BME 2901-BIOCHEMISTRY

Protein Functions and Enzymes

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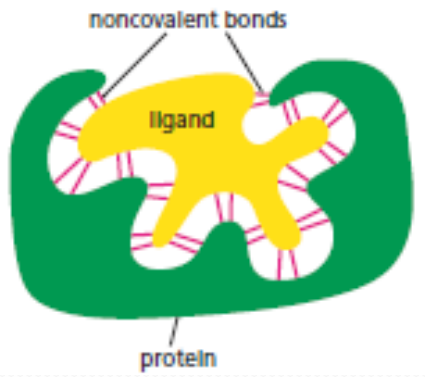
Fall 2019

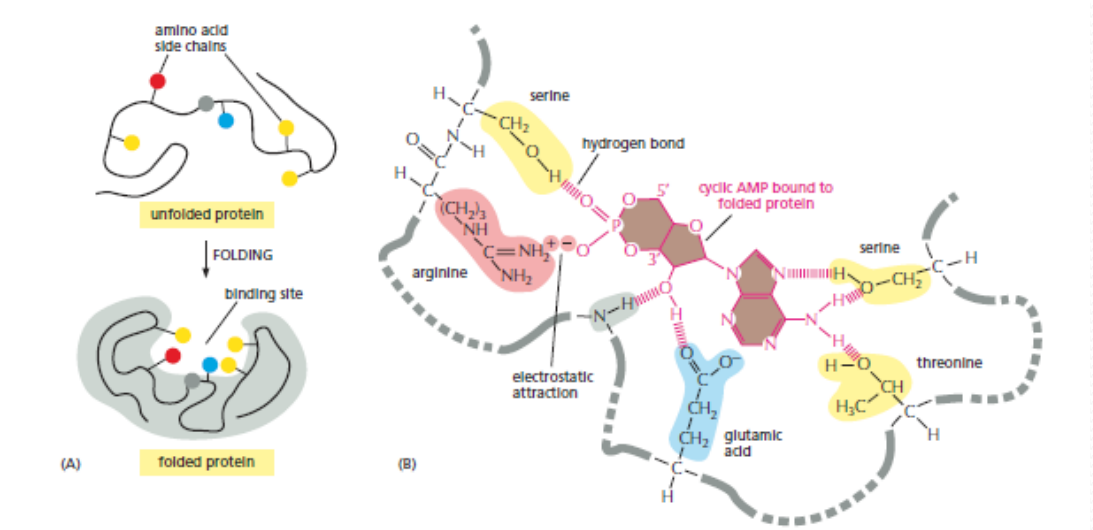
Protein Functions

- Proteins are made from an enormous variety of amino acid sequences and can fold into a unique shape.
- The surface topography of a protein's side chains endows each protein with a unique function, based on its chemical properties.
- The union of **structure, chemistry, and function** gives proteins the extraordinary ability to orchestrate the large number of dynamic processes that occur in cells.
- Thus, for proteins, form and function are strongly linked.

- The biological properties of a protein molecule depend on its physical interaction with other molecules.
- The binding of a protein to other biological molecules always shows great **specificity**.
- Any substance that is bound by a protein is referred to as a **ligand** for that protein.
- The ability of a protein to bind selectively and with high affinity to a ligand is due to the formation of a set of weak, noncovalent interactions—hydrogen bonds, electrostatic attractions, van der Waals attractions and hydrophobic forces.
- Each individual noncovalent interaction is weak, so that effective binding requires many such bonds to be formed simultaneously.



- The region of a protein that associates with a ligand, known as its **binding site**, usually consists of a cavity in the protein surface formed by a particular arrangement of amino acid side chains.
- These side chains can belong to amino acids that are widely separated on the linear polypeptide chain, but are brought together when the protein folds.
- Other regions on the surface often provide binding sites for different ligands that regulate the protein's activity.
- Yet other parts of the protein may be required to attract or attach the protein to a particular location in the cell.



ENZYMES

function: Catalyze covalent bond breakage or formation.

examples: Living cells contain thousands of different enzymes, each of which catalyzes (speeds up) one particular reaction. Examples include: *tryptophan synthetase*—makes the amino acid tryptophan; *pepsin*—degrades dietary proteins in the stomach; *ribulose bisphosphate carboxylase*—helps convert carbon dioxide into sugars in plants; *DNA polymerase*—copies DNA; *protein kinase*—adds a phosphate group to a protein molecule.

STRUCTURAL PROTEINS

function: Provide mechanical support to cells and tissues.

examples: Outside cells, *collagen* and *elastin* are common constituents of extracellular matrix and form fibers in tendons and ligaments. Inside cells, *tubulin* forms long, stiff microtubules, and *actin* forms filaments that underlie and support the plasma membrane; *keratin* forms fibers that reinforce epithelial cells and is the major protein in hair and horn.

TRANSPORT PROTEINS

function: Carry small molecules or ions.

examples: In the bloodstream, *serum albumin* carries lipids, *hemoglobin* carries oxygen, and *transferrin* carries iron. Many proteins embedded in cell membranes transport ions or small molecules across the membrane. For example, the bacterial protein *bacteriorhodopsin* is a light-activated proton pump that transports H^+ ions out of the cell; *glucose carriers* shuttle glucose into and out of cells; and a *Ca^{2+} pump* clears Ca^{2+} from a muscle cell's cytosol after the ions have triggered a contraction.

MOTOR PROTEINS

function: Generate movement in cells and tissues.

examples: *Myosin* in skeletal muscle cells provides the motive force for humans to move; *kinesin* interacts with microtubules to move organelles around the cell; *dynein* enables eukaryotic cilia and flagella to beat.

STORAGE PROTEINS

function: Store amino acids or ions.

examples: Iron is stored in the liver by binding to the small protein *ferritin*; *ovalbumin* in egg white is used as a source of amino acids for the developing bird embryo; *casein* in milk is a source of amino acids for baby mammals.

SIGNAL PROTEINS

function: Carry extracellular signals from cell to cell.

examples: Many of the hormones and growth factors that coordinate physiological functions in animals are proteins; *insulin*, for example, is a small protein that controls glucose levels in the blood; *netrin* attracts growing nerve cell axons to specific locations in the developing spinal cord; *nerve growth factor* (*NGF*) stimulates some types of nerve cells to grow axons; *epidermal growth factor* (*EGF*) stimulates the growth and division of epithelial cells.

RECEPTOR PROTEINS

function: Detect signals and transmit them to the cell's response machinery.

examples: *Rhodopsin* in the retina detects light; the *acetylcholine receptor* in the membrane of a muscle cell is activated by acetylcholine released from a nerve ending; the *insulin receptor* