly, at very high pH values SH^+ will ionize and lose its positive charge.

$$E^- + SH^+ \text{ ------}\rightarrow \quad ESH$$

acidic pH

$$E^- + SH^+ \text{ ------}\rightarrow \quad EH + SH^+ \text{ ---------}\rightarrow \quad \text{No ESH formation}$$

alkaline pH

$$SH^+ \text{ ----------}\rightarrow \quad S + H^+ + E^- \text{ --------}\rightarrow \quad \text{No ESH formation}$$

Another important factor is the change in conformation (denaturation) of enzyme at extreme pH values.

Inhibitors

Some compounds have the ability to combine with certain enzymes but do not serve as substrates and therefore block catalysis. These compounds are called inhibitors. The important type of inhibitors are competitive and noncompetitive inhibitors.

Competitive inhibitor

Any compound which possessess a close structural resemblance to a particular substrate and which competes with that of substrate for the same active site on the enzyme is called as competitive inhibitor. The inhibitor is not acted upon by the enzyme and so remains bound to the enzyme preventing the substrate to bind. This is a reversible process. It depends upon the relative concentration of substrate and

inhibitor. Competitive inhibition can be completely reversed by addition of large excess of substrate.

$$E + I \underset{\text{high substrate concn.}}{\overset{\text{high inhibitor concn.}}{\rightleftharpoons}} E I$$

The enzyme, succinate dehydrogenase converts succinate to fumarate. For this reaction, malonic acid is a competitive inhibitor as it structurally resembles that of succinate (Figure 5.11).

Non-competitive inhibitor

Non-competitive inhibitors bind to a site other than the active site on the enzyme often to deform the enzyme, so that, it does not form the ES complex at its normal rate. Once formed, the ES complex does not decompose at the normal rate to yield products. These effects are not reversed by increasing the substrate concentration.

$$E + I \text{ -------- } EI$$
$$ES + I \text{ ------- } ESI$$

Some enzymes possessing an essential -SH group are non-competitively inhibited by heavy metal ions (Hg^{2+}, Pb^{2+}). Some metalloenzymes are inhibited non competitively by metal chelating agents like ethylene diamine tetraacetic acid (EDTA).

Inhibitors are used as tools to probe the mechanism of enzyme - catalysed reactions and as therapeutic agents.

5.6. Isoenzymes

Enzymes which exist in multiple forms within a single species of organism or even in a single cell are called isoenzymes or isozymes. Such multiple forms can be detected and separated by gel electrophoresis of cell extracts. Since they are coded by different genes, they differ in amino acid composition and thus in their isoelectric pH values. Lactate dehydrogenase is an example for the isoenzymes which occur as five different forms in the tissues of the human and other vertebrates. All the five isozymes catalyze the same reaction.

$$\text{Lactate} + NAD^+ \text{----------}\rightarrow \text{Pyruvate} + NADH + H^+$$

They have the molecular weight of about 134,000 and contain four polypeptides. The five isozymes consist of five different combinations of two different kinds of polypeptides M and H. Kinetic study of lactate dehydrogenase isozymes has revealed that although they catalyze the same reaction, they differ significantly in their Km values for their substrates as well as Vmax values. The two polypeptide chains in LDH are coded by two different genes. (Figure 5.12). Skeletal muscle contains four identical M chains and designated as M_4; whereas heart muscle contains four identical H chains and designated as H_4. LDH of other tissues are a mixture of the five possible forms H_4, H_3M, H_2M_2, HM_3 and M_4. A determination of the relative amounts of the five LDH isozymes and the total concentration of LDH in a serum sample can provide valuable diagnostic information about which tissues have been damaged and the extent of the damage.

5.7.Immobilization of enzymes

Methods of immobilisation

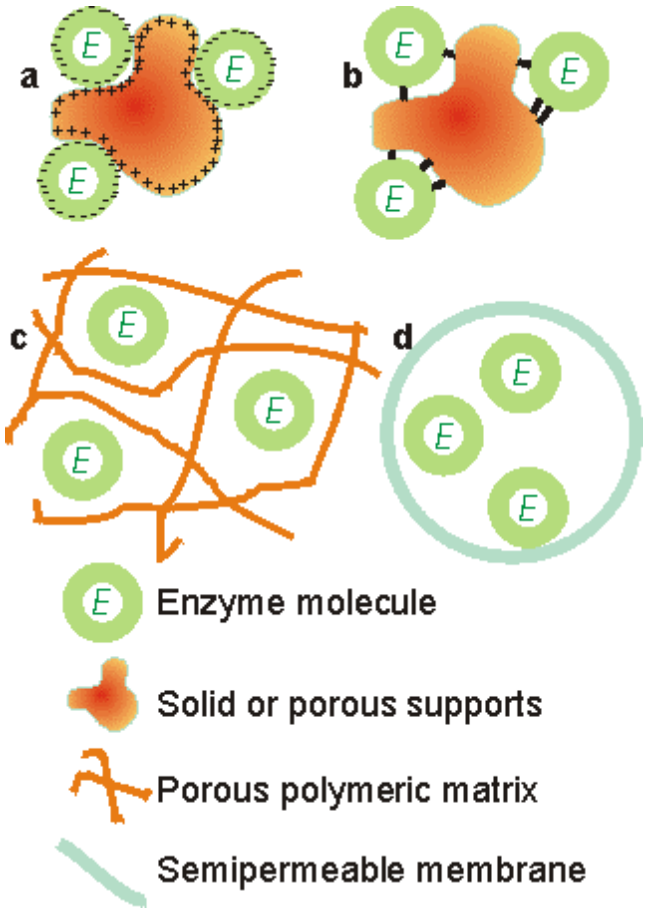
There are four principal methods available for immobilising enzymes

a.adsorption

b.Covalent binding

c.entrapment

d.membrane confinement



. Immobilised enzyme systems. (a) enzyme non-covalently adsorbed to an insoluble particle; (b) enzyme covalently attached to an insoluble particle; (c) enzyme entrapped within an insoluble particle by a cross-linked polymer; (d) enzyme confined within a semipermeable membrane.

Carrier matrices for enzyme immobilisation by adsorption and covalent binding must be chosen with care. Of particular relevance to their use in industrial processes is their cost relative to the overall process costs; ideally they should be cheap enough to discard. The manufacture of high-valued products on a small scale may allow the use of relatively expensive supports and immobilisation techniques whereas these would not be economical

in the large-scale production of low added-value materials. A substantial saving in costs occurs where the carrier may be regenerated after the useful lifetime of the immobilised enzyme. The surface density of binding sites together with the volumetric surface area sterically available to the enzyme, determine the maximum binding capacity. The actual capacity will be affected by the number of potential coupling sites in the enzyme molecules and the electrostatic charge distribution and surface polarity (i.e. the hydrophobic-hydrophilic balance) on both the enzyme and support. The nature of the support will also have a considerable affect on an enzyme's expressed activity and apparent kinetics. The form, shape, density, porosity, pore size distribution, operational stability and particle size distribution of the supporting matrix will influence the reactor configuration in which the immobilised biocatalyst may be used. The ideal support is cheap, inert, physically strong and stable. It will increase the enzyme specificity (k_{cat}/K_m) whilst reducing product inhibition, shift the pH optimum to the desired value for the process, and discourage microbial growth and non-specific adsorption. Some matrices possess other properties, which are useful for particular purposes such as ferromagnetism (e.g. magnetic iron oxide, enabling transfer of the biocatalyst by means of magnetic fields), a catalytic surface (e.g. manganese dioxide, which catalytically removes the inactivating hydrogen peroxide produced by most oxidases), or a reductive surface environment (e.g. titania, for enzymes inactivated by oxidation). Clearly most supports possess only some of these features, but a thorough understanding of the properties of immobilised enzymes does allow suitable engineering of the system to approach these optimal qualities.

Adsorption of enzymes onto insoluble supports is a very simple method of wide applicability and capable of high enzyme loading (about one gram per gram of matrix). Simply mixing the enzyme with a suitable adsorbent, under appropriate conditions of pH and ionic strength, followed, after a sufficient incubation period, by washing off loosely bound and unbound enzyme will produce the immobilised enzyme in a directly usable form (Figure 3.2). The driving force causing this binding is usually due to a combination of hydrophobic effects and the formation of several salt links per enzyme molecule. The particular choice of adsorbent depends principally upon minimising leakage of the enzyme during use. Although the physical links between the enzyme molecules and the support are often very strong, they may be reduced by many factors including the introduction of the substrate. Care must be taken that the binding forces are not weakened during use by inappropriate changes in pH or ionic strength. Examples of suitable adsorbents are ion-exchange matrices (Table 3.1), porous carbon, clays, hydrous metal oxides, glasses and polymeric aromatic resins. Ion-exchange matrices, although more expensive than these other supports, may be used economically due to

the ease with which they may be regenerated when their bound enzyme has come to the end of its active life; a process which may simply involve washing off the used enzyme with concentrated salt solutions and re-suspending the ion exchanger in a solution of active enzyme.

Entrapment of enzymes within gels or fibres is a convenient method for use in processes involving low molecular weight substrates and products. Amounts in excess of 1 g of enzyme per gram of gel or fibre may be entrapped. However, the difficulty which large molecules have in approaching the catalytic sites of entrapped enzymes precludes the use of entrapped enzymes with high molecular weight substrates. The entrapment process may be a purely physical caging or involve covalent binding. As an example of this latter method, the enzymes' surface lysine residues may be derivatised by reaction with acryloyl chloride (CH_2=CH-CO-Cl) to give the acryloyl amides. This product may then be copolymerised and cross-linked with acrylamide (CH_2=CH-CO-NH_2) and bisacrylamide (H_2N-CO-CH=CH-CH=CH-CO-NH_2) to form a gel. Enzymes may be entrapped in cellulose acetate fibres by, for example, making up an emulsion of the enzyme plus cellulose acetate in methylene chloride, followed by extrusion through a spinneret into a solution of an aqueous precipitant. Entrapment is the method of choice for the immobilisation of microbial, animal and plant cells, where calcium alginate is widely used.

Membrane confinement of enzymes may be achieved by a number of quite different methods, all of which depend for their utility on the semipermeable nature of the membrane. This must confine the enzyme whilst allowing free passage for the reaction products and, in most configurations, the substrates. The simplest of these methods is achieved by placing the enzyme on one side of the semipermeable membrane whilst the reactant and product stream is present on the other side. Hollow fibre membrane units are available commercially with large surface areas relative to their contained volumes (> 20 m^2 l^{-1}) and permeable only to substances of molecular weight substantially less than the enzymes. Although costly, these are very easy to use for a wide variety of enzymes (including regenerating coenzyme systems, without the additional research and development costs associated with other immobilisation methods. Enzymes, encapsulated within small membrane-bound droplets or liposomes may also be used within such reactors. As an example of the former, the enzyme is dissolved in an aqueous solution of 1,6-diaminohexane. This is then dispersed in a solution of hexanedioic acid in the immiscible solvent, chloroform. The resultant reaction forms a thin polymeric (Nylon-6,6) shell around the aqueous droplets which traps the enzyme. Liposomes are concentric spheres of lipid membranes, surrounding the soluble enzyme. They are formed by the addition of phospholipid to enzyme solutions. The micro-capsules and liposomes are washed free of non-confined enzyme and transferred back to aqueous solution before use.

All glucose isomerases are used in immobilised forms. Although differerent immobilisation methods have been used for enzymes from differerent organisms, the principles of use are

very similar. Immobilisation is generally by cross-linking with glutaraldehyde, plus in some cases a protein diluent, after cell lysis or homogenisation.

Originally, immobilised glucose isomerase was used in a batch process. This proved to be costly as the relative reactivity of fructose during the long residence times gave rise to significant by -product production. Also, difficulties were encountered in the removal of the added Mg^{2+} and Co^{2+} and the recovery of the catalyst. Nowadays most isomerisation is performed in PBRs. They are used with high substrate concentration (35-45% dry solids, 93-97% glucose) at 55-60°C. The pH is adjusted to 7.5-8.0 using sodium carbonate and magnesium sulphate is added to maintain enzyme activity (Mg^{2+} and Co^{2+} are cofactors). The Ca^{2+} concentration of the glucose feedstock is usually about 25 μm, left from previous processing, and this presents a problem. Ca^{2+} competes successfully for the Mg^{2+} binding site on the enzyme, causing inhibition. At this level the substrate stream is normally made 3 mM with respect to Mg^{2+}. At higher concentrations of calcium a Mg^{2+} : Ca^{2+} ratio of 12 is recommended. Excess Mg^{2+} is uneconomic as it adds to the purification as well as the isomerisation costs. The need for Co^{2+} has not been eliminated altogether, but the immobilisation methods now used fix the cobalt ions so that none needs to be added to the substrate streams.

Use of immobilised raffinase

The development of a raffinase (α-D-galactosidase) suitable for commercial use is another triumph of enzyme technology. Plainly, it would be totally unacceptable to use an enzyme preparation containing invertase to remove this material during sucrose production. It has been necessary to find an organism capable of producing an α-galactosidase but not an invertase. A mould, *Mortierella vinacea* var. *raffinoseutilizer*, fills the requirements. This is grown in a particulate form and the particles harvested, dried and used directly as the immobilised-enzyme preparation. It is stirred with the sugar beet juice in batch stirred tank reactors. When the removal of raffinose is complete, stirring is stopped and the juice pumped off the settled bed of enzyme. Enzyme, lost by physical attrition, is replaced by new enzyme added with the next batch of juice. The galactose released is destroyed in the alkaline conditions of the first stages of juice purification and does not cause any further problems while the sucrose is recovered. This process results in a 3% increase in productivity and a significant reduction in the costs of the disposal of waste molasses.

Immobilised raffinase may also be used to remove the raffinose and stachyose from soybean milk. These sugars are responsible for the flatulence that may be caused when soybean milk is used as a milk substitute in special diets.

Use of immobilised invertase

Invertase was probably the first enzyme to be used on a large scale in an immobilised form (by Tate & Lyle). In the period 1941 -1946 the acid, previously used in the manufacture of Golden Syrup, was unavailable, so yeast invertase was used instead.

Yeast cells were autolysed and the autolysate clarified by adjustment to pH 4.7, followed by filtration through a bed of calcium sulphate and adsorption into bone char. A layer of the bone char containing invertase was included in the bed of bone char already used for decolourising the syrup. The scale used was large, the bed of invertase-char being 2 ft (60 cm) deep in a bed of char 20 ft (610 cm) deep. The preparation was very stable, the limiting factors being microbial contamination or loss of decolourising power rather than loss of enzymic activity. The process was cost-effective but, not surprisingly, the product did not have the subtlety of flavour of the acid-hydrolysed material and the immobilised enzyme process was abandoned when the acid became available once again. Recently, however, it has been relaunched using Brimac^{TM}, where the invertase - char mix is stabilised by cross-linking and has a half-life of 90 days in use (pH 5.5, 50°C). The revival is due, in part, to the success of HFCS as a high-quality low-colour sweetener. It is impossible to produce inverted syrups of equivalent quality by acid hydrolysis. Enzymic inversion avoids the high-colour, high salt-ash, relatively low conversion and batch variability problems of acid hydrolysis. Although free invertase may be used (with residence times of about a day), the use of immobilised enzymes in a PBR (with residence time of about 15 min) makes the process competitive; the cost of 95% inversion (at 50% (w/w)) being no more than the final evaporation costs (to 75% (w/w)). A productivity of 16 tonnes of inverted syrup (dry weight) may be achieved using one

Use of immobilised lactase

Lactase is one of relatively few enzymes that have been used both free and immobilised in large-scale processes. The reasons for its utility has been given earlier, but the relatively high cost of the enzyme is an added incentive for its use in an immobilised state.

Immobilised lactases are important mainly in the treatment of whey, as the fats and proteins in the milk emulsion tend to coat the biocatalysts. This both reduces their apparent activity and increases the probability of microbial colonisation.

Yeast lactase has been immobilised by incorporation into cellulose triacetate fibres during wet spinning, a process developed by Snamprogetti S.p.A. in Italy. The fibres are cut up and used in a batchwise STR process at 5°C (*Kluyveromyces lactis*, pH optimum 6.4 -6.8, 90 U g^{-1}). Fungal lactases have been immobilised on 0.5 mm diameter porous silica (35 nm mean

pore diameter) using glutaraldehyde and γ-aminopropyltriethoxysilane (*Asperigillus niger*, pH optimum 3.0 -3.5, 500 U g^{-1}; *A. oryzae*, pH optimum 4.0 -1.5, 400 U g^{-1}). They are used in PBRs. Due to the different pH optima of fungal and yeast lactases, the yeast enzymes are useful at the neutral pH of both milk and sweet whey, whereas fungal enzymes are more useful with acid whey.

Immobilised lactases are particularly affected by two inherent short-comings. Product inhibition by galactose and unwanted oligosaccharide formation are both noticeable under the diffusion-controlled conditions usually prevalent. Both problems may be reduced by an increase in the effectiveness factor and a reduction in the degree of hydrolysis or initial lactose concentration, but such conditions also lead to a reduction in the economic return. The control of microbial contamination within the bioreactors is the most critical practical problem in these processes. To some extent, this may be overcome by the use of regular sanitation with basic detergent and a dilute protease solution.

5.8.Industrial uses of enzymes

The use of enzymes in starch hydrolysis

Starch is the commonest storage carbohydrate in plants. It is used by the plants themselves, by microbes and by higher organisms so there is a great diversity of enzymes able to catalyse its hydrolysis. Starch from all plant sources occurs in the form of granules which differ markedly in size and physical characteristics from species to species. Chemical differences are less marked. The major difference is the ratio of amylose to amylopectin; e.g. corn starch from waxy maize contains only 2% amylose but that from amylomaize is about 80% amylose. Some starches, for instance from potato, contain covalently bound phosphate in small amounts (0.2% approximately), which has significant effects on the physical properties of the starch but does not interfere with its hydrolysis. Acid hydrolysis of starch has had widespread use in the past. It is now largely replaced by enzymic processes, as it required the use of corrosion resistant materials, gave rise to high colour and saltash content (after neutralisation), needed more energy for heating and was relatively difficult to control.

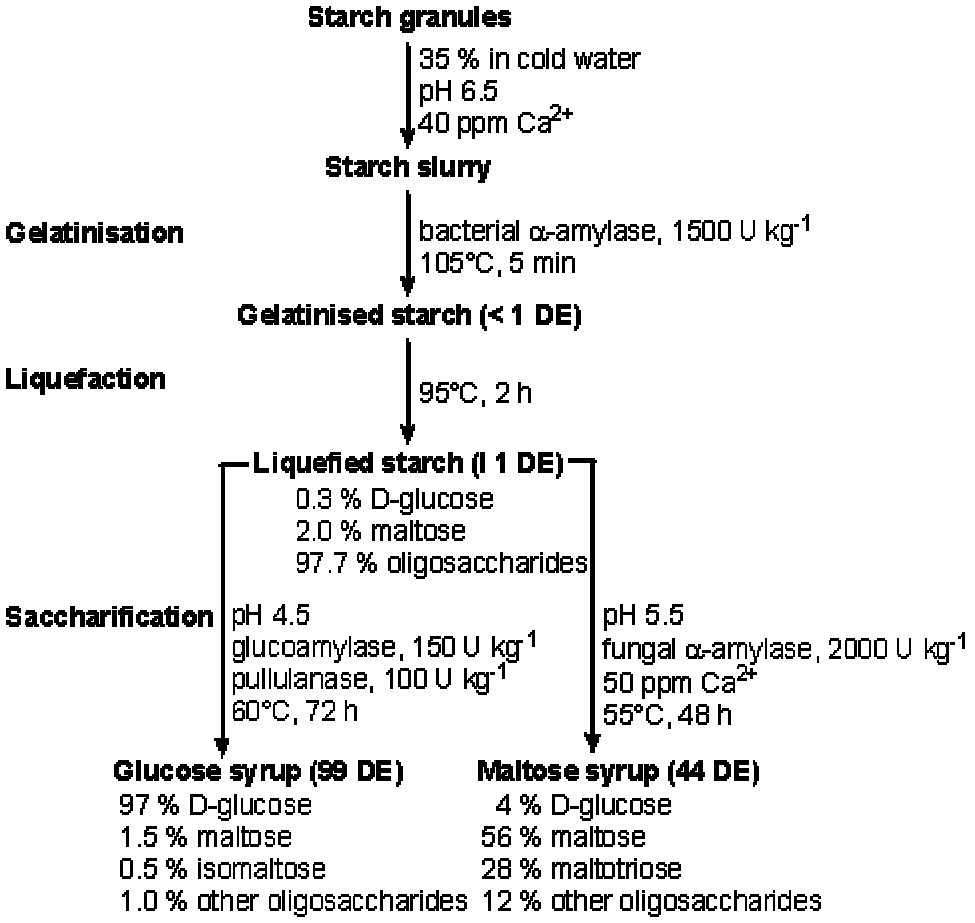


Figure. The use of enzymes in processing starch. Typical conditions are given.

Of the two components of starch, amylopectin presents the great challenge to hydrolytic enzyme systems. This is due to the residues involved in α-1,6-glycosidic branch points which constitute about 4 - 6% of the glucose present. Most hydrolytic enzymes are specific for α-1,4-glucosidic links yet the α-1,6-glucosidic links must also be cleaved for complete hydrolysis of amylopectin to glucose. Some of the most impressive recent exercises in the development of new enzymes have concerned debranching enzymes.

It is necessary to hydrolyse starch in a wide variety of processes which m be condensed into two basic classes:

1. processes in which the starch hydrolysate is to be used by microbes or man, and
2. processes in which it is necessary to eliminate starch.

In the former processes, such as glucose syrup production, starch is usually the major component of reaction mixtures, whereas in the latter processes, such as the processing of

sugar cane juice, small amounts of starch which contaminate non-starchy materials are removed. Enzymes of various types are used in these processes. Although starches from diverse plants may be utilised, corn is the world's most abundant source and provides most of the substrate used in the preparation of starch hydrolysates.

There are three stages in the conversion of starch gelatinisation, involving the dissolution of the nanogram-sized starch granules to form a viscous suspension;

1. liquefaction, involving the partial hydrolysis of the starch, with concomitant loss in viscosity; and
2. saccharification, involving the production of glucose and maltose by further hydrolysis.

Gelatinisation is achieved by heating starch with water, and occurs necessarily and naturally when starchy foods are cooked. Gelatinised starch is readily liquefied by partial hydrolysis with enzymes or acids and saccharified by further acidic or enzymic hydrolysis.

The starch and glucose syrup industry uses the expression dextrose equivalent or DE, similar in definition to the DH units of proteolysis, to describe its products, where:

$$DE = 100x\left(\frac{\text{Number of glycosidic bonds cleaved}}{\text{Initial number of glycosidic bonds present}}\right)$$

In practice, this is usually determined analytically by use of the closely related, but not identical, expression:

$$DE = 100x\left(\frac{\text{Reducing sugar, expressed as glucose}}{\text{Total carbohydrate}}\right)$$

Thus, DE represents the percentage hydrolysis of the glycosidic linkages present. Pure glucose has a DE of 100, pure maltose has a DE of about 50 (depending upon the analytical methods used; see equation (4.3)) and starch has a DE of effectively zero. During starch hydrolysis, DE indicates the extent to which the starch has been cleaved. Acid hydrolysis of starch has long been used to produce 'glucose syrups' and even crystalline glucose (dextrose monohydrate). Very considerable amounts of 42 DE syrups are produced using acid and are used in many applications in confectionery. Further hydrolysis using acid is not satisfactory because of undesirably coloured and flavoured breakdown products. Acid hydrolysis appears to be a totally random process which is not influenced by the presence of α-1,6-glucosidic linkages.

Table Enzymes used in starch hydrolysis

me	C number	Source	Action

ylase	3.2.1.1	*Bacillus amyloliquefaciens*	α-1,4-oligosaccharide links are cleaved to α-dextrins and predominantly maltose G3, G6 and G7 oligosaccharides
		B. licheniformis	α-1,4-oligosaccharide links are cleaved to α-dextrins and predominantly maltose, G4 and G5 oligosaccharides
		pergillus oryzae, *A. niger*	α-1,4 oligosaccharide links are cleaved to α-dextrins and predominantly maltose and ligosaccharides
harifying α-ase	3.2.1.1	*B. subtilis mylosaccharitic*us)	α-1,4-oligosaccharide links are cleaved to α-dextrins with maltose, G3, G4 and up to (w/w) glucose
ylase	3.2.1.2	Malted barley	α-1,4-links are cleaved, from non-cing ends, to give limit dextrins and β-ose
bamylase	3.2.1.3	*A. niger*	and α-1,6-links are cleaved, from the educing ends, to give β-glucose
lanase	3.2.1.41	*. acidopullulyticus*	α-1,6-links are cleaved to give straight-maltodextrins

The nomenclature of the enzymes used commercially for starch hydrolysis is somewhat confusing and the EC numbers sometimes lump together enzymes with subtly different activities For example, α-amylase may be subclassified as liquefying or saccharifying amylases but even this classification is inadequate to encompass all the enzymes that are used in commercial starch hydrolysis. One reason for the confusion in the nomenclature is the use of the anomeric form of the released reducing group in the product rather than that of the bond being hydrolysed; the products of bacterial and fungal α-amylases are in the α-configuration and the products of β-amylases are in the β-configuration, although all these enzymes cleave between α-1,4-linked glucose residues.

The α-amylases (1,4-α-D-glucan glucanohydrolases) are endohydrolases which cleave 1,4-α-D-glucosidic bonds and can bypass but cannot hydrolyse 1,6-α-D-glucosidic branchpoints. Commercial enzymes used for the industrial hydrolysis of starch are produced by *Bacillus amyloliquefaciens* (supplied by various manufacturers) and by *B. licheniformis* (supplied by Novo Industri A/S as Termamyl). They differ principally in their tolerance of high temperatures, Termamyl retaining more activity at up to 110°C, in the presence of starch, than the *B. amyloliquefaciens* α-amylase. The maximum DE obtainable using bacterial α-amylases is around 40 but prolonged treatment leads to the formation of maltulose (4-α-D-glucopyranosyl-D-fructose), which is resistant to hydrolysis by glucoamylase and α-amylases. DE values of 8-12 are used in most commercial processes where further

saccharification is to occur. The principal requirement for liquefaction to this extent is to reduce the viscosity of the gelatinised starch to ease subsequent processing.

Various manufacturers use different approaches to starch liquefaction using α-amylases but the principles are the same. Granular starch is slurried at 30-40% (w/w) with cold water, at pH 6.0-6.5, containing 20-80 ppm Ca^{2+} (which stabilises and activates the enzyme) and the enzyme is added (via a metering pump). The α-amylase is usually supplied at high activities so that the enzyme dose is 0.5-0.6 kg $tonne^{-1}$ (about 1500 U kg^{-1} dry matter) of starch. When Termamyl is used, the slurry of starch plus enzyme is pumped continuously through a jet cooker, which is heated to 105°C using live steam. Gelatinisation occurs very rapidly and the enzymic activity, combined with the significant shear forces, begins the hydrolysis. The residence time in the jet cooker is very brief. The partly gelatinised starch is passed into a series of holding tubes maintained at 100-105°C and held for 5 min to complete the gelatinisation process. Hydrolysis to the required DE is completed in holding tanks at 90-100°C for 1 to 2 h. These tanks contain baffles to discourage backmixing. Similar processes may be used with *B. amyloliquefaciens* α-amylase but the maximum temperature of 95°C must not be exceeded. This has the drawback that a final 'cooking' stage must be introduced when the required DE has been attained in order to gelatinise the recalcitrant starch grains present in some types of starch which would otherwise cause cloudiness in solutions of the final product.

The liquefied starch is usually saccharified but comparatively small amounts are spray-dried for sale as 'maltodextrins' to the food industry mainly for use as bulking agents and in baby food. In this case, residual enzymic activity may be destroyed by lowering the pH towards the end of the heating period.

Fungal α-amylase also finds use in the baking industry. It often needs to be added to bread-making flours to promote adequate gas production and starch modification during fermentation. This has become necessary since the introduction of combine harvesters. They reduce the time between cutting and threshing of the wheat, which previously was sufficient to allow a limited sprouting so increasing the amounts of endogenous enzymes. The fungal enzymes are used rather than those from bacteria as their action is easier to control due to their relative heat lability, denaturing rapidly during baking.

Certain proteases have been used in food processing for centuries and any record of the discovery of their activity has been lost in the mists of time. Rennet (mainly chymosin), obtained from the fourth stomach (abomasum) of unweaned calves has been used traditionally in the production of cheese. Similarly, papain from the leaves and unripe fruit of the pawpaw (*Carica papaya*) has been used to tenderise meats. These ancient discoveries have led to the development of various food applications for a wide range of available

proteases from many sources, usually microbial. Proteases may be used at various pH values, and they may be highly specific in their choice of cleavable peptide links or quite non-specific. Proteolysis generally increases the solubility of proteins at their isoelectric points.

The action of rennet in cheese making is an example of the hydrolysis of a specific peptide linkage, between phenylalanine and methionine residues (-Phe_{105}-Met_{106}-) in the κ-casein protein present in milk (see reaction scheme. The κ-casein acts by stabilising the colloidal nature of the milk, its hydrophobic N-terminal region associating with the lipophilic regions of the otherwise insoluble α- and β-casein molecules, whilst its negatively charged C-terminal region associates with the water and prevents the casein micelles from growing too large. Hydrolysis of the labile peptide linkage between these two domains, resulting in the release of a hydrophilic glycosylated and phosphorylated oligopeptide (caseino macropeptide) and the hydrophobic para-κ-casein, removes this protective effect, allowing coagulation of the milk to form curds, which are then compressed and turned into cheese (Figure 4.1). The coagulation process depends upon the presence of Ca^{2+} and is very temperature dependent (Q_{10} = 11) and so can be controlled easily. Calf rennet, consisting of mainly chymosin with a small but variable proportion of pepsin, is a relatively expensive enzyme and various attempts have been made to find cheaper alternatives from microbial sources These have ultimately proved to be successful and microbial rennets are used for about 70% of US cheese and 33% of cheese production world-wide.

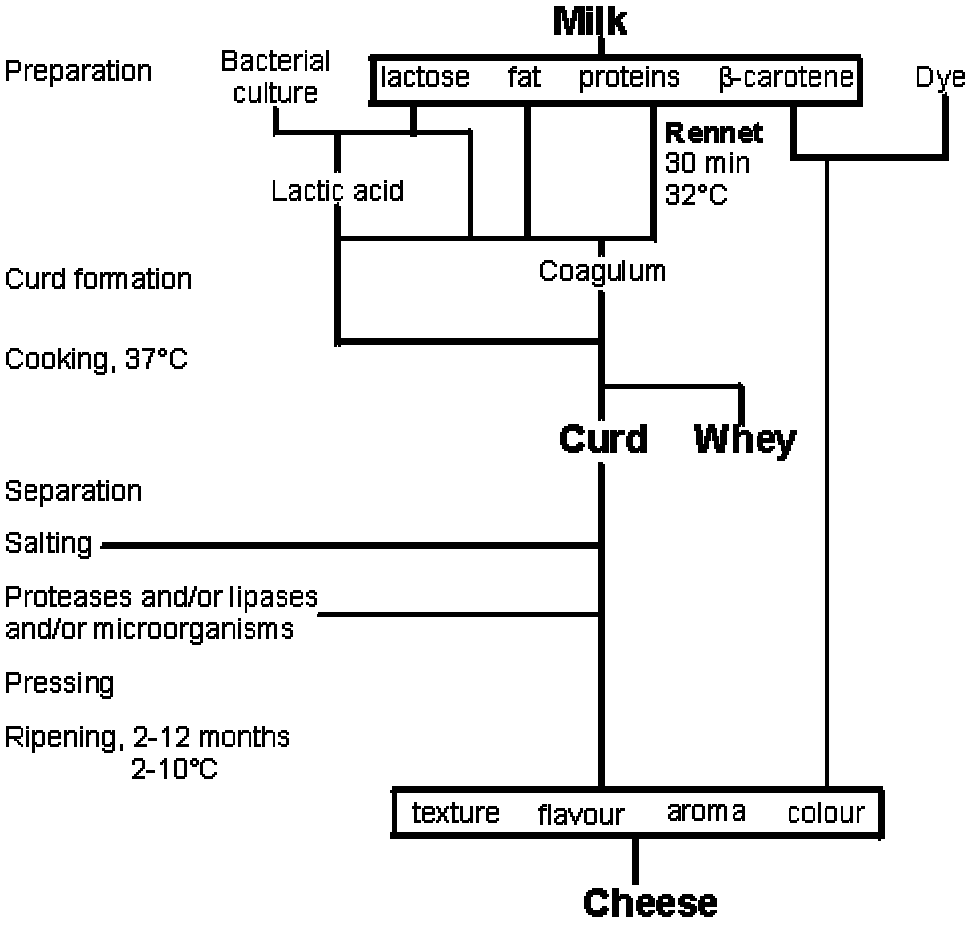


Figure Outline method for the preparation of cheese.

The major problem that had to be overcome in the development of the microbial rennets was temperature lability. Chymosin is a relatively unstable enzyme and once it has done its major job, little activity remains. However, the enzyme from *Mucor miehei* retains activity during the maturation stages of cheese-making and produces bitter off-flavours. Treatment of the enzyme with oxidising agents (e.g. H_2O_2, peracids), which convert methionine residues to their sulfoxides, reduces its thermostability by about 10°C and renders it more comparable with calf rennet. This is a rare example of enzyme technology being used to destabilise an enzyme Attempts have been made to clone chymosin into *Escherichia coli* and *Saccharomyces cerevisiae* but, so far, the enzyme has been secreted in an active form only from the latter.

The development of unwanted bitterness in ripening cheese is an example of the role of proteases in flavour production in foodstuffs. The action of endogenous proteases in meat

after slaughter is complex but 'hanging' meat allows flavour to develop, in addition to tenderising it. It has been found that peptides with terminal acidic amino acid residues give meaty, appetising flavours akin to that of monosodium glutamate. Non-terminal hydrophobic amino acid residues in medium-sized oligopeptides give bitter flavours, the bitterness being less intense with smaller peptides and disappearing altogether with larger peptides. Application of this knowledge allows the tailoring of the flavour of protein hydrolysates. The presence of proteases during the ripening of cheeses is not totally undesirable and a protease from *Bacillus amyloliquefaciens* may be used to promote flavour production in cheddar cheese. Lipases from *Mucor miehei* or *Aspergillus niger* are sometimes used to give stronger flavours in Italian cheeses by a modest lipolysis, increasing the amount of free butyric acid. They are added to the milk (30 U l^{-1}) before the addition of the rennet.

When proteases are used to depolymerise proteins, usually non-specifically, the extent of hydrolysis (degree of hydrolysis) is described in DH units where:

$$DH = 100x\left(\frac{\text{Number of peptide - bonds cleaved}}{\text{Initial number of peptide bonds present}}\right)$$

Commercially, using enzymes such as subtilisin, DH values of up to 30 are produced using protein preparations of 8-12% (w/w). The enzymes are formulated so that the value of the enzyme : substrate ratio used is 2-4% (w/w). At the high pH needed for effective use of subtilisin, protons are released during the proteolysis and must be neutralised:

$$H_2N\text{-aa-aa-aa-aa-aa-}COO^- \xrightarrow{\text{subtilisin (pH 8.5)}} H_2N\text{-aa-aa-aa-}COO^- + H_2N\text{-aa-aa-}COO^- + H^+$$

where aa is an amino acid residue.

Correctly applied proteolysis of inexpensive materials such as soya protein can increase the range and value of their usage, as indeed occurs naturally in the production of soy sauce. Partial hydrolysis of soya protein, to around 3.5 DH greatly increases its 'whipping expansion', further hydrolysis, to around 6 DH improves its emulsifying capacity. If their flavours are correct, soya protein hydrolysates may be added to cured meats. Hydrolysed proteins may develop properties that contribute to the elusive, but valuable, phenomenon of 'mouth feel' in soft drinks.

Proteases are used to recover protein from parts of animals (and fish) would otherwise go to waste after butchering. About 5% of the meat can be removed mechanically from bone. To recover this, bones are mashed incubated at 60°C with neutral or alkaline proteases for up to 4 h. The meat slurry produced is used in canned meats and soups.

Large quantities of blood are available but, except in products such black puddings, they are not generally acceptable in foodstuffs because of their colour. The protein is of a high quality

nutritionally and is de-haemed using subtilisin. Red cells are collected and haemolysed in water. Subtilisin is added and hydrolysis is allowed to proceed batchwise, with neutralisation of the released protons, to around 18 DH, when the hydrophobic haem molecules precipitate. Excessive degradation is avoided to prevent the formation of bitter peptides. The enzyme is inactivated by a brief heat treatment at 85°C and the product is centrifuged; no residual activity allowed into meat products. The haem-containing precipitate is recycled and the light-brown supernatant is processed through activated carbon beads to remove any residual haem. The purified hydrolysate, obtained in 60% yield, may be spray-dried and is used in cured meats, sausages and luncheon meats.

Meat tenderisation by the endogenous proteases in the muscle after slaughter is a complex process which varies with the nutritional, physiological and even psychological (i.e. frightened or not) state of the animal at the time of slaughter. Meat of older animals remains tough but can be tenderised by injecting inactive papain into the jugular vein of the live animals shortly before slaughter. Injection of the active enzyme would rapidly kill the animal in an unacceptably painful manner so the inactive oxidised disulfide form of the enzyme is used. On slaughter, the resultant reducing conditions cause free thiols to accumulate in the muscle, activating the papain and so tenderising the meat. This is a very effective process as only 2 - 5 ppm of the inactive enzyme needs to be injected. Recently, however, it has found disfavour as it destroys the animals heart, liver and kidneys that otherwise could be sold and, being reasonably heat stable, its action is difficult to control and persists into the cooking process.

Proteases are also used in the baking industry. Where appropriate, dough may be prepared more quickly if its gluten is partially hydrolysed. A heat-labile fungal protease is used so that it is inactivated early in the subsequent baking. Weak-gluten flour is required for biscuits in order that the dough can be spread thinly and retain decorative impressions. In the past this has been obtained from European domestic wheat but this is being replaced by high-gluten varieties of wheat. The gluten in the flour derived from these must be extensively degraded if such flour is to be used efficiently for making biscuits or for preventing shrinkage of commercial pie pastry away from their aluminium dishes.

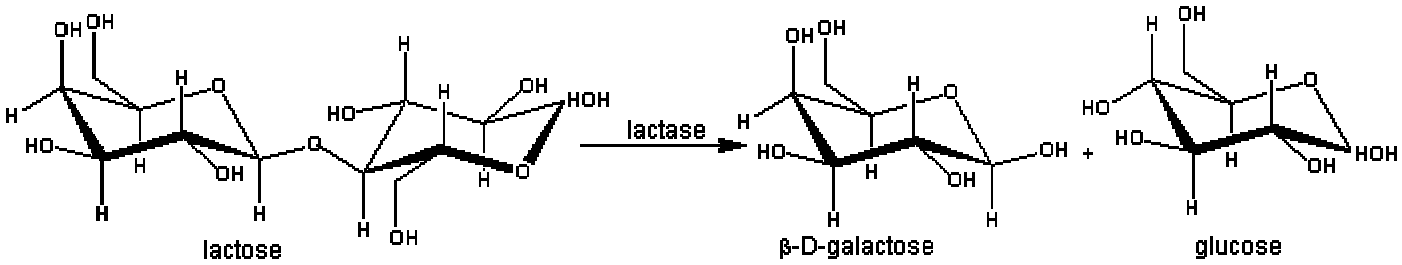
The use of lactases in the dairy industry

Lactose is present at concentrations of about 4.7% (w/v) in milk and the whey (supernatant) left after the coagulation stage of cheese-making. Its presence in milk makes it unsuitable for the majority of the world's adult population, particularly in those areas which have traditionally not had a dairy industry. Real lactose tolerance is confined mainly to peoples whose origins lie in Northern Europe or the Indian subcontinent and is due to 'lactase persistence'; the young of all mammals clearly are able to digest milk but in most cases this ability reduces after weaning. Of the Thai, Chinese and Black American populations, 97%, 90% and 73% respectively, are reported to be lactose intolerant, whereas 84% and 96% of the US White and Swedish populations, respectively, are tolerant. Additionally, and only very rarely some

individuals suffer from inborn metabolic lactose intolerance or lactase deficiency, both of which may be noticed at birth. The need for low-lactose milk is particularly important in food-aid programmes as severe tissue dehydration, diarrhoea and even death may result from feeding lactose containing milk to lactose-intolerant children and adults suffering from protein-calorie malnutrition. In all these cases, hydrolysis of the lactose to glucose and galactose would prevent the (severe) digestive problems.

Another problem presented by lactose is its low solubility resulting in crystal formation at concentrations above 11 % (w/v) (4°C). This prevents the use of concentrated whey syrups in many food processes as they have a unpleasant sandy texture and are readily prone to microbiological spoilage. Adding to this problem, the disposal of such waste whey is expensive (often punitively so) due to its high biological oxygen demand. These problems may be overcome by hydrolysis of the lactose in whey; the product being about four times as sweet. much more soluble and capable of forming concentrated, microbiologically secure, syrups (70% (w/v)).

Lactose may be hydrolysed by lactase, a β-galactosidase.



Commercially, it may be prepared from the dairy yeast *Kluyveromyces fragilis* (*K. marxianus* var. marxianus), with a pH optimum (pH 6.5-7.0) suitable for the treatment of milk, or from the fungi *Aspergillus oryzae* or *A. niger,* with pH optima (pH 4.5-6.0 and 3.0-4.0, respectively) more suited to whey hydrolysis. These enzymes are subject to varying degrees of product inhibition by galactose. In addition, at high lactose and galactose concentrations, lactase shows significant transferase ability and produces β-1,6-linked galactosyl oligosaccharides.

Lactases are now used in the production of ice cream and sweetened flavoured and condensed milks. When added to milk or liquid whey (2000 U kg^{-1}) and left for about a day at 5°C about 50% of the lactose is hydrolysed, giving a sweeter product which will not crystallise if condensed or frozen. This method enables otherwise-wasted whey to replace some or all of the skim milk powder used in traditional ice cream recipes. It also improves the 'scoopability' and creaminess of the product. Smaller amounts of lactase may be added to long-life sterilised milk to produce a relatively inexpensive lactose-reduced product (e.g. 20 U kg^{-1}, 20°C, 1 month of storage). Generally, however, lactase usage has not reached its full potential, as present enzymes are relatively expensive and can only be used at low temperatures.

Enzymes in the fruit juice, wine, brewing and distilling industries

One of the major problems in the preparation of fruit juices and wine is cloudiness due primarily to the presence of pectins. These consist primarily of α-1,4-anhydrogalacturonic acid polymers, with varying degrees of methyl esterification. They are associated with other plant polymers and, after homogenisation, with the cell debris. The cloudiness that they cause is difficult to remove except by enzymic hydrolysis. Such treatment also has the additional benefits of reducing the solution viscosity, increasing the volume of juice produced (e.g. the

yield of juice from white grapes can be raised by 15%), subtle but generally beneficial changes in the flavour and, in the case of wine-making, shorter fermentation times. Insoluble plant material is easily removed by filtration, or settling and decantation, once the stabilising effect of the pectins on the colloidal haze has been removed.

Commercial pectolytic enzyme preparations are produced from *Aspergillus niger* and consist of a synergistic mixture of enzymes:

a. polygalacturonase (EC 3.2.1.15), responsible for the random hydrolysis of 1,4-α-D-galactosiduronic linkages;
b. pectinesterase (EC 3.2.1.11), which releases methanol from the pectyl methyl esters, a necessary stage before the polygalacturonase can act fully (the increase in the methanol content of such treated juice is generally less than the natural concentrations and poses no health risk);
c. pectin lyase (EC 4.2.2.10), which cleaves the pectin, by an elimination reaction releasing oligosaccharides with non-reducing terminal 4-deoxymethyl-α-D-galact-4-enuronosyl residues, without the necessity of pectin methyl esterase action; and
d. hemicellulase (a mixture of hydrolytic enzymes including: xylan endo-1,3-β-xylosidase, EC 3.2.1.32; xylan 1,4-β-xylosidase, EC 3.2.1.37; and α-L-arabinofuranosidase, EC 3.2.1.55), strictly not a pectinase but its adventitious presence is encouraged in order to reduce hemicellulose levels.

The optimal activity of these enzymes is at a pH between 4 and 5 and generally below 50°C. They are suitable for direct addition to the fruit pulps at levels around 20 U l^{-1} (net activity). Enzymes with improved characteristics of greater heat stability and lower pH optimum are currently being sought.

In brewing, barley malt supplies the major proportion of the enzyme needed for saccharification prior to fermentation. Often other starch containing material (adjuncts) are used to increase the fermentable sugar and reduce the relative costs of the fermentation. Although malt enzyme may also be used to hydrolyse these adjuncts, for maximum economic return extra enzymes are added to achieve their rapid saccharification. It not

necessary nor desirable to saccharify the starch totally, as non-fermentable dextrins are needed to give the drink 'body' and stabilise its foam 'head'. For this reason the saccharification process is stopped, by boiling the 'wort', after about 75% of the starch has been converted into fermentable sugar.

The enzymes used in brewing are needed for saccharification of starch (bacterial and fungal α-amylases), breakdown of barley β-1,4- and β-1,3- linked glucan (β-glucanase) and hydrolysis of protein (neutral protease) to increase the (later) fermentation rate, particularly in the production of high-gravity beer, where extra protein is added. Cellulases are also occasionally used, particularly where wheat is used as adjunct but also to help breakdown the barley β-glucans. Due to the extreme heat stability of the *B. amyloliquefaciens* α-amylase, where this is used the wort must be boiled for a much longer period (e.g. 30 min) to inactivate it prior to fermentation. Papain is used in the later post-fermentation stages of beer-making to prevent the occurrence of protein- and tannin-containing 'chill-haze' otherwise formed on cooling the beer.

Recently, 'light' beers, of lower calorific content, have become more popular. These require a higher degree of saccharification at lower starch concentrations to reduce the alcohol and total solids contents of the beer. This may be achieved by the use of glucoamylase and/or fungal α-amylase during the fermentation.

A great variety of carbohydrate sources are used world wide to produce distilled alcoholic drinks. Many of these contain sufficient quantities of fermentable sugar (e.g. rum from molasses and brandy from grapes), others contain mainly starch and must be saccharified before use (e.g. whiskey from barley malt, corn or rye). In the distilling industry, saccharification continues throughout the fermentation period. In some cases (e.g. Scotch malt whisky manufacture uses barley malt exclusively) the enzymes are naturally present but in others (e.g. grain spirits production) the more heat-stable bacterial α-amylases may be used in the saccharification.

Glucose oxidase and catalase in the food industry

Glucose oxidase is a highly specific enzyme, from the fungi *Aspergillus niger* and *Penicillium*, which catalyses the oxidation of β-glucose to glucono-1,5-lactone (which spontaneously hydrolyses non-enzymically to gluconic acid) using molecular oxygen and releasing hydrogen peroxide (see reaction scheme). It finds uses in the removal of either glucose or oxygen from foodstuffs in order to improve their storage capability. Hydrogen peroxide is an effective bacteriocide and may be removed, after use, by treatment with catalase (derived from the same fungal fermentations as the glucose oxidase) which converts it to water and molecular oxygen:

catalase
$2H_2O_2 \longrightarrow 2H_2O + O_2$ For most large-scale applications the two enzymic activities are

not separated. Glucose oxidase and catalase may be used together when net hydrogen peroxide production is to be avoided.

A major application of the glucose oxidase/catalase system is in the removal of glucose from egg-white before drying for use in the baking industry. A mixture of the enzymes is used (165 U kg^{-1}) together with additional hydrogen peroxide (about 0.1 % (w/w)) to ensure that sufficient molecular oxygen is available, by catalase action, to oxidise the glucose. Other uses are in the removal of oxygen from the head-space above bottled and canned drinks and reducing non-enzymic browning in wines and mayonnaises.

Glucose from cellulose

There is very much more cellulose available, as a potential source of glucose, than starch, yet cellulose is not a significant source of pure glucose. The reasons for this are many, some technical, some commercial. The fundamental reason is that starch is produced in relatively pure forms by plants for use as an easily biodegradable energy and carbon store. Cellulose is structural and is purposefully combined and associated with lignin and pentosans, so as to resist biodegradation; dead trees take several years to decay even in tropical rainforests. A typical waste cellulolytic material contains less than half cellulose, most of the remainder consisting of roughly equal quantities of lignin and pentosans. A combination of enzymes is needed to degrade this mixture. These enzymes are comparatively unstable of low activity against native lignocellulose and subject to both substrate and product inhibition. Consequently, although many cellulolytic enzymes exist and it is possible to convert pure cellulose to glucose using only enzymes, the cost of this conversion is excessive. The enzymes might be improved by strain selection from the wild or by mutation but problems caused by the physical nature of cellulose are not so amenable to solution. Granular starch is readily stirred in slurries containing 40% (w/v) solids and is easily solubilised but, even when pure, fibrous cellulose forms immovable cakes at 10% solids and remains insoluble in all but the most exotic (and enzyme denaturing) solvents. Impure cellulose often contains almost an equal mass of lignin, which is of little or no value as a by-product and is difficult an expensive to remove.

Summary

Enzymes are biomolecules that catalyse and regulate thousands of chemical reactions occurring in biological systems. In the absence of enzymes, these reactions would proceed too slowly. Enzymes, being proteins exhibit all properties of proteins. Like proteins, they are denatured by changes in pH and temperature. Enzymes undergo physical changes during the reaction but revert to their original form at the end of reaction. Enzyme activity is regulated in a variety of ways involving inhibitors, activators and through isoenzymes.

According to the International Union of Biochemists, enzymes are classified into six major classes as oxidoreductases, transferases, hydrolases, lyases, isomerases and synthases. Each major class is divided into subclass, which in turn is subdivided into sub-sub class.

All enzymes contain one or more active sites at which substrates bind for catalytic reaction. The specific folding of polypeptide chains creates the active sites and they usually consist of chiral groves, shape and polarity to accommodate the substrates. Enzymes combine transiently with the substrate to produce a transition state intermediate having a lower energy of activation than the uncatalysed reaction thus they accelerate chemical reactions by lowering the energy of activation than the uncatalysed reaction.

Two theories were proposed to explain the mechanism of enzyme action- Lock and Key theory and Induced-Fit theory. The drawback in the feature of Lock and Key model is the implied rigidity of the catalytic site. In the induced-fit model, the substrate induces a conformational change in the enzyme, which aligns the amino acid residues or other groups for substrate binding, catalysis or both.

Enzymatic reaction is affected by factors like substrate concentration, enzyme concentration, temperature, pH and inhibitors. With respect to substrate concentration, Michaelis

-Menten derived an equation to explain enzyme-substrate behaviour and according to them, the kinetics for an enzyme-catalysed reaction is given as

$$v = \frac{V_{max}(S)}{K_m + (S)}$$

Where Km, known as Michaelis-Menten constant, is defined as the substrate concentration at half maximal velocity. Each enzyme needs optimum pH and temperature to exhibit its maximal velocity of enzymatic reaction.

Enzyme action may be inhibited by compounds that have the ability to block catalysis. These compounds are called inhibitors. The important type of inhibitors include competitive and noncompetitive. Heavy metal ions, certain pesticides, structural analogues of substrates and a wide variety of drugs function as inhibitors. Competitive inhibitors are usually structurally similar to substrates and compete for active site whereas noncompetitive inhibitors bind at sites distant from the active sites, so it is possible for both substrate and inhibitor to bind separately or simultaneously. Inhibitors are used as tools to probe the mechanism of enzyme- catalysed reactions and as therapeutic agents.

Enzymes are routinely employed as analytical tools, diagnostic agents and therapeutic agents. Their function lies in their unique value of their specificity and enormous catalytic power.

Isozymes are physically distinct enzymes that catalyse the same reaction. Some isozymes are oligomeric proteins that differ from one another in subunit compositions. The determination of LDH isoenzyme levels helps in the diagnosis of myocardial infarctions.

Review questions

A. Multiple choice questions

1. The competitive inhibitor for succinate dehydrogenase is
   a. Malic acid b. Malonic acid c. Citric acid d. Ascorbic acid
2. Which one of the statement is not true with respect to non-competitive inhibition?
   a. Inhibitors bind to a site other than the active site
   b. ES complex does not decompose at the normal rate to yield products.
   c. Inhibition is not reversed by increasing the substrate concentration.
   d. Inhibition is reversed by increasing the substrate concentration.
3. Km is expressed as
   a. Mole/litre b.Mole c.mg d .No units
4. At maximal velocity the reaction follows a kinetics of
   a. Zero order b.First order c. Second order d. Mixed order
5. Which one of the following is an iron-containing enzyme?
   a. Carbonic anhydrase b. Pyruvate kinase
   c. Hexokinase d. Cytochrome oxidase

B. Fill up the blanks

6. A coenzyme that is covalently bound to the enzyme protein is ----------.
7. The non-protein components of enzymes are called as ----------.
8. Enzyme increase the rate of reaction by ----------.
9. In the presence of non-competitive inhibitor the concentration of active enzyme ----------.
10. The stage at which the substrate possess enough energy to attain activated condition is ----

C. Write short answers for the following

11. Define the following
    a. Coenzyme b. Cofactor c. Km value d. Optimum temperature
    e. Optimum pH f. Isoenzyme g. Holoenzyme h. Metalloenzymes i. Active site
    j. Competitive inhibition k. Non-competitive inhibition l. Activation energy
    m. Enzyme Commission number
12. Explain enzyme specificity with examples.
13. What are metalloenzymes? How is it different from metal-activated enzymes?
14. Explain allosteric inhibition with example.
15. What is activation energy? How does enzyme reduces the activation energy?

D. Write in detail on the following.

16. How are enzymes classified according to IUB system? Explain the function of each class with suitable example.
17. Explain the mechanism of enzyme action with energy diagram.
18. Describe the different factors influencing enzyme-catalysed reaction.
19. Explain competitive and non-competitive inhibition with examples.
20. Explain the function of isozymes with an example.
21. Explain lock and key and induced- fit models proposed for enzyme action.
22. Give an account of enzyme kinetics

E. Solve the following problems.

23. Answer the questions given below from the reaction

$$\text{Glucose + ATP} \xrightarrow[Mg^{++}]{\text{glucokinase}} \text{Glucose 6P + ADP}$$

a. What is the substrate for glucokinase?
b. Which group is transferred?
c. What is the function of Mg++?
d. Is this reaction reversible? Why?

24. Complete and balance the equation.

$$CH_3\,CH(OH)\text{-}COOH + NAD^+ \longrightarrow CH_3\text{ - }CO\text{- }COOH^+$$

25. Why enzyme detergents (Protease) fail to work in boiling water?
26. Show the major products obtained when the following peptide is treated with trypsin.
Phe - Tyr - Arg - Phe - Leu - Lys - Tyr.
27. You are provided with saliva, starch and iodine solution. How will you prove that saliva contains amylase enzyme.

F. Gain additional knowledge by further reading

28. Enzyme engineering
29. Enzyme regulation
30. Location of active site in enzymes.
31. Ribozymes and abzymes

Table 5.1 Classification and functions of enzymes

Main classification number	Major classes and sub-classes	Type of reaction catalysed
1.	**Oxidoreductases** Dehydogenases Oxidases Reductases Peroxidases Catalases Hydroxylases	Transfer of electrons usually in the form of hydrogen atoms or hydride ions
2.	**Transferases** Kinases Acyltransferases Transaldolases Methyl transferases	Transfer of functional groups form one molecule to another
3.	**Hydrolases** Esterases Glycosidases Peptidses Phosphatases Thiolases Phospholipases Amidases	Cleavage of bonds by hydrolysis
4.	**Lyases** Decarboxylases Aldolases Hydratases Dehydratases	Removal of groups by a mechanism other than hydrolysis leaving a double bond in one of the product.
5.	**Isomerases** Racemases Epimerases Isomerases Mutases	Transfer of groups within a molecule to yield isomeric forms

6.	**Ligases** Synthetases Carboxylases	Formation of new bonds(C-C, C-S, C-O C-N etc.) by condensation coupled with hydrolysis of high energy molecules like ATP).

Table 5.2 Enzymes requiring metal ions as cofactors

Enzymes	Cofactors
Cytochrome oxidase	Fe^{2+} and Cu^{2+}
Peroxidase, catalase	Fe^{2+} or Fe^{3+}
Carbonic anhydrase	Zn^{2+}
Alcohol dehydrogenase	Zn^{2+}
Hexokinase	Mg^{2+}
Pyruvate kinase	Mg^{2+}
Glucose 6-phosphatase	Mg^{2+}
Pyruvate kinase	K^{+}
Nitrogenase, nitrate reductase	Mo
Glutathione peroxidase	Se

Table 5.3 Km Values for some enzyme- substrate systems

Enzyme	Substrate	Km(mM)
Catalase	H_2O_2	0.001
Hexokinase from brain	**ATP**	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	9
Chymotrypsin	N-Benzoyl tyrosinamide	2.5
	Glycyltyrosinylglycine	108
β-Galactosidase	Lactose	4.0
Pyruvate carboxylase	Pyruvate	1.0

CHAPTER 6

VITAMINS AND COENZYMES

Lecture.24

6.1 Introduction

Vitamins are low molecular weight organic compounds required in small amounts in the diet. Most of the vitamins are not synthesized in the human body but are synthesized by the plants. Hence these essential nutrients are mainly obtained through the food. Though most of them are present in the diet as such, some are present as precursors known as provitamins.

Vitamins are divided into two major categories. They are fat-soluble (A, D, E and K) and water-soluble vitamins (B-complex and vitamin C). B complex vitamins include thiamine (B_1), riboflavin (B_2), niacin, pyridoxine (B_6), biotin, folic acid, pantothenic acid and cobalamin (B_{12}). Inositol, cholic and para-aminobenzoic acid are vitamin-like substances sometimes classified as part of the B complex, but no convincing evidence has been shown so far to be included as vitamins. All the fat-soluble vitamins and some B vitamins exist in multiple forms. The active forms of vitamin A are retinol, retinal and retinoic acid and vitamin D is available as ergocalciferol (D_2) and cholecalciferol (D_3). The vitamin E family includes four tocopherols and four tocotrienols but α-tocopherol being the most abundant and active form. The multiple forms of vitamins are interconvertible and some are interchangeable.

6.2 Fat-soluble vitamins

The fat-soluble vitamins are soluble in fat and other nonpolar solvents. All are synthesized fully or partly from isoprene units and excess quantities are stored in fat containing cells. The fat-soluble vitamins appear not to function as components of coenzymes but to serve other important roles. The important dietary sources, functions and deficient diseases associated with fat- soluble vitamins are presented in table 6.1

6.3 Water-soluble vitamins

The water-soluble vitamins include B-complex group and vitamin C. The important dietary sources and deficient symptoms associated with them are presented in table 6.2.

6.4 Coenzymes

Mechanism of coenzyme action

Coenzyme accelerates the enzymatic reaction by helping the formation of the product (s) by acting as acceptor for one of the products (Figure 6.1)

The substrate combines with the apoenzyme to form activated complex. But this combination takes place in the presence of coenzyme. The bond in the substrate is strained and ruptured when one of the cleavage products is directly transferred to the coenzyme, which has suitable receptor site in its structure. The other cleavage product now dissociates from the apoenzyme liberating the enzyme protein for fresh reaction. The cleavage product attached to the coenzyme is next released from the surface of the coenzyme after the completion of enzyme action. Now both apoenzyme and coenzyme are regenerated to their original form and are ready for fresh reaction. A prosthetic group also acts in a similar fashion with the difference that the prosthetic group is firmly attached to the surface of the apoenzyme**.**

The structure and coenzyme functions of B-complex group vitamins are described below.

6.4.1 Thiamine

The coenzyme form of thiamine, thiamine pyrophosphate (TPP), is shown in figure 6.2.

The two important reactions in which TPP functions as coenzyme are

i. Oxidative decarboxylation of α-keto acids such as pyruvate and α-ketoglutarate.

ii) Transketolase reaction

TPP provides a reactive carbon on the thiazole ring and forms a carbanion stabilized by positively charged ring nitrogen. The carbanion is then free to add the carbonyl carbon of pyruvate (or α-ketoglutarate). The addition compound is then decarboxylated eliminating

CO_2 and generating hydroxyethyl-TPP. This reaction occurs in a multienzyme complex known as pyruvate dehydrogenase complex (or α-ketoglutarate dehydrogenase complex). The acetaldehyde (decarboxylated product) moiety is then transferred to the lipoamide in the complex.

The role of TPP as a coenzyme in the transketolase reaction is very similar to that of oxidative decarboxylation. The carbanion of TPP combines with the carbonyl carbon of xylulose 5P. Carbon 1 and 2 of xylulose 5P are retained to form hydroxyethyl derivative of TPP. Then it is transferred to the carbonyl carbon of ribose 5P to form sedoheptulose 7P (Figure 6.3).

6.4.2 Riboflavin

The flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are the two-coenzyme forms of riboflavin (Figure 6.4).

FMN and FAD serve as prosthetic groups of oxidation-reduction enzymes known as flavoenzymes or flavoproteins. They are usually tightly, but not covalently, bound to the protein. Many flavoproteins contain one or more metals as additional cofactors and are known as the metalloflavoproteins. In the catalytic cycle of flavoproteins the flavin moiety of the flavin nucleotides undergoes reversible reduction of the isoalloxazine ring to yield the reduced nucleotides $FMNH_2$ and $FADH_2$. L-amino acid oxidase contains tightly bound FMN as the prosthetic group. Succinate dehydrogenase and D-amino acid oxidase contain FAD as prosthetic group.

6.4.3 Niacin

The coenzyme forms of niacin are NAD^+ and $NADP^+$ which function as the coenzymes of a large number of oxidoreductases collectively called as pyridine-linked dehydrogenases. These coenzymes are bound to the dehydrogenase protein relatively loosely during the catalytic cycle and therefore serve as substrate than as prosthetic group.

They function as electron acceptors during the enzymatic removal of hydrogen atoms from specific substrate molecules. One hydrogen atom from the substrate is transferred as a hydride ion to the nicotinamide portion of the oxidised forms of these coenzymes. The other hydrogen atom from the substrate becomes a hydrogen ion (Figure 8.5). Pyridine linked dehydrogenases are specific for either NAD^+ or $NADP^+$, but a few will function with both. Isocitrate dehydrogenase and lactate dehydrogenase are NAD-specific. Glucose 6-P dehydrogenase is NADP-specific. Glutamate dehydrogenase functions with NAD^+ or $NADP^+$.

6.4.4 Pyridoxine

The coenzyme form of pyridoxine is known as pyridoxal phosphate (PP) (Figure 6.6).

The most common type of reaction requiring PP as a coenzyme is transamination. Enzymes catalysing such reactions are known as transaminases or aminotransferases The coenzyme binds to its apoenzyme via Schiff's base between its aldehyde group and the epsilon amino group of a lysine in the enzyme. Additional ionic bond is also formed between its phosphate and the enzyme. During reaction, α-amino group of amino acid displaces the epsilon amino group forming a new Schiff's base. By a series of electron shifts and rearrangements, the pyridoxal phosphate becomes pyridoxamine phosphate. The amino acid is oxidatively deaminated to form the corresponding α-keto acid. The α-amino group is then transferred to a new α-keto acid to change it to an amino acid. PP also acts as coenzyme in the decarboxylation, desulfuration, transulfuration reactions associated with amino acid metabolism.

6.4.5.Pantothenic acid

The coenzyme form of pantothenic acid is coenzyme A and is represented as CoASH (Figure 8.7). The thiol group (-SH) acts as a carrier of acyl group. The acyl-sulphur bond formed between coenzyme A and the acyl moiety is a high-energy bond, equivalent to the high-energy bond of ATP. The function of coenzyme A is to serve as a carrier of acyl group in reactions associated with fatty acid oxidation, fatty acid synthesis, pyruvate

oxidation and biological acetylations. It is also involved in many biosynthetic processes such as synthesis of cholesterol, terpenes and steroids.

6.4.6. Biotin

The important function of biotin is its role as coenzyme for carboxylase, which catalyses carbon dioxide fixation or carboxylation reaction. The epsilon amino group of lysine in carboxylase enzymes combines with the carboxyl group of biotin to form covalently linked biotinyl carboxyl carrier protein (BCCP or biocytin) (Figure 6.8). This serves as an intermediate carrier of carbon dioxide. The carboxylation of acetyl CoA to malonyl CoA in presence of acetyl CoA carboxylase requires biotin as coenzyme. Propionyl carboxylase and pyruvate carboxylase are also associated with biotin.

6.4.7 Folic acid

The coenzyme form of folic acid is tetra hydro folic acid (Figure 6.9). Tetrahydro folic acid is associated with one carbon metabolism. The tetrahydro folic acid serves as a carrier of single carbon moieties such as formyl, methenyl, methylene, formyl or methyl group. (Figure 6.10). It is involved in the biosynthesis of purines, pyrimidines, serine, methionine and glycine.

6.4.8 Lipoic acid

The oxidised and reduced forms of lipoic acid are given in figure 6.11. Lipoic acid functions as a coenzyme in pyruvate and α-ketoglutarate dehydrogenase multienzyme complexes.

6.4.9.Vitamin B_{12} (cobalamin)

The 5-deoxyadenosyl cobalamin and methyl cobalamin function as coenzyme forms and are required for the action of several enzymes. Methyl malonyl CoA mutase uses 5-deoxyadenosyl cobalamin as coenzyme. Methyl cobalamin functions as a carrier of methyl group to homocysteine and convert it to methionine

The functions of important coenzymes and their precursors are presented in table 6.3.

Summary

Vitamins are classified as fat-soluble and water-soluble. Fat-soluble vitamins include vitamins A, D, E and K. B-complex group and vitamin C belong to water-soluble vitamins. B-complex group of vitamins function as coenzymes and play a key role in biochemical reactions. The coenzyme from of thiamine is thiamine pyrophosphate (TPP). It is associated with oxidative decarboxylation of α-keto acids such as pyruvate and α-ketoglutarate as well as transketolase enzyme. The carbanion formed in the thiazole ring of TPP condenses with the carbonyl carbon – pyruvate and then decarboxylation occurs. The flavin mononucleotide (FMN) and flavin adnine dinucleotide (FAD) are the two coenzymes forms of riboflavin. FMV and FAD serve as coenzymes for contain and oxidation reaction and under reversible reduction of the isoalloxazine ring to yield $FMNH_2$ and $FADH_2$. The coenzyme forms of niacin are NAD^+ and $NADP^+$, which function as the coenzymes for a large number of oxidoreductases. They function as electron acceptors. One hydrogen atom from the substrate is transferred as a hydride ion to the nicotinamide portion of the oxidized forms of these coenzymes. The coenzyme form of pyridoxine is pyridoxal phosphate. It function as coenzyme in transamination reaction as well as decarboxylation, desulfuration reaction in amino acid metabolism.

The coenzyme form of pantothenic acid is coenzyme A and is represented as CoASH. The thiol group acts as a carrier of acyl group. It is an important coenzyme involved in fatty acid oxidation, pyruvate oxidation and is also biosynthesis of terpenes. The epsilon amino group of lysine in carboxylase enzymes combines with the carboxyl carrier protein (BCCP or biocytin) and server as an intermediate carrier of CO_2. Acetyl CoA pyruvate and propionyl carboxylayse require the participation of BCCP. The coenzyme form of folic acid is tetrahydro folic acid. It is associated with one carbon metabolism. The oxidised and reduced forms of lipoic acid function as coenzyme in

pyruvate and α-ketoglutarate dehydrogenase complexes. The 5-deoxy adenosyl and methyl cobalamins function as coenzyme forms of vitamin B12. Methyl cobalamin is involved in the conversion of homocysteine to methionine.

Review questions

A. Multiple choice questions

1. The coenzyme form of pantothenic acid is
   a. Acetyl CoA b. Coenzyme A c. Cofactor A . d. Pantothenyl phosphate
2. The coenzyme involved in transamination reaction is
   a. Pyridoxal phosphate b Thiamine pyrophosphate. c. Flavin monophosphate
   d. Biocytin.
3. Which one of the following is not the active form of pyridoxine?
   a) Pyridoxol b) Pyridoxal c) Pyridoxamine d) Pyridine
4. The active ring system involved in the functioning of TPP is
   a) Pyrimidine b) Thiazole c) Pyridine d) Imidazole
5. Para amino benzoic acid (PABA) is a constituent of
   a) Folic acid b) Coenzyme A c) Lipoic acid d) Cyanocobalamin

B. Fill up the blanks

6. The coenzyme required for transketolase is ----------
7. The coenzyme form of folic acid is ----------.
8. The coenzyme form of Vit B_6 is ----------
9. The ε-amino group of lysine in carboxylase combine with carboxyl group of biotin to form ----------
10. The coenzyme involved in the carboxylation reaction is ----------

C. Write short answers for the following

11. Draw the structure and mention the metabolic role of folic acid.

12. Draw the structure and mention the metabolic role of lipoic acid

13. Differentiate coenzyme from isoenzyme

14. Draw the structure and indicate the metabolic role of pantothenic acid

15. State the biochemical role of Vit.C

D. Write in detail on the following.

16. Draw the structure and coenzyme form of

a) Thiamine b) Pyridoxine c) Niacin d) Folic acid

17. Explain the mechanism of action of the following coenzymes

a) TPP b) PP c) NAD^+ d) FAD

18. Mention two enzymes associated with the following coenzymes

a) TPP b) PP c) NAD^+ d) FAD

e) NADP f) FMN g) BCCP h) Tetrahydrofolic acid

19.Name the deficiency diseases associated with B-complex vitamins.

20. What are the important sources for fat and water-soluble vitamins?

E. Solve the following problems.

21. The Coenzyme NADH (reduced form) has an absorption maximum at 340nm. Can you suggest a suitable method of assay for NAD-linked dehydrogenases?

22. The parboiled rice is better than raw rice based on B-complex vitamins content. Why?

F. Gain additional knowledge by further reading

23. Vitamin antagonists

24. Changes in vitamin content during processing of foods
25. Golden rice
26. Vitamin toxicity
27. RDA for vitamins

Table 6.1 Fat-soluble vitamins

Vitamin	Functions	Some common dietary sources	Deficiency symptoms
Vitamin A	Visual cycle and maintaining epithelial cells	Fruits, vegetables, fish-liver oils	Night blindness and eventually total blindness, anorexia (appetite loss), dermatitis, recurrent infections; in children, cessation of skeletal growth and lesions in the central nervous system

Vitamin D	Calcium metabolism	Fish-liver oil	Bone pain and skeletal deformities such as bowlegs (Rickets) and knock-knee in children. Osteomalacia in adults.
Vitamin E	Antioxidant	Plant oils, green leafy vegetables, milk, eggs, meat	Symptoms in humans, if any, are controversial; possibly anaemia
Vitamin K	Blood clotting	Leafy vegetables, soybeans, vegetable oils	Impaired blood clotting

Table 6.2 Water-soluble vitamins

	Vitamin	Some common dietary sources	Deficiency symptoms in humans
1.	Thiamine (Vitamin B_1)	Liver, meat, milk, vegetables, whole grains, nuts	Dry and wet beri-beri. Weight loss-, muscle wasting, sensory changes, mental confusion, enlargement of heart, constipation
2.	Riboflavin (Vitamin B_2)	Liver, wheat germ, eggs, milk, green leafy vegetables, meat	Magenta-coloured tongue, fissuring at the corners of mouth and lips, dermatitis

3. Niacin (nicotinic acid)	Meat, liver, cereals, legumes	Pellagra. Dermatitis when exposed to sunlight, weakness, insomnia, impaired digestion, diarrhea, dementia, irritability, memory loss, headaches
4. Pyridoxine (vitamin B_6 or pyridoxol)	Egg yolk, fish, meat, lentils, nuts, fruits, vegetables	Convulsions, dermatitis, weight loss, irritability, weakness in infants
5. Pantothenic acid	Eggs, peanuts, liver, meat, milk, cereals, vegetables	Vomiting, abdominal distress, cramps, fatigue, insomnia
6. Biotin	Liver, yeast, meat, peanuts, eggs, chocolate, dairy products, grains fruits, vegetables	Dermatitis, skin dryness, depression, muscle pain, nausea, anorexia (appetite loss)
7. Folic acid	Yeast, liver, green vegetables, some fruits	Anemia leading to weakness, tiredness, sore tongue, diarrhea, irritability, headache, heart palpitations
8. Cobalamin (vitamin B_{12})	Meat, shellfish, fish, milk, eggs	Neurological disorders, anemia leading to tiredness, sore tongue, constipation, headache, heart palpitations.
9 Ascorbic acid (Vitamin C)	Vegetables and citrus fruits	Sore gums, loose teeth, joint pain, edema, anaemia, fatigue, depression, impaired iron absorption, impaired wound healing.

Table 6.3 Functions of important coenzymes and their precursors

Coenzyme	Short form(s)	Chemical groups transferred	Vitamin precursor
Thiamine pyro-phosphate	TPP	Two carbon aldehydes	Thiamine
Flavin adenine dinucleotide	FAD	Electrons	Riboflavin
Flavin mono nucleotide	FMN		

Nicotinamide adenine dinucleotide	NAD	Hydride ion	Nicotinic acid
Nicotinamide adenine dinucleotide Phosphate	NADP		
Coenzyme A	CoASH	Acyl group	Pantothenic acid
Pyridoxal phosphate	PP	Amino group	Pyridoxal (Pyridoxamine)
Coenzyme B_{12}	Cobamide	Hydrogen atoms and alkyl groups	Vitamin B_{12}
Biocytin	BCCP	Carbon dioxide	Biotin
Tetrahydrofolate	THF (FH_4)	One-carbon groups	Folate
Lipoamide	-	Electrons and acyl groups	Lipoate

CHAPTER 7

INTRODUCTION TO METABOLISM

Lectures 25

7.1 Introduction

The reaction pathways that comprise metabolism are divided into two categories.

i. Those involved in degradation called catabolism
ii. Those involved in biosynthesis called anabolism.

In catabolic reactions, complex substances are broken down to simpler compounds with a concomitant release of free energy. The released free energy during these catabolic reactions is conserved in the form of ATP or NADPH. The major nutrients

such as carbohydrates, lipids and proteins are converted to common intermediate and further metabolised in a central oxidative pathway.

The opposite process occurs during biosynthesis. Simple organic molecules such as pyruvic acid, acetyl unit or intermediate compounds of citric acid cycle serve as starting molecules for varied biosynthetic products. The energy rich molecules such as ATP or NADPH derived from catabolic reactions are utilized in the biosynthetic reactions (Figure 7.1).

7.2 Experimental approaches to study metabolism

The elucidation of metabolic pathway is a complex process involving contributions from a variety of disciplines. The important techniques employed to elucidate metabolic pathways are the use of

i. Metabolic inhibitors
ii. Genetic defects (abnormalities)
iii. Mutants
iv. Isotope labeling

7.2.1 Metabolic inhibitors

Metabolic inhibitors were used to elucidate glycolytic pathway - conversion of glucose to ethanol in yeast. The inhibitors block the pathway at specific points thereby causing preceding intermediates to build-up.

Iodoacetate causes accumulation of fructose 1,6-bisphosphate whereas fluoride causes buildup of 3-phophoglycerate and 2-phosphoglycerate. The isolation, identification of these intermediates was vital to the tracing of glycolytic pathway

Glucose

↓

↓

Fructose 1,6-bisphosphate (accumulate)

↓ Iodoacetate

↓

3-Phosphoglycerate

↓

2-Phosphoglycerate (accumulate)

↓ Fluoride

↓

Ethanol

7.2.2 Genetic defects

Archi Garrod invented that human genetic diseases are the result of deficiencies in specific enzymes. This invention also contributed to the enumeration of metabolic sequences. For instance, ingestion of either phenylalanine or tyrosine causes the excretion of homogentisic acid in the urine of certain individuals but not in normal. This disease condition was known as alkaptonuria. Hence, the affected individual lacks an enzyme that catalyses the degradation of homogentisic acid. Another genetic defect, phenylketonuria results in the buildup of phenylpyruvate in the urine. In these individuals, tyrosine was metabolised normally. Hence, the pathway of phenylalanine was tentatively deduced as shown below:

Phenylalanine

Defective in ↓

phenylketonuria

Tyrosine

↓

Homogentisic acid

Defective in ↓

alkaptonuria ↓

$CO_2 + H_2O$

7.2.3 Metabolic blocks (Mutants)

Mutation inactivates or deletes an enzyme in a metabolic pathway. It can be readily generated in rapidly reproducing microorganisms through the use of mutagens. Desired mutants are identified by their requirement of the pathway end product for growth. The pathway of arginine was traced by using mutant of *Neurospora crassa* by Beadle and Tatum.

--------→ Ornithine --------→ citrulline ---------→ arginine

Mutant 1 Mutant 2 Mutant 3

All three mutants grow in arginine but mutant 1 grows in presence of citrulline or ornithine. Mutant 2 grows in citrulline. In mutant 1, an enzyme leading to the formation of ornithine is absent while other enzymes of the pathway are normal.

7.2.4 Isotope labeling studies

The metabolic fate of a specific atom (label) in a metabolite can be elucidated by isotopically labeling that position and following its progress through the metabolic pathway of interest.

Starting material* -----→ Intermediate A*------→ Intermediate B*------→ Products

Isotope tracers are useful in establishing the sequence of metabolic intermediates and the precursor product relationships.

CHAPTER 8

METABOLISM OF CARBOHYDRATE

Lectures 26-28

8.1 Introduction

Carbohydrates are major sources of energy for living organisms. The chief source of carbohydrate in human food is starch, which is the storage form of glucose in plants. Plants may store relatively large amounts of starch within their own cells in time

of abundant supply, to be used later by the plant itself when there is a demand for energy production. Glycogen is the glucose storage polysaccharide of animals. It accounts for upto 10% of the mass of the liver and one percent of the mass of the muscle. Glycogen is larger and highly branched than amylopectin. By the action of several enzymes, such as α-amylase, β-amylase, amylo $\alpha(1\rightarrow6)$ glucosidase and $\propto(1\rightarrow4)$ glucosidase, starch and glycogen from dietary intake are degraded finally to glucose.

Carbohydrate is utilized by cells mainly in the form of glucose. The three principal monosaccharides resulting from the digestive processes are glucose, fructose and galactose. Both fructose and galactose are readily converted to glucose by the liver. Pentose sugars such as xylose, arabinose and ribose may be present in the diet, but their fate after absorption is obscure. Since glucose is the compound formed from starch and glycogen, the carbohydrate metabolism commences with this monosaccharide.

The major metabolic processes in carbohydrates are:

i. Glycolysis:

Glycolysis is the sequence of reactions that convert glucose into pyruvate with the concomitant trapping of the energy as ATP.

ii. The citric acid cycle:

It is the final common oxidative pathway for carbohydrates, fats and proteins. It is also a source of precursors for biosynthesis of various biomolecules. The acetyl CoA that enters in this pathway is completely oxidised to carbon dioxide and water with concomitant production of reducing equivalents, namely NADH and $FADH_2$.

iii. The hexose monophosphate shunt:

It is an alternative pathway to the glycolytic pathway and the citric acid cycle for the oxidation of glucose to carbon dioxide and water with the generation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) molecules and ribose 5-phosphate.

iv. Gluconeogenesis:

It is a biosynthetic pathway that generates glucose from non-carbohydrate precursors.

v. Glycogenesis:

It is a pathway by which glycogen is synthesised from glucose.

vi. Glycogenolysis:

The breakdown of glycogen to glucose is known as glycogenolysis.

An overview of carbohydrate metabolism is shown in figure 10.1.

Figure 10.1

8.2 Glycolysis

Glycolysis, also called as Embden-Meyerhof-Parnas pathway (EMP pathway), consists of a series of reactions through which glucose is converted to pyruvate with the concomitant production of relatively small amounts of adenosine triphosphate (ATP). It is derived from the Greek stem 'glykys' meaning sweet and 'lysis' meaning splitting. It is the primary pathway occurring in the cytoplasm of all the tissues of biological systems. All the enzymes responsible for the catalysis are found in the extra-mitochondrial soluble fraction of the cells (cytoplasm).

In plants, glucose and fructose are the main monosaccharides catabolised by glycolysis although others are also converted into these sugars. Glucose entering the glycolysis is derived from starch or sucrose, and fructose is derived from sucrose. The starch is either from seeds or chloroplasts of matured plants. Glycolysis normally takes place in the presence of O_2 in higher plant cells.

The enzymes in the cytoplasm catalyse the reactions involved in the conversion of glucose to pyruvate (Figure 8.2).

The series of reactions indicated take place in 3 stages.

Stage 1: Conversion of glucose to fructose 1,6-bisphosphate

The formation of fructose 1,6-bisphosphate takes place in three steps catalysed by enzymes as indicated in figure 10.2 and the purpose of these reactions is to form a compound that can be readily cleaved into phosphorylated three carbon units from which, through a series of reactions, ATP is formed.

After the first phosphorylation reaction to form glucose 6-phosphate, isomerisation of glucose 6-phosphate to fructose-6-phosphate occurs which is conversion of an aldose into a ketose. A second phosphorylation reaction follows the isomerization, catalysed by phosphofructokinase resulting in the formation of fructose 1,6-bisphosphate. Phosphofructokinase is the key enzyme in the control of glycolysis.

Stage 2: Conversion of fructose 1,6-bisphosphate to 3-phosphoglycerate.

The splitting of fructose 1,6-bisphosphate occurs in the second stage of glycolysis resulting in the formation of a molecule of glyceraldehyde 3-phosphate and a molecule of dihydroxyacetone phosphate catalysed by aldolase. The dihydroxyacetone phosphate is isomerised to glyceraldehyde 3-phosphate by phosphotriose isomerase. The isomerisation reaction is rapid and reversible. In the next step, glyceraldehyde 3-phosphate is oxidised to 1,3-bisphosphoglycerate catalyzed by glyceraldehyde 3-phosphate dehydrogenase. The product is further converted into 3-phosphoglycerate and a molecule of ATP is formed. The phosphorylation of ADP to ATP is called substrate level phosphorylation since the phosphate group from a substrate molecule is transferred to ADP.

Stage 3: Formation of pyruvate

An intramolecular rearrangement of the phosphoryl group occurs resulting in the formation of 2-phosphoglycerate from 3-phosphoglycerate catalyzed by phosphoglycerate mutase. The 2-phosphoglycerate formed undergoes dehydration forming phosphoenolpyruvate which gives rise to pyruvate and a molecule of ATP

(substrate level phosphorylation). The reaction is irreversible and catalyzed by pyruvate kinase.

The net reaction in the transformation of glucose to pyruvate is

$$\text{Glucose} + 2\,Pi + 2ADP + 2\,NAD^{+} \longrightarrow 2\,\text{pyruvate} + 2\,ATP + 2\,NADH + 2\,H^{+} + H_2O$$

Once pyruvate is formed, further degradation is determined by the presence or absence of oxygen (Figure 8.3).

Under anaerobic conditions, in one of the pathways, pyruvate undergoes reduction yielding lactic acid. The formation of lactic acid is very rare in plants with exception of potato tubers maintained under anaerobic condition and some green algae.

In the second pathway, pyruvate is converted to ethyl alcohol and carbon dioxide. The alcoholic fermentation is the basis of the beer and wine-making industries.

Under aerobic conditions, pyruvate is oxidatively decarboxylated to acetyl CoA which is then completely oxidised to CO_2 and water through the citric acid cycle (See later).

8.2.1 Energetics of glycolysis

From glucose, two molecules of glyceraldehyde 3-phosphate are formed in the second stage of glycolysis from which two molecules of pyruvate are obtained as end products of glycolysis. Hence energetic of glycolysis is calculated by taking into account two molecules of glyceraldehyde 3-phosphate.

Energetics of glycolysis

Stages/steps	Enzyme	Method of high energy bond formation	No. of ATP formed
Stage 1			
Formation of 1,3-bisphospho glycerate from glyceraldehyde 3-phosphate	Glyceraldehyde 3-phosphate dehydrogenase	Respiratory chain oxidation of 2 NADH	5
Stage 2			
Formation of 3 phosphoglycerate from 1,3 bisphospho glycerate	Phosphoglycerate kinase	Phosphorylation at subtrate level	2
Stage 3			
Formation of pyruvate from phosphoenol pyruvate	Pyruvate kinase	Phosphorylation at subrate level	2
Allowance for consumption of ATP by reactions catalysed by hexokinase and phosphofructokinase			2
Number of ATP molecules generated by the catabolism of one molecule of glucose under aerobic conditions			7
Number of ATP molecules generated by the catabolism of one molecule			2

of glucose under anaerobic conditions

8.2.2 Significance of glycolysis

Glycolysis is an almost universal central pathway of glucose catabolism occurring in the cytoplasm of all the tissues of biological systems leading to generation of energy in the form of ATP for vital activities. It is the pathway through which the largest flux of carbon occurs in most cells. Some plant tissues which are modified for the storage of starch such as potato tubers and some plants adapted to growth in inundated water such as water cress derive most of their energy from glycolysis. In plants, glycolysis is the key metabolic component of the respiratory process, which generates energy in the form of ATP in cells where photosynthesis is not taking place. Many types of anaerobic microorganisms are entirely dependent on glycolysis. Mammalian tissues such as renal medulla and brain solely dependent on glycolysis for major sources of metabolic energy.

8.3. The tricarboxylic acid cycle

In 1937, Sir Hans Krebs, an English biochemist proposed a pathway consisting of a cycle of reactions through which acetyl CoA is converted to carbon dioxide and water and hence the cycle was named as Kreb's cycle. All the enzymes catalyzing the reactions of this cycle occur inside mitochondria (mitochondrial matrix) in contrast with those of glycolysis, which occur in the cytosol (Figure 2.2).

Before pyruvate can enter the citric acid cycle, it must be oxidatively decarboxylated to acetyl CoA (active acetate). Three different enzymes working sequentially in a multienzyme complex catalyse this reaction. This formation of acetyl CoA from pyruvate by alpha-oxidative decarboxylation occurs in the mitochondrion following the formation of pyruvate in the cytosol during glycolysis. The reaction involves six cofactors: coenzyme A, NAD^+, lipoic acid, FAD, thiamine pyrophosphate (TPP) and Mg^{2+}.

$$CH_3\text{-}CO\text{-}COOH + CoASH + NAD^+ \xrightarrow[\text{Lipoate, } Mg^2]{\text{TPP, FAD}} CH_3\text{-}CO\text{-}S\text{-}CoA + NADH + H^+ + CO_2$$

8.3.1 Reactions of the TCA cycle

Acetyl CoA, derived mainly from the oxidation of carbohydrates, lipids and proteins, combines with oxaloacetate to form citrate which is the first reaction of the citric acid cycle. Subsequently, citrate is oxidised in a series of reactions liberating carbon dioxide and reducing equivalents (NADH, $FADH_2$). The oxaloacetate is regenerated and functions therefore in a catalytic manner in the oxidation of acetyl CoA to two molecules of carbon dioxide (Figure 8.4).

Figure 8.4

The citric acid cycle has eight steps as described below:

i. Formation of citrate

The first step is the reaction between the four-carbon unit, oxaloacetate and the two-carbon unit, acetyl CoA resulting in the formation of citrate and coenzyme A catalysed by citrate synthase. The coenzyme A formed in this reaction is recycled.

ii. Formation of isocitrate via cis-aconitate

The isomerization of citrate to isocitrate catalysed by aconitase occurs in two steps with the formation of cis-aconitate as an intermediate. This formation of isocitrate involves both dehydration and hydration. The result is an interchange of hydrogen and a hydroxyl group. In this reaction, fluoroacetate acts as an inhibitor to the enzyme, aconitase.

iii. Oxidation of isocitrate to α-ketoglutarate

The enzyme, isocitrate dehydrogenase oxidatively decarboxylates isocitrate to α-ketoglutarate with simultaneous liberation of carbon dioxide. The intermediate in this reaction is oxalosuccinate, an unstable β-ketoacid. While bound to the enzyme, it loses carbon dioxide to form α-ketoglutarate. There are two different forms of isocitrate dehydrogenase (isozymes), one requiring NAD^+ and other requiring $NADP^+$.

iv. Oxidation of α-ketoglutarate to succinyl CoA

α-Ketoglutarate, undergoes oxidative decarboxylation forming succinyl-CoA and carbon dioxide in the presence of α-ketoglutarate dehydrogenase complex, an assembly consisting of three kinds of enzymes. The mechanism of this reaction is very similar to the reaction catalyzed by pyruvate dehydrogenase complex. This reaction is irreversible. Arsenite acts as an inhibitor of TCA cycle by inhibiting the action of α-ketoglutarate dehydrogenase complex.

v. Conversion of succinyl CoA to succinate

Succinate is formed in a reversible reaction from succinyl CoA catalysed by the enzyme, succinyl CoA synthetase or succinate thiokinase with the simultaneous formation of GTP and coenzyme A. Succinate thiokinase utilises GDP in animal tissues whereas it uses ADP predominantly in plants and bacteria. The formation of GTP in this reaction is a substrate level phosphorylation reaction.

vi. Formation of fumarate by oxidation of succinate

The succinate formed from succinyl CoA is oxidised to fumarate by succinate dehydrogenase with the participation of FAD. Malonate, an analogue of succinate being a strong competitive inhibitor of succinate dehydrogenase, blocks the citric acid cycle.

vii. Formation of malate by hydration of fumarate

The reversible hydration of fumarate to L-malate is catalysed by fumarase.

viii. Oxidation of malate to oxaloacetate

This reaction forms the last reaction of the citric acid cycle. NAD-linked malate dehydrogenase catalyses the oxidation of L-malate to oxaloacetate.

8.3.1 Energetics of tricarboxylic acid cycle

From one molecule of glucose, two molecules of pyruvate are formed which in turn give rise to two molecules of acetyl CoA. When two molecules of acetyl-CoA undergo oxidation through TCA cycle, the following number of high-energy bonds (ATPs) are produced.

Reaction	Enzyme which catalyses the reaction	Mode of ATP production	No. of ATP formed
i. Pyruvate to acetyl CoA	Pyruvate dehydro-genase complex	Respiratory chain oxidation of 2 NADH	5
ii. Citric acid cycle			
Isocitrate to α-ketoglutarate	Isocitrate dehydrogenase	Respiratory chain oxidation of 2 NADH	5
α- Ketoglutarate to succinyl CoA	α-Ketoglutarate dehydrogenase complex	Respiratory chain oxidation of 2 NADH	5
Succinyl CoA to succinate	Succinate thiokinase	Phosphorylation at substrate level	2
Succinate to fumarate	Succinate dehydro-genase	Respiratory chain oxidation of 2 $FADH_2$	3
Malate to oxaloacetate	Malate dehydro-genase	Respiratory chain oxidation of 2 NADH	5
iii. Glycolytic pathway (Refer above)			7
Total number of high energy phosphate bonds formed per molecule of glucose under aerobic conditions			32

8.3.3 Significance of the TCA cycle

i) The major significance of the citric acid cycle is to act as the final common pathway for the oxidation of carbohydrates, lipids and proteins, since glucose, fatty acids and many amino acids are all metabolised to acetyl CoA.

ii) This cycle serves as the mechanism by which much of the free energy liberated during the oxidation of carbohydrate, lipids and amino acids is made available.

iii) TCA cycle is of further significance since it has dual or amphibolic role thus providing precursor compounds for biosynthesis of other biomolecules (amino acids, fatty acids, and glucose.

8.4 Electron transport chain and oxidative phosphorylation

The mitochondrion is the aerobic organelle in which the final stage of the oxidation of food occurs. It is the site of the citric acid cycle, fatty acid oxidation and oxidative phosphorylation, processes that are responsible for the formation of ATP under aerobic condition. The two most important energy transductions in the biological systems are the oxidative phosphorylation (ATP synthesis driven by electron transfer to oxygen) and photophosphorylation (ATP synthesis driven by light).

Oxidative phosphorylation is the process in which ATP molecules are formed as a result of the transfer of electrons from the reducing equivalents, NADH or $FADH_2$ (produced by glycolysis, the citric acid cycle and fatty acid oxidation) to oxygen by a series of electron carriers in the form of a chain located in the inner membrane of mitochondria. This is the final reaction sequence of respiration. Since the electrons are transferred by a series of electron carriers in the form of a chain, it is known as electron transport chain (ETC). In plants, ATP is mainly derived through photosynthesis utilizing the energy derived from the sun. In non-photosynthetic tissues, ATPs are derived through respiration.

The electrons are transferred along a set of cytochromes in the form of a chain in steps from the more electronegative components ($NADH/FADH_2$) to the more electropositive oxygen (Figure 8.7).

The respiratory chain consists of a number of protein complexes that are remarkably complicated in nature. They are known as NADH- ubiquinone reductase, succinate-ubiquinone reductase, ubiquinone-cytochrome c reductase and cytochrome c oxidase These complexes are also called as NADH dehydrogenase, succinate dehydrogenase, cytochrome b-c complex and cytochrome c oxidase respectively or as complexes I - IV.

All the three reductases are also known as iron-sulphur proteins since they contain Fe-S centres as their critical components. Iron in these enzyme complexes can exist in two forms as Fe^{2+} and Fe^{3+}. Each cytochrome in its oxidised form (Fe^{3+}) accepts one electron and becomes reduced to Fe^{2+} form. Fe^{2+} donates electron to the next carrier.

The oxidation-reduction reactions involve the loss of electrons by one chemical species and the gain by another. The flow of electrons in oxidation-reduction reactions is responsible for all of the work done by living organisms. Electrons move from various metabolic intermediates to specialised electron carriers in enzyme-catalysed reactions. Those carriers in turn donate electrons to acceptors with higher electron affinities with the release of energy. The electron-donating molecule in an oxidation-reduction reaction is called the reducing agent or reductant; the electron-accepting molecule is the oxidising agent or oxidant. A given agent, such as iron cation in the ferrous (Fe^{2+}) and the ferric (Fe^{3+}) state, functions as a conjugate reductant-oxidant pair (redox pair). The transfer of electrons occurs depending upon the relative affinity of the electron acceptor of each redox pair for electrons. The standard reduction potential Eo is a measure of this affinity.

e.g. the standard reduction potential of $NAD^+ + 2H^+ + 2e^-$ -----> NADH+H+ is -0.320 and that of coenzyme Q + 2e- ------> Q H_2 is + 0.045. The electron flows from NADH to CoQ. The free energy change for the oxidation-reduction reaction is 70.4 kJ.mole ($\Delta G^o = -nF\Delta E_o$ where n is number of electrons, F is Faraday's constant, 96.48 kJ/V.mol; i.e -2 (96.5 kJ/V.mol) 0.365 = 70.4 kJ.mol). During the transport of electrons to O_2 along the carrier, ATP molecules are formed.

Not all the substrates are linked to the respiratory chain through NAD-specific dehydrogenases. Substrate like succinate, because of their more positive redox potential, is linked directly to flavoprotein dehydrogenases, which in turn are linked to the cytochromes of the respiratory chain.

Oxidation of one molecule of NADH results in generation of 2.5 molecules of ATP whereas oxidation of one molecule of $FADH_2$ generates 1.5 molecules of ATP. The sites of ATP formation are indicated in figure 10.7.

10.5.1. Sites of ATP formation

When electrons are transported along the respiratory chain, due to high amount of energy released, ATP molecules are synthesised at the following three sites.

i) transfer of electrons from NADH to ubiquinone via flavoprotein (FMN).
ii) transfer of electrons from cyt b to cyt c.
iii) transfer of electrons from cyt a to cyt a_3.

10.5.2. Mechanism of ATP formation

Two principal hypotheses have been proposed for the mechanism of oxidative phosphorylation.

i. Chemical hypothesis
ii. Chemiosmotic theory

Chemical hypothesis

Many attempts have been made since 1920 to identify an energy-rich metabolite linking oxidation and phosphorylation. No such intermediates was isolated and in 1960, Peter Mitchell suggested that no possibility of existence of such an intermediate compound. So, the chemical hypothesis has become discredited.

Chemiosmotic theory

The chemiosmotic theory states that the coupling of oxidation to phosphorylation is indirect. According to this, the hydrogen ions (protons) generated by the oxidation of components in the respiratory chain are ejected to the outside (matrix) of the inner membrane. The electrochemical potential difference resulting from the asymmetric distribution of the hydrogen ions (protons or H^+) is used to drive a membrane-located ATP synthase which in the presence of Pi + ADP forms ATP.

Inhibitors of respiratory chain

Inhibitors, which inhibit respiratory chain, may be grouped as follows:

i. Inhibitors of electron transfer

ii. Inhibitors of ATP synthase

iii. Uncouplers of oxidative phosphorylation

Inhibitors that arrest respiration by blocking the respiratory chain act at three sites.

Compounds such as barbiturates, amytal, rotenone prevent the transfer of electron from FeS centre to ubiquinone. Carboxin specifically inhibits transfer of reducing equivalents from succinate dehydrogenase to ubiquinone.

Antimycin A blocks electron transfer from cytochrome b to cytochrome c_1.

Substances such as cyanide (CN^-), azide (N_3^-) and carbon monoxide inhibit cytochrome c oxidase by binding to heme group and are extremely poisonous. Oligomycin inhibits ATP synthase.

In the presence of the uncouplers such as dicoumarol and 2,4-dinitrophenol, oxidation proceeds without phosphorylation (dissociation of oxidation in the respiratory

chain from phosphorylation) releasing energy in the form of heat rather than in the form of ATP.

Summary

The major metabolic processes in carbohydrates are glycolysis, citric acid cycle, hexose monophosphate shunt and gluconeogenesis. Glycolysis, also called as Embden -Meyerhof-Parnas pathway consists of a series of reactions through which glucose is converted to two molecules of pyruvate involving three stages. In the first stage glucose is converted to fructose 1,6-bisphosphate and two ATP molecules are utilised in this stage. During the second stage, fructose 1,6 bisphosphate is converted to three molecules of phosphoglycerate. During this stage one molecule of ATP is synthesised by substrate level phosphorylation. In the final stage, 3-phosphoglycerate is converted to pyruvate. During this stage also, one more molecule of ATP is formed by substrate level phosphorylation. Under anaerobic conditions, pyruvate is reduced to lactic acid or ethyl alcohol. Under aerobic conditions, pyruvate is oxidatively decarboxylated to acetyl Co A which is then oxidised to CO_2 and H_20 through citric acid cycle. The number of net ATP molecules produced under anaerobic condition from one molecule of glucose is two whereas seven net ATP molecules are produced under aerobic conditions via glycolytic reactions.

The major significance of the citric aid cycle is to act as the final common pathway for the oxidation of carbohydrates, lipids and proteins. TCA cycle also serves as an amphibolic pathway. It functions not only as an oxidative pathway but also provides precursor compounds for the biosynthesis of other biomolecules.

Plants employ a modification of the TCA cycle called the glyoxalate cycle to produce four carbon dicarboxylic acids and bypass the oxidative decaboxylation reaction of the TCA

cycle. Isocitrate lyase and malate synthase are two additional enzymes required for this cycle, which are located in glyoxysome.

The final stage of oxidation of biomolecules occurs at the mitochondria through electron transport chain. Oxidative phosphorylation is the process in which ATP molecules are formed as a result of transfer of electrons from NADH or $FADH_2$ to molecular oxygen.. Oxidation of one molecule of NADH results in generation of 2.5 molecules of ATP while $FADH_2$ generates 1.5 molecules of ATP.

Review questions

A. Multiple choice questions

1. Which one of the following pathway produces NADPH?
   a. Glycolysis b. TCA cycle c. Glyoxalate cycle d. HMP shunt
2. ATP molecule formed during the conversion of phosphoenol pyruvate to pyruvate is through
   a. Oxidative phosphorylation b. Photophosphorylation
   c. Substrate level phosphorylation d. Product phosphorylation
3. The reaction, which is irreversible in TCA cycle, is
   a. Isocitrate to α-ketoglutarate b. α- Ketoglutarate to succinyl CoA
   c. Succinyl CoA to succinate d. Malate to oxaloacetate
4. Glyoxysomes contain the enzyme
   a. Isocitrate lyase b. Succinate dehydrogenase
   c. Fumarase d. Malate dehydrogenase
5. Which one of the following compounds is uncoupler of oxidative phosphorylation
   a. 2,4- dinitrophenol b. Cyanide c. Rotenone d. Antimycin A

B. Fill up the blanks

6. During conversion of pyruvic acid to acetaldehyde, one carbon is removed as ---------
-
7. The coenzyme attached to succinate dehydrogenase is ----------.
8. Biosynthesis of glucose from non-carbohydrate precursors is known as ----------
.
9. The breakdown of glycogen to glucose is called as ----------.
10. NADPH is produced in ---------- pathway of glucose catabolism.

C. Write short answers for the following

11. Define the following
    a. Gluconeogenesis b. Oxidative phosphorylation c. Substrate level phosphorylation d. Uncouplers e. Glycolysis f. Glycogenolysis.
12. How is glycolytic pathway different from HMP shunt?
13. Write the other names of the TCA cycle and why is it named so?
14. What is the significance of glycolysis?
15. What are the irreversible reactions that occur when glucose is convertd into CO_2 and water?

D. Write in detail on the following.

16. Enumerate the reactions of glycolytic pathway indicating the enzymes and cofactors.
17. How is pyruvic acid oxidatively decarboxylated and get oxidised in TCA cycle? Indicate the enzymes and cofactors at appropriate places.
18. Write the components of electron transport chain and indicate the flow of electrons and the site of ATP formation.
19. What is the importance of glyoxylate cycle in plants? Sketch the reactions of

the cycle.

20. Name the inhibitors of ETC and their mode of action.
21. Explain the mechanism of ATP formation.
22. Enumerate the reactions of HMP shunt. What are the functions of this pathway?

E. Solve the following problems.

23. Study the glycolytic pathway and answer the following
    a. Which reactions are energy consuming?
    b. Which reactions are energy yielding?
    c. Why few reactions are not reversible while others are reversible?
24. Prepare a cell free extract and dialyse it. If you add glucose to the dialysed extract, does glycolysis occur? What are the components you have to add to restore it? If you heat the original extract, what will be its effect on glycolytic reactions?
25. During glycolysis, one molecule of glucose is converted into two molecules of pyruvic acid. Which carbon number of glucose form methyl, carbonyl and carboxylic carbon of pyruvic acid?
26. What would be the effect of vitamin B-complex deficiency on carbohydrate metabolism? Which one of the TCA-cycle reaction is badly affected? Why?
27. Arrange the following molecules on the basis of their energy content
    a. $FADH_2$ b. NADH c. Ascorbate d. Succinate.
28. Calculate the number of ATP molecules when one molecule of glucose undergo catabolic reactions via
    a. Anaerobic condition b. Aerobic condition
    c. Alcoholic fermentation d. Pentose phosphate pathway

F. Gain additional knowledge by further reading

29. ATP synthase
30. Mechanism of ATP synthesis

31. Regulation of TCA cycle
32. Pyruvate dehydrogenase multienzyme complex.
33. Malting

CHAPTER 9

METABOLISM OF LIPIDS

Lectures29 &30

9.1 Introduction

Lipids constitute one of the four major classes of compounds that are found in living systems. The lipids of metabolic significance include triacylglycerol, phospholipids and the products of lipid metabolism such as free fatty acids and glycerol. An overview of lipid metabolism is shown in figure 9.1.

9.2 Lipolysis

9.2.1. Lipases

Triacylglycerols or triglycerides undergo hydrolysis by lipases to form glycerol and fatty acids, which undergo further oxidation generating energy. Lipases have been reported to be present in dry seeds of some species, e.g. castor bean, Scots pine and Douglas fir but at a low level, or absent in others e.g. apple. In most cases of seeds, following imbibitions, there appears to be a rise in lipase activity but whether this increase is due to the *de novo* synthesis of the enzyme or activation of existing lipases has not been determined. A decline in lipase activity is always associated with decline in acylglycerol reserves. In castor bean, as in many other fat-storing seeds, free fatty acids do not accumulate, but are rapidly degraded and converted to carbohydrate within the endosperm. In other seeds such as germinating seeds of oil palm (*Elaeis guineensis*), a different pattern of fat mobilization can be observed. The products of

lipid catabolism are transported via specialized structures called haustorium through its vascular system. Lipases are generally non-specific and can hydrolyse a wide variety of triacylglycerols (Figure 12.2). They initiate digestion by hydrolyzing triacylglycerols to form free fatty acids and 1,2-diacylglycerols. Complete hydrolysis of triacylglycerols produces glycerol and fatty acids. Lipase hydrolyses easily the terminal fatty acids to produce 2-monoacyl glycerol as major end product and it can digest the secondary linkage only with difficulty. In plants, 99% of total lipase activity is associated with the acid lipase (pH 4.6). The precise location of the acid lipase is still undetermined but it could be associated with the glyoxysomes. In castor bean, within two days of the start of imbibitions, the acid lipase of pH 5.0 is activated to the peak level followed by decline in activity on the 4th day by which time the alkaline lipase of pH 9.0 shows maximal activity. The acid lipase is associated with oil bodies and capable of hydrolysing tri-, di- and mono acylglycerols whereas the alkaline lipase is specific only for monoacylglycerols.

9.2.2. Phospholipases

Phospholipases are the hydrolytic enzymes acting on phospholipids and splitting into different products. There are four types of phospholipases known as phospholipase A_1, phospholipase A_2 or B_1, phospholipase C and phospholipase D. The action of phospholipases on phospholipids is shown in figure 9.3.

Phospholipase A

Phospholipase A is present in large amounts in snake venom and human pancreas. It is also designated as phospholipase A_1. It catalyses the hydrolysis of the fatty acids in the 2 or β-position of the phospholipids. Though this enzyme attacks on glycerophosphatides, it is fairly specific for phosphatidyl choline (lecithin). The enzyme is relatively stable to heat (below pH 7.0). The product of the hydrolysis, a lysolecithin, (monoacylphosphoryl choline) has a powerful hemolytic activity.

Phospholipase B (A_2)

It is otherwise termed as lysophospholipase and widely distributed in nature often in association with phospholipase A. Phospholipase B is also designated as phospholipase A_2 since it acts on the lysolecithin (the product obtained from phospholipid by the action of phospholipase A_1). The action of this enzyme following that of phospholipase A yields glycerophosphorylcholine as the final product.

Phospholipase C

Phospholipase C is mostly found in the plant kingdom but it may also be present in some animal tissues and venoms. It catalyses the liberation of a 1,2-diacylglycerol and phosphorylcholine from phosphatidylcholine. Phosphorylcholine is also liberated from sphingomyelin by this enzyme.

Phospholipase D

Phospholipase D, an enzyme described mainly in plants catalyses the hydrolysis of choline from phosphatidylcholine leaving phosphatidic acid.

9.4 Oxidation of fatty acids

Fatty acids obtained by hydrolysis of fats undergo different oxidative pathways designated as alpha (α), beta (β) and omega (ω) pathways.

9.4.1 α-oxidation

α-Oxidation of fatty acids has been found in certain tissues especially in brain tissue of mammals and plant systems. It does not require CoA intermediates and no high-energy phosphates are generated. This type of oxidation results in the removal of one carbon at a time from the carboxyl end of the fatty acid. The physiological role of α-oxidation in plants is not yet fully established but it has been suggested that it may be involved in the degradation of long chain fatty acids as observed in many animal

tissues. α-Oxidation is clearly the main source of the odd-carbon fatty acids and their derivatives that occur in some plant lipids. In this process, sequential removal of one carbon at a time from free fatty acids of chain length ranging from C_{13} to C_{18} occur.

9.4.2.ω-Oxidation

ω-Oxidation is normally a very minor pathway brought about by hydroxylase enzymes involving cytochrome P-450 in the endoplasmic reticulum. Fatty acids with oxygen function (alcoholic or carboxyl) at the methyl terminal end (ω-end) are formed by ω-oxidation and frequently occur as constituents of cutin and suberin. The requirements for the oxygenase-mediated conversion of a ω-methyl fatty acyl CoA into a ω-hydroxymethyl fatty acyl CoA are molecular oxygen, reduced pyridine nucleotide and a non-heme iron protein in higher plants.

9.4.3. β-Oxidation of fatty acids

In 1904, Franz Knoop made a critical contribution to the elucidation of the mechanism of fatty acid oxidation and demonstrated that most of the fatty acids are degraded by oxidation at the β-carbon. β-Oxidation of fatty acids takes place in mitochondria. Fatty acids are activated before they enter into mitochondria for oxidation.

Activation of fatty acids

Fatty acids are converted into active intermediate in a reaction with ATP and coenzyme A. A thioester linkage between the carboxyl group of a fatty acid and the sulfhydryl group of coenzyme A is formed with the hydrolysis of ATP. This activation reaction takes place on the outer mitochondrial membrane catalysed by acyl CoA synthetase. Several acyl CoA synthetases each specific for fatty acids of different chain length are present in the membrane of mitochondria (Figure 9.4).

Penetration of long chain fatty acids into mitochondria

Long chain acyl-CoA molecules do not readily get into the inner mitochondrial membrane and are carried across the inner membrane by conjugating with carnitine (β-hydroxy γ-trimethyl ammonium butyrate), a zwitterionic compound formed from lysine. Activation of lower fatty acids and their oxidation within the mitochondria occur independently of carnitine, but long-chain acyl CoA will become oxidised unless they form acylcarnitines. The acyl CoA combines with carnitine in the presence of carnitine acyltransferase I, which is bound to the outer mitochondrial membrane. Acylcarnitine is transported in, coupled with the transport out of one molecule of carnitine. The acylcarnitine then reacts with coenzyme A catalyzed by carnitine palmitoyl transferase II, located on the inside of the inner membrane. Acyl CoA is reformed in the mitochondrial matrix and carnitine is liberated (Figure 9.5).

Oxidation

A saturated acyl CoA is oxidised by a recurring sequence of four reactions: oxidation in presence of FAD, hydration, oxidation in presence of NAD^+, and thiolysis by CoASH. In β-oxidation, 2 carbons are cleaved at a time from acyl CoA molecules, starting from the carboxyl end. The chain is broken between the α-and β-carbon atoms. The two-carbon units formed are acetyl CoA (Figure 9.6).

i) The first reaction in β-oxidation of acyl CoA is the formation of *trans* Δ^2-enoyl CoA or α, β-unsaturated acyl CoA in presence of acyl-CoA dehydrogenase and the coenzyme, FAD.

ii) The next step is the hydration of the double bond between C-2 and C-3 by enoyl CoA hydratase with the formation of β-hydroxy acyl CoA.

iii) In the third step, the β-hydroxy acyl CoA is dehydrogenated in the presence of β-hydroxy acyl CoA dehydrogenase and NAD^+ forming β-ketoacyl CoA.

iv) In the last step of β-oxidation, β-ketoacyl CoA reacts with coenzyme A in the presence of the enzyme, thiolase. The products of this reaction are acetyl CoA and an acyl CoA containing two carbons less than the original acyl CoA molecule that underwent oxidation.

By the above steps of β-oxidation fatty acids are completely degraded to acetyl CoA units. The acetyl CoA formed from fatty acids can be oxidised to carbon dioxide and water via citric acid cycle.

Energetics of β oxidation

The energetics or the energy conserved in terms of ATP by oxidation of a molecule of palmitic acid is given below:

Palmitic acid (16 carbons) undergoes β-oxidation forming eight molecules of acetyl CoA by undergoing seven β-oxidation spirals. When one cycle of β-oxidation takes place, one molecule of $FADH_2$, one molecule of NADH and one molecule of acetyl CoA are produced. Electrons from these reducing equivalents ($FADH_2$ and NADH) are transported through the respiratory chain in mitochondria with simultaneous regeneration of high-energy phosphate bonds. Mitochondrial oxidation of $FADH_2$ eventually results in the net formation of about 1.5 ATP. Likewise, oxidation of electrons from NADH yields 2.5 molecules of ATP. Hence, a total of four ATP molecules are formed per cycle and ten molecules of ATP are formed through Krebs's cycle from each molecule of acetyl CoA.

8 Acetyl CoA through TCA cycle yield (8x10) = 80 ATP

7 β-oxidation spiral reactions yield (7x4) = 28 ATP

Total 108 ATP

ATP utilized in the initial step = 2 ATP

Hence, complete oxidation of palmitic acid yields 106 ATP.

9.5.Biosynthesis of fatty acids

For years, it was thought that fatty acid biosynthesis occurred by reversal of the β-oxidation pathway. On the contrary, it occurs by a separate pathway that differs from β-oxidation in several ways.

i. Synthesis takes place in the cytosol, in contrast with degradation or oxidation, which occurs in the mitochondrial matrix.

ii. Intermediates in fatty acid synthesis are covalently linked to the sulfhydryl group of an acyl carrier protein (ACP) whereas intermediates in fatty acid breakdown are bonded to coenzyme A.

iii. The enzymes of fatty acid synthesis in animals are joined in a single polypeptide chain called fatty acid synthase. In contrast, the degradative enzymes do not seem to be associated. Plants employ separate enzymes to carry out the biosynthetic reactions.

iv. The reductant in fatty acid synthesis is NADPH, whereas the oxidants in fatty acid oxidation are NAD^+ and FAD.

Elongation by the fatty acid synthase complex stops upon formation of palmitate (16 C). Further elongation and the formation of double bonds are carried out by other enzyme systems. The following seven steps are involved in fatty acid biosynthesis (Figure 9.9).

Formation of malonyl CoA

The synthesis of malonyl CoA from acetyl CoA is catalyzed by acetyl CoA carboxylase having biotin as prosthetic group. The production of malonyl CoA is the initial and controlling step in fatty acid synthesis. In this reaction, bicarbonate serves as a source of CO_2. The reaction takes place in two steps, namely carboxylation of biotin involving ATP and transfer of the carboxyl group to acetyl CoA resulting in malonyl CoA.

Biotin - enzyme + ATP + HCO^-_3 $\dashrightarrow$ CO_2 - biotin - enzyme + ADP + Pi

CO_2 - biotin - enzyme + acetyl CoA $\dashrightarrow$ malonyl CoA + biotin - enzyme

Acetyl CoA carboxylase plays a key role in regulating fatty acid metabolism and the same is inactivated by phosphorylation.

ii) Formation acetyl and malonyl ACP

Acetyl transacylase and malonyl transacylase catalyze the formation of acetyl ACP and malonyl ACP respectively. Acetyl transacylase can transfer acetyl as well acyl groups whereas malonyl transacylase is highly specific.

$$\text{Acetyl CoA + ACP} \xrightarrow[\text{transacylase}]{} \text{acetyl - ACP + COASH}$$

$$\text{Malonyl CoA + ACP} \xrightarrow[\text{transacylase}]{} \text{Malonyl - ACP + COASH}$$

iii) Formation of acetoacetyl - ACP (β-ketoacyl ACP)

Acetyl ACP condenses with malonyl ACP to form acetoacetyl ACP. Carbondioxide is eliminated from malonyl ACP.

iv) Reduction of β-ketoacyl ACP to β-hydroxyl acyl ACP. The β- keto group in acetoacetyl ACP is reduced by NADPH- dependent β-ketoacyl reductase.

v) Formation of unsaturated acyl ACP.

The β-hydroxyl group combines with the hydrogen atom attached to the γ-carbon and a water molecule is removed to form α, β-unsaturated acyl ACP.

vi) Formation of Acyl ACP

The unsaturated acyl ACP is converted in the next step to a saturated acyl ACP by the enzyme α,β-unsaturated acyl ACP reductase using NADPH as the coenzyme. The resultant product contains two carbon atoms more than the starting material. Addition of subsequent acetyl units through malonyl ACP leads to the formation of 16-carbon palmitate.

Stoichiometry of fatty acid synthesis

The stoichiometry of the synthesis of palmitate is given below:

$$\text{Acetyl CoA} + 7 \text{ malonyl CoA} + 14 \text{ NADPH} + 20\ H^{+} \text{ ----------}\rightarrow$$

$$\text{Palmitate} + 7\ CO_2 + 14\ NADP^{+} + 8\ \text{CoASH} + 6\ H_2O$$

The equation for the synthesis of the malonyl CoA used in the above reaction is

$$7 \text{ Acetyl CoA} + 7\ CO_2 + 7 \text{ ATP } \text{---------}\rightarrow 7 \text{ malonyl CoA} + 7\text{ADP} + 7 \text{ Pi} + 14\ H^{+}$$

The overall stoichiometry for the synthesis of palmitate is

$$8 \text{ Acetyl CoA} + 7 \text{ ATP} + 14 \text{ NADPH} + 6H^{+} \text{ --------}\rightarrow \text{Palmitate} + 14 \text{ NADP} + 8 \text{ CoASH} + 6\ H_2O + 7 \text{ ADP} + 7 \text{ Pi}$$

Fatty acid synthesis and degradation are reciprocally regulated so that both are not simultaneously active.

9.6. Biosynthesis of triacylglycerols

Triacylglycerols are not synthesised by reversal of lipolysis. They are synthesisd by a different mechanism in which both glycerol and fatty acids are activated by ATP before they are incorporated into acylglycerols (Figure 9.11).

i) Activation of glycerol

Glycerol kinase catalyses the activation of glycerol to glycerol 3-phosphate. If glycerol kinase is found in low quantity or absent, glycerol 3-phosphate will be formed from dihydroxyacetone phosphate obtained from glycolysis and this reaction is catalysed by the enzyme glycerol 3-phosphate dehydrogenase.

ii) Activation of fatty acids

Fatty acids are activated to acyl CoA by the enzyme acyl CoA synthetase, utilizing ATP and CoASH. Two molecules of acyl CoA combine with glycerol 3-phosphate to form 1,2-diacylglycerol phosphate. Formation of 1,2-diacyl glycerol

phosphate takes place in two stages, catalysed by glycerol 3-phosphate acyl transferase and then by 1-acyl glycerol 3- phosphate acyl transferase. The phosphate group is removed from 1,2-diacyl glycerol phosphate by phosphatidate phosphatase to form 1,2-diacyl glycerol. Triacylglycerols are finally formed by esterification of one or more molecule of acyl CoA with the diacylglycerol.

Alternative pathway for triacylglycerol biosynthesis

In this pathway, dihydroxyacetone phosphate from glycolysis is reduced by NADPH, acylated and converted to lysophosphatidate. This pathway accounts for less than 10% of total triacylglycerol synthesis.

Summary

Triacylglycerols are the highly concentrated form of energy stored in seeds. They provide up to six times the metabolic energy of an equal weight of hydrated glycogen. Triaylglycerols are hydrolysed by lipases to glycerol and fatty acids, which then undergo further oxidation generating energy.

Phospholipases are the hydrolytic enzymes acting on phospholipids and splitting into different products. There are four types of phospholipases. Phospholipase A catalyses the hydrolysis of the ester bonds and releases the fatty acids in the 2 or β-position of the phospholipids. This enzyme is fairly specific to lecithin. Phopholipase C catalyses the liberation of a 1,2-diacyl glycerol and phosphoryl choline from a phosphatidyl choline. Phopholipase D catalyses the removal of choline from phosphatidyl choline.

Fatty acids obtained by hydrolysis undergo different oxidative pathways called as α, β and ω. Most of the fatty acids are oxidised through β oxidation pathway, which takes place in mitochondria. Before fatty acids are oxidized, they are converted to the acyl CoA derivatives by acyl CoA synthase in an ATP requiring process, transported into mitochondria as carnitine esters, reconverted inside the mitochondrial matrix to acyl CoA. β-Oxidation of fatty acyl CoA

occurs in 2-carbon units so as to convert even-chain fatty acyl CoAs completely to acetyl CoA. The pathway involves FAD-dependent dehydrogenation of all alkyl group, hydration of the resulting double bond, oxidation of this alcohol to ketone, and C-C bond cleavage to form acetyl CoA and a new fatty acyl CoA with two carbon atoms less than the parent compound. Complete oxidation of the acyl CoA is achieved through the citric acid cycle.

α Oxidation does not require CoA intermediates and no high-energy phosphates are generated. This type of oxidation results in the removal of one carbon at a time from the carboxyl end of the fatty acid.

ω-Oxidation is a very minor pathway. Fatty acids with oxygen function at the methyl terminal end are formed by ω-oxidation. Oxidation of unsaturated fatty acids follows many reactions of fatty acids but require the participation of additional enzymes, an isomerase and a novel reductase.

Fatty acid biosynthesis differs from fatty acid oxidation in several aspects. Synthesis takes place in cytosol. The intermediates in fatty acid synthesis are covalently linked to acyl-carrier protein (ACP). Malonyl CoA is the precursor in biosynthesis. The pathway involves formation of acetyl and malonyl ACP. Palmitate is the primary product of fatty acid biosynthesis. Longer chain fatty acids and unsaturated fatty acid biosynthesis from palmitate occur by elongation and desaturation reaction. The unsaturated fatty acid linoleate and α-linolenate cannot be synthesised by mammals, but plants can synthesise both.

Triacylglycerols are synthesized from both glycerol and fatty acid, which are activated by ATP before they are incorporated into acylglycerol. An alternate pathway for the synthesis of triacyl glycerol also exists but this pathway accounts for less than 10% of total triacylglycerol synthesis.

Review questions

A. Multiple choice questions

1. The quantum of energy produced when the same weight of lipid and hydrated glycogen oxidised is

   a. Equal b. Six times higher for lipid c. Six times higher for hydrated glycogen d. Two times higher for lipid.

2. The enzyme which catalyzes the hydrolysis of the fatty acids in the 2 or β-position of phospholipids is

   a. Phospholipase A b. Phospholipase B c. Phospholipase C d. Phospholipase D.

3. The fatty acid oxidation pathway in which oxidation results in the removal of one carbon at a time is

   a. β-oxidation b. ω-oxidation c. α-oxidation d. None of the above

4. Triacylglycerols are synthesized through

   a. Reversal of lipolysis b. Reversal of β-oxidation pathway c. Glycerol and fatty acid activation d. ω- oxidation.

5. Fatty acid with 18-carbon atoms are synthesized with the help of

   a. Fatty acid synthase complex b. Elongase c. Fatty acid synthase complex plus elongase

   d. Desaturase.

B. Fill up the blanks

6. Triacylglycerols undergo hydrolysis by the enzyme----------to form glycerol and fatty acids
7. Lysolecithin is produced by the action of the enzyme---------- on phospholipid.

8. Fatty acids are transported across the inner mitochondrial membrane with the help of ----------
.
9. Oxidation of fatty acids occur in ----------.
10. The reductant in fatty acid synthesis is ----------.

C. Write short answers for the following

11. Define the following
    a. Lipases b. Phospholipases c. α-Oxidation d. ACP e. β-Oxidation
    f. ω-Oxidation .
12. How did fatty acid biosynthetic pathway differs from oxidation pathway?
13. How is long chain fatty acids transported inside mitochondria?
14. What are the roles of α and ω-oxidation of fatty acids?
15. What is the role of biotin in biosynthesis of fatty acids?

D. Write in detail on the following.

16. Explain the biosynthesis of fatty acids.
17. Give an account of β-oxidation of saturated fatty acids.
18. Explain the β-oxidation of polyunsaturated fatty acids.
19. Describe the biosynthesis of triacylglycerol.
20. Give an account of phospholipases.
21. Describe the oxidation pathway of monounsaturated fatty acids.

E. Solve the following problems.

22. What is the ATP yield from the complete oxidation of one molecule of stearic acid?

23. What is the energy price in ATP equivalents of breaking down of palmitic acid to acetyl Co A and then resynthesizing it?
24. Compare the oxidation of one molecule of glucose with that of palmitic acid and the amount of ATP produced.
25. Write the action of phospholipases on phosphatidyl choline. Name the products formed.
26. The oxidative metabolism of fats yield approximately twice the energy on equal weight of hydrated glycogen. How?

G. Gain additional knowledge by further reading

27. Regulation of fatty acid metabolism
28. Cholesterol metabolism - good and bad aspects
29. Atherosclerosis
30. Ketosis
31. Lipid metabolism during germination of seeds

CHAPTER 10

METABOLISM OF PROTEINS AND AMINO ACIDS

Lectures 31-33

10.1. Introduction

Protein metabolism is a key physiological process in all forms of life. Proteins are converted to amino acids and then catabolised. The complete hydrolysis of a polypeptide requires mixture of peptidases because individual peptidases do not cleave all peptide bonds. Both exopeptidases and endopeptidases are required for complete conversion of protein to amino acids.

10.2 Peptidases

13.2.1. Exopeptidases

Exopeptidases hydrolyse the polypeptides either from the carboxyl terminus (carboxypeptidases) or from the amino terminus (amino peptidase). Some of the important exopeptidases and their sources are given in table 13.1.

Table 10.1 Sources of some exopeptidases

Exopeptidase	Source
Carboxypeptidase A	Bovine pancreas
Carboxypeptidase B	Bovine Pancreas
Carboxypeptidase C	Citrus leaves
Amino peptidase M	Porcine kidney
Leucine amino peptidase	Porcine kidney

10.2.2.Endopeptidases

Enzymes, which hydrolyse the internal peptide bonds, are called as endopeptidases. Endopeptidases have side chain requirements for the residues flanking the scissile peptide bond. (Peptide bond to be cleaved) (Figure10.1). The important endopeptidases, their sources and their specificities are listed in table 10.2.

Table 10.2 Sources and specificities of some endopeptidases

Enzyme	Source	Specificity	
		Rn-1	Rn
Trypsin	Bovine pancreas	Positively charged residues Arg, Lys	No proline
Chymotrypsin	Bovine pancreas	Bulky hydrophobic residues Phe,Trp,Tyr	No proline

Pepsin	Bovine gastric mucosa	No proline	Leu, Phe, Trp, Tyr
Elastase	Bovine pancreas	Small neutral residues Ala, Gly, Ser, Val	No proline
Papain	Papaya latex	Lys, Leu, Arg, Gly	-
Bromelain	Pineapple	Basic and aromatic amino acids	-

Trypsin

It is a proteolytic enzyme, present in the intestine in its inactive form (zymogen), trypsinogen. Trypsinogen is converted into its active form, trypsin, by enteropeptidase, a specialized proteolytic enzyme secreted by intestinal cells. Some free trypsin formed also catalyses the conversion of trypsinogen into trypsin. Trypsin can also convert chymotrypsinogen and procarboxypeptidase into chymotrypsin and carboxypeptidase, respectively. Trypsin has different amino acid specificity when compared with other proteolytic enzymes. Trypsin hydrolyses those peptide bonds whose carboxyl groups are contributed by Lys or Arg residues and if the next residue is not proline. The number of smaller peptides resulting from trypsin action is equal to the total number of Arg and Lys residues in the protein plus one.

Papain

Papain is widely used in brewing industry, for tenderization in meat industry, fish, food, laundry, detergents, pharmaceutical and allied industries. Papain consists of a single polypeptide chain of 212 amino acid residues, cross-linked by four disulfide bonds. The active site of papain contains a cysteine residue whose sulfhydryl group is required for catalysis. It has a broad specificity for peptide bonds with wide pH range.

Pepsin

The precursor of pepsin is pepsinogen (MW 40,000) and is converted into active pepsin in the gastric juice by the enzymatic action of pepsin itself. In this conversion, 42 amino acid residues are removed from the amino-terminal end of the polypeptide chain. The portion of the molecule that remains intact is enzymatically active pepsin of molecular weight 33,000 Dalton. Pepsin hydrolyses proteins at peptide bonds on the amino terminal side of tyrosine, phenylalanine and tryptophan and converts it into a mixture of smaller peptides.

Chymotrypsin

Chymotrypsin is secreted from the pancreas in the zymogen form as chymotrypsinogen and it is converted to the active form by trypsin. It reacts with the substrates protein, proteoses and peptones cleaving the peptide bonds whose carboxyl groups are furnished by aromatic amino acids.

10.3 Amino acid metabolism

The amino acids not only function as energy metabolites but also used as precursors of many physiologically important compounds such as heme, bioactive amines, small peptides, nucleotides and nucleotide coenzymes. In normal human beings about 90% of the energy requirement is met by oxidation of carbohydrates and fats. The remaining 10% comes from oxidation of the carbon skeleton of amino acids. Since the 20 common protein amino acids are distinctive in terms of their carbon

skeletons, amino acids require unique degradative pathway. The degradation of the carbon skeletons of 20 amino acids converges to just seven metabolic intermediates namely.

i. Pyruvate
ii. Acetyl CoA
iii. Acetoacetyl CoA
iv. α-Ketoglutarate
v. Succinyl CoA
vi. Fumarate
vii. Oxaloacetate

Pyruvate, α-ketoglutarate, succinyl CoA, fumarate and oxaloacetate can serve as precursors for glucose synthesis through gluconeogenesis. Amino acids giving rise to these intermediates are termed as glucogenic. Those amino acids degraded to yield acetyl CoA or acetoacetate are termed ketogenic since these compounds are used to synthesize ketone bodies. Some amino acids are both glucogenic and ketogenic (For example, phenylalanine, tyrosine, tryptophan and threonine (Figure 10.2).

10.3.1.Catabolism of amino acids

The important reaction commonly employed in the breakdown of an amino acid is always the removal of its α-amino group. The product ammonia is excreted after conversion to urea or other products and the carbon skeleton is degraded to CO_2 releasing energy. The important reaction involved in the deamination of amino acids is

i. Transamination
ii. Oxidative deamination
iii. Non oxidative deamination

Transamination

Most amino acids are deaminated by transamination reaction catalysed by aminotransferases or transaminases. The α-amino group present in an amino acid is transferred to an α-keto acid to yield a new amino acid and the α-keto acid of the

original amino acid. (Figure 10.3). The predominant amino group acceptor is α-keto glutarate.

Glutamate's amino group is then transferred to oxaloacetate in a second transamination reaction yielding aspartate.

Glutamate + oxaloacetate ----------→ α-ketoglutarate + aspartate

pyridoxal phosphate

Pyridoxal phosphate, the coenzyme of pyridoxine (vitamin B6) plays an important role in these reactions. Amino transferase reactions occur in two stages.

Pyridoxal phosphate is covalently attached to the amino transferases via a Schiff's base linkage formed between the aldehyde group of pyridoxal phosphate and the epsilon amino group of lysine residue of the enzyme. Pyridoxal phosphate is converted to pyridoxamine phosphate. In the second stage, the amino group attached to pyridoxamine phosphate is transferred to a different keto acid to yield a new amino acid and releases pyridoxal phosphate (Figure 10.4).

Oxidative deamination

Transamination does not result in net deamination, since one amino acid is replaced by another amino acid. The function of transamination is to funnel the amino nitrogen into one or a few amino acids. For glutamate to play a role in the net conversion of amino groups to ammonia, a mechanism for glutamate deamination is needed so that α-ketoglutarate can be regenerated for further transamination. The generation is accomplished by the oxidative deamination of glutamate by glutamate dehydrogenase. Glutamate is oxidatively deaminated in the mitochondrion by glutamate dehydrogenase. NAD^+ or $NADP^+$ functions as the coenzyme. Oxidation is thought to occur with the transfer of a hydride ion from glutamate's α carbon to $NAD(P)^+$ to form α-iminoglutarate, which is then hydrolysed to α-ketoglutarate and ammonia. The ammonia produced is then converted to urea in mammals (Figure 10.5).

Amino acid oxidase

Two non-specific amino acid oxidases namely, L-amino acid and D-amino acid oxidases catalyse the oxidation of L and D-amino acids utilizing FAD as their coenzymes.

$$\text{Amino acid} + FAD + H_2O \xrightarrow{\quad\quad} \alpha\text{-Keto acid} + NH_3 + FADH_2$$

Non-oxidative deamination

Amino acids such as serine and histidine are deaminated non-oxidatively (Figure 10.6).

The other reactions involved in the catabolism of amino acids are decarboxylation, transulfuration, desulfuration, dehydration etc. The decarboxylation process is important since the products of decarboxylation reactions give rise to physiologically active amines.

Decarboxylation

The enzymes, amino acid decarboxylases are pyridoxal phosphate- dependent enzymes. Pyridoxal phosphate forms a Schiff's base with the amino acid so as to stabilise the α-carbanion formed by the cleavage of bond between carboxyl and α-carbon atom. The physiologically active amines epinephrine, nor-epinephrine, dopamine, serotonin, γ-amino butyrate and histamine are formed through decarboxylation of the corresponding precursor amino acids (Figure 10.7).

Summary

Proteins are hydrolysed by exo-and endo peptidases to amino acids and then undergo catabolic reactions. Exopeptidases hydrolyse the polypeptides either from the carboxyl or amino terminus. Enzymes, which hydrolyse the internal peptide bonds are called as endopeptidases. Trypsin, chymotrypsin, pepsin and papain are a few important endopeptidases. These endopeptidases show specificities towards the peptide bonds they hydrolyse.

The amino acids not only function as energy metabolites (about 10%) but also used as precursors for many physiologically important compounds. The degradation of the carbon skeletons of 20 amino acids converges to seven metabolites.

Most amino acids are deaminated by transamination catalysed by amino transferases. Oxidative deamination is accomplished by the glutamate dehydrogenase. The major route involved in ammonia assimilation in plants is through glutamate synthase and glutamine synthase. An additional enzyme glutamate dehydrogenase is widely distributed but is not significantly involved in ammonia assimilation. The synthesis of non-essential amino acids require only one or two-step reactions, while the synthesis of essential amino acids in plants require multi-step reactions.

Review questions

A. Multiple choice questions

1. Trypsin hydrolyses a peptide bond on the carboxyl side of
   a. Arginine b. Proline c. Leucine d. Phenylalanine

2. The source for bromelain is
   a. Human b. Bacteria c. Fungi d. Pineapple

3.The amino acids are attached to the tRNA through
   a. 3′OH group of terminal adenosine
   b. 5′ OH group of terminal adenosine
   c. 3′ OH group of terminal cytosine
   d. 5′ OH group of terminal cytosine

4. High fidelity in protein synthesis is maintained by
   a. Peptidyl transferase
   b. Amino acyl tRNA synthetase
   c. Terminating codons
   d. Elongation factors

5.Tetracycline inhibits binding of amino acyl tRNA to the

e. A site on the prokaryotic ribosome
f. A site on the eukaryotic ribosome
g. P site on the eukaryotic ribosome
h. P site on the prokaryotic ribosome

B. Fill up the blanks

6. Enzymes which hydrolyse the internal peptide bonds are called as ----------

7.Amino acids giving rise to the precursors for glucose synthesis are termed as----------

8.Amino acids that are degraded to acetyl CoA or acetoacetyl CoA are termed as----------

9.The coenzyme ---------- is essential for transamination reaction.

10.---------- is an initiating codon in protein biosynthesis.

C. Write short answers for the following

11.Define the following

a. Exopeptidases b. Endopeptidases c. Transamination
d. Translation e. Ketogenic amino acids f. Glucogenic amino acids

12.How is carboxypeptidase different from aminopeptidase?

13.Differentiate the action of endo- and exo-peptidases.

14.What is a zymogen? Give examples.

15.How are amino acids deaminated?

16.What is transamination? Give an example.

17.How is fidelity maintained in protein biosynthesis?

18.What are terminating codons?

D. Write in detail on the following.

19.Explain the specificity involved in the hydrolysis of peptide bonds by trypsin, chymotrypsin and

pepsin.

20.Describe the steps involved in protein biosynthesis.

21.What are the different post-translational modifications occurring in proteins?

22.Explain the mechanism of action of puromycin, streptomycin, chloramphenicol, diphtheria toxin and ricin.

E. Solve the following problems.

23.Calculate the energy requirement for the synthesis of a protein with 200 amino acids.

24.How many mRNAs are possible for a tetra peptide containing Met-Arg-Leu-Lys? (use the codon table). Justify your answer.

25.The sense strand of DNA contains 5′ACCCTTAACCCTTGG3′

i) What amino acid sequence could be coded from this portion?

ii) Whether the complimentary stand also synthesise the same protein? Justify your answer (use the codon table).

26.What is the anticodon for 5′UCA, UCC, UCG and UCU? Which of these codon- anticodon pairings have a wobble base pair?

27.A protein has 122 amino acids. How many bases will be present in the reading frame?

F. Gain additional knowledge by further reading

28.Signal sequence

29.Shine – Dalgarno sequence

30.Protein targeting

31.Protein degradation

32.Industrial uses of papain

CHAPTER 11

METABOLIC INTERRELATIONSHIP

Lecture 34

11.1.Introduction

Several of the metabolic pathways so far discussed are either catabolic or anabolic. Catabolic reactions serve to generate chemical energy and are useful to drive the synthesis of essential biomolecules. Despite their opposing purposes, these reactions typically occur at the same time so that food molecules are broken down to provide the building blocks and energy for ongoing biosynthesis. The interrelationship between different metabolic pathways is shown in figure 11.1.

The typical metabolic processes can be interrelated by a scheme involving metabolic blocks such as photosynthesis, catabolism, anabolism and secondary metabolism.

11.1. Photosynthesis

The carbon source for almost all organic molecules in the living system is derived through photosynthesis. The ATP and NADPH derived from light reactions of photosynthesis are utilized for the fixation of CO_2 in dark reaction. Different forms of carbohydrates are derived through these reactions.

11.2. Catabolism

In animals and plants, carbohydrates and fats are oxidised to CO_2 and H_2O and most of the electrons liberated are passed to oxygen via an electron transport pathway

coupled to oxidative phosphorylation so that ATP is formed. Some electrons go to reduce $NADP^+$, the source of reducing power in anabolic reactions. Glycolysis, citric acid cycle, hexose monophosphate shunt (or pentose phosphate pathway), electron transport and oxidative phosphorylation are the major pathways in catabolism. The metabolic intermediates in these pathways serve as substrates for anabolic reactions.

11.3. Anabolism

The biosynthetic reactions are included in anabolism. The NADPH and ATP molecules derived from catabolic reactions and the intermediate compounds of catabolic pathways are utilized for the synthesis of major biomolecules such as carbohydrates, fatty acids, lipids, amino acids, proteins, nucleotides, nucleic acids and secondary metabolites.

11.4. Secondary metabolism

The phosphoenolpyruvate derived from glycolysis and erythrose 4-phosphate derived from phosphate pathway are utilized for the synthesis of aromatic amino acids (shikimate pathway) which are then deaminated and hydroxylated into different phenolic compounds. The acetyl CoA derived through fatty acids or carbohydrates are utilized for the synthesis of fatty acids (acetate-malonate pathway) which are then converted into alkanes, oxygenated alkanes, esters, long chain alcohols aldehydes, cutins and suberins, the components of plant cuticle. The acetyl CoA is also converted into isopentenyl pyrophosphate through mevalonate (acetate-mevalonate pathway), which are then converted into different terpenoid, compounds such as monoterpenes, sesquiterpenes, diterpenes, steroids, carotenoids and polyterpenes. The amino acids are also utilized for the synthesis of alkaloids.

Appendix 1. Moisture, protein, fat, carbohydrate, fibre and mineral contents of some common foodstuffs (g/100g edible portion)

Name of the foodstuff		Moisture	Crude protein	Fat	Carbohydrate	Fibre	Minerals
I. Cereals							
1.	Rice (raw milled)	13.7	8.0	0.5	77.0	0.2	0.6
2.	Wheat (whole)	12.8	11.8	1.5	71.2	1.2	1.5
3.	Pearl millet	12.4	11.6	5.0	67.5	1.2	2.3
4.	Maize, dry	14.9	11.1	3.6	66.2	2.7	1.5
5.	Ragi	13.1	8.2	1.3	71.1	3.6	2.7
6.	Sorghum	11.9	10.4	1.9	72.6	1.6	1.6
II. Pulses							
7.	Red gram (dhal)	13.4	22.3	1.7	57.6	1.5	3.5
8.	Bengal gram (dhal)	9.9	20.8	5.6	59.8	1.2	2.7
9.	Green gram(dhal)	10.1	24.5	1.2	59.9	0.8	3.5
10	Black gram (dhal)	10.9	24.0	1.4	59.6	0.9	3.2
11	Cow pea	13.4	24.1	1.0	54.5	3.8	3.2
12	Horse gram (whole)	11.8	22.0	0.5	57.2	5.3	3.2
13	Peas (dry)	16.0	19.7	1.1	56.5	4.5	2.2
14	Soybean	8.1	43.2	19.5	20.2	3.7	4.6
III. Nuts and Oilseeds							
15	Groundnut	3.0	25.3	40.1	26.1	3.1	2.4
16	Gingelly seeds	5.3	18.3	43.3	25.0	2.9	5.2
17	Coconut dry	4.3	6.8	62.3	18.4	6.6	1.6
18	Mustard	8.5	20.0	39.7	23.8	1.8	4.2
19	Sunflower (dehulled)	5.5	19.8	52.1	17.9	1.0	3.7
20	Safflower	5.5	13.5	25.6	17.9	34.9	2.6
21	Almond	5.2	20.8	58.9	10.5	1.7	2.9
IV Fruits							
22	Amla	81.8	0.5	0.1	13.7	3.4	0.5
23	Apple	84.6	0.2	0.5	13.4	1.0	0.3
24	Banana	70.1	1.2	0.3	27.2	0.4	0.8
25	Grapes (blue)	82.2	0.6	0.4	13.1	2.8	0.9
26	Dates, dry	15.3	2.5	0.4	75.8	3.9	2.1
27	Guava	81.7	0.9	0.3	11.2	5.2	0.7
28	Jack fruit	76.2	1.9	0.1	19.8	1.1	0.9
29	Lemon	85.0	1.0	0.9	11.1	1.7	0.3
30	Mango	81.0	0.6	0.4	16.9	0.7	0.4
31	Orange	87.6	0.7	0.2	10.9	0.3	0.3
32	Papaya	90.8	0.6	0.1	7.2	0.8	0.5

33	Pineapple	87.8	0.4	0.1	10.8	0.5	0.4
34	Plum	86.9	0.7	0.5	11.1	0.4	0.4
35	Sapota	73.7	0.7	1.1	21.4	2.6	0.5
36	Tomato	94.0	0.9	0.2	3.6	0.8	0.5
37	Wood apple	64.2	7.1	3.7	18.1	5.0	1.9
38	Strawberry	87.8	0.7	0.2	9.8	1.1	0.4
39	Custard apple	70.5	1.6	0.4	23.5	3.1	0.9
V	**Roots and tubers**						
40	Beet root	87.7	1.7	0.1	8.8	0.9	0.8
41	Carrot	86.0	0.9	0.2	10.6	1.2	1.1
42	Colocasia	73.1	3.0	0.1	21.1	1.0	1.7
43	Garlic	62.0	6.3	0.1	29.8	0.8	1.0
44	Onion (big)	86.6	1.2	0.1	11.1	0.6	0.4
45	Potato	74.7	1.6	0.1	22.6	0.4	0.6
46	Radish (pink)	90.8	0.6	0.3	6.8	0.6	0.9
47	Tapioca	59.4	0.7	0.2	38.1	0.6	1.0
48	Turnip	91.6	0.5	0.2	6.2	0.9	0.6
49	Sweet potato	68.5	1.2	0.3	28.2	0.8	1.0
50	Yam	78.7	1.2	0.1	18.4	0.8	0.8
VI	Vegetables						
51	Agathi	73.1	8.4	1.4	11.8	2.2	3.1
52	Amaranth (tender)	85.7	4.0	0.5	6.1	1.0	2.7
53	Cabbage	91.9	1.8	0.1	4.6	1.0	0.6
54	Chekkur manis	73.6	6.8	3.2	11.6	1.4	3.4
55	Coriander leaves	86.3	3.3	0.6	6.3	1.2	2.3
56	Curry leaves	63.8	6.1	1.0	18.7	6.4	4.0
57	Drumstic leaves	75.9	6.7	1.7	12.5	0.9	2.3
58	Fenugreek leaves	86.1	4.4	0.9	6.0	1.1	1.5
59	Manathakkali leaves	82.1	5.9	1.0	8.9	-	2.1
60	Ash gourd	96.5	0.4	0.1	1.9	0.8	0.3
61	Bitter gourd	83.2	2.1	1.0	10.6	1.7	1.4
62	Bottle gourd	96.1	0.2	0.1	2.5	0.6	0.5
63	Brinjal	92.7	1.4	0.3	4.0	1.3	0.3
64	Cauliflower	90.8	2.6	0.4	4.0	1.2	1.0
65	Cluster beans	81.0	3.2	0.4	10.8	3.2	1.4
66	Cowpea pods	85.3	3.5	0.2	8.1	2.0	0.9
67	Cucumber	96.3	0.4	0.1	2.5	0.4	0.3
68	Drumstick	86.9	2.5	0.1	3.7	4.8	2.0
69	Knol-khol	92.7	1.1	0.2	3.8	1.5	0.7
70	Ladies fingers	89.6	1.9	0.2	6.4	1.2	0.7

71	Plantain green	83.2	1.4	0.2	14.0	0.7	0.5
72	Pumpkin	92.6	1.4	0.1	4.6	0.7	0.6
73	Ridge gourd	95.2	0.5	0.1	3.4	0.5	0.3
74	Snake gourd	94.6	0.5	0.3	3.3	0.8	0.5
75	French beans	91.4	1.7	0.1	4.5	1.8	0.5

Source: Nutritive value of Indian foods, National Institute of Nutrition, ICMR, Hyderabad.

Appendix 2. Vitamin contents of important plant foodstuffs

		Carotene (µg)	Thiamine **(mg)**	**Riboflavin (mg)**	**Niacin (mg)**	**Pyridoxine (mg)**	**Folic acid (µg)**	**Vit C (mg)**
		(Per 100g edible portion)						
I. Cereals								
1.	Rice	0	0.06	0.06	1.9	-	8.0	0
2.	Wheat	64	0.45	0.17	5.5	0.57	36.6	0
3.	Pearl millet	132	0.33	0.25	2.3	-	45.5	0
4.	Maize	90	0.42	0.10	1.8	-	20.0	6
5.	Ragi	42	0.42	0.19	1.1	-	18.3	0
6.	Sorghum	47	0.37	0.13	3.1	0.21	20.0	0
II. Pulses								
7.	Red gram (dhal)	132	0.45	0.19	2.9	0.54	103.0	0
8.	Bengal gram (dhal)	129	0.48	0.18	2.4	-	147.5	1
9.	Soybean	426	0.73	0.39	3.2	-	100.0	-
III. Nuts and Oilseeds								
10	Groundnut	37	0.90	0.13	19.9	-	20.0	0
11	Gingelly	60	1.01	0.34	4.4	-	134.0	0
12	Mustard	162	0.65	0.26	4.0	-	-	0
IV Fruits								
13	Amla	9	0.03	0.01	0.2	-	-	600
14	Apple							
15	Banana	78	0.05	0.08	0.5	-	-	7
16	Dates dry	26	0.01	0.02	0.09	-	-	3
17	Grapes (blue)	3	0.04	0.03	0.2	-	-	1
18	Guava	0	0.03	0.03	0.4	-	-	212
19	Jack fruit	175	0.03	0.13	0.4	-	-	7
20	Mango	2743	0.08	0.09	0.9	-	-	16
21	Orange	11.4	-	-	-	-	-	30
22	Papaya	666	0.04	0.25	0.2	-	-	57
23	Pineapple	18	0.20	0.12	0.1	-	-	39
24	Plum	166	0.04	0.1	0.3	-	-	5
25	Sapota	97	0.02	0.03	0.2	-	-	6

26	Custard apple	0	0.07	0.17	1.3	-	-	37
27	Strawberry	18	0.03	0.02	0.2	-	-	52
28	Wood apple	61	0.04	0.17	0.8	-	-	3
29	Jamun (Naval)	48	0.03	0.01	0.2	-	-	18
V	Vegetables							
30	Agathi	5400	0.21	0.09	1.2	-	-	169
31	Amaranth (tender)	5520	0.03	0.30	1.2	-	149	99
32	Cabbage	120	0.06	0.09	0.4	-	23	124
33	Chekkur manis	5706	0.48	0.32	2.6	-	-	247
34	Coriander leaves	6918	0.05	0.06	0.8	-	-	135
35	Curry leaves	7560	0.08	0.21	2.3	-	93.9	4
36	Drumstic leaves	6072	0.05	0.05	0.8	-	-	220
37	Fenugreek leaves	2340	0.04	0.31	0.8	-	-	52
38	Manathakkali leaves	-	-	0.59	0.9	-	-	11
39	Ash gourd	0	0.06	0.01	0.4	-	-	1
40	Beet root	0	0.04	0.09	0.4	-	-	10
41	Carrot	1890	0.04	0.02	0.6	-	15	3
42	Onion, big	0	0.08	0.01	0.4	-	6	11
43	Potato	24	0.10	0.01	1.2	-	7	17
44	Radish, pink	3	0.06	0.02	0.4	-	-	17
45	Sweet potato	6	0.08	0.04	0.7	-	-	24
46	Tapioca	-	0.05	0.10	0.3	-	-	25
47	Turnip	0	0.04	0.04	0.5	-	-	43
48	Yam, ordinary	78	0.07	-	0.7	-	17.5	-
49	Bitter gourd	126	0.07	0.09	0.5	-	-	88
50	Bottle gourd	0	0.03	0.01	0.2	-	-	0
51	Brinjal	74	0.04	0.11	0.9	-	34	12
52	Cauliflower	30	0.04	0.10	1.0	-	-	56
53	Cluster beans	198	0.09	0.03	0.6	-	144	49
54	Cowpea pods	564	0.07	0.09	0.9	-	-	14
55	Cucumber	0	0.03	0	0.2	-	14.7	7
56	Drumstick	110	0.05	0.07	0.2	-	-	120
57	French beans	132	0.08	0.06	0.3	-	45.5	24
58	Knol-khol	21	0.05	0.09	0.5	-	-	85
59	Ladies fingers	52	0.07	0.10	0.6	-	105.1	13
60	Plantain green	30	0.05	0.02	0.3	-	16.4	24
61	Pumpkin	50	0.06	0.04	0.5	-	13	2
62	Ridge gourd	33	-	0.01	0.2	-	-	5
63	Snake gourd	96	0.04	0.06	0.3	-	15.5	0
64	Tomato	351	0.12	0.06	0.4	-	30	27

Source: Nutritive value of Indian foods, National Institute of Nutrition, ICMR, Hyderabad.

Appendix-3

Textbooks for further reading

1. Bonner J and JE Varner 1976. Plant Biochemistry, Second edition, Academic Press, San Diego.
2. Devlin TM 2001. Textbook of Biochemistry with Clinical Correlations Fifth edition, John Wiley & Sons, Inc. Publication, New York.
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4. Goodwin TW and EI Mercer 1983. Introduction to Plant Biochemistry, Second edition, Pergamon Press, New York.
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6. Heldt, HW 1997. Plant Biochemistry and Molecular Biology, First edition, Oxford University Press, London.
7. Lea PJ and RC Leagood 1999. Plant Biochemistry and Molecular biology, Wiley, San Diego.
8. Murray RK, DK Granner, PA Mayes and VW Rodwell 2000. Harper's Biochemistry, Twenty fifth editions, Prentice-Hall International, New York.
9. Plummer DT 1998. An Introduction to Practical Biochemistry, Third edition, Mc Graw-Hill, New Delhi.
10. Sawhney SK and Randhir Singh 2000. Introductory Practical Biochemistry, Narosa Publishing House Ltd, New Delhi.
11. Segel H 1976. Biochemical Calculations, Second edition, John Wiley and Sons, New York.
12. Stryer L, J Berg, J Tymoczko 2000. Biochemistry. Fifth edition, W.H. Freeman and Company, New York.
13. Voet D and JG Voet 2001. Biochemistry. Third edition, John Wiley & Sons, New York.
14. Wilson, K and J Walker 2000. Principles and Techniques of Practical Biochemistry, Fifth edition, Cambridge University Press, London.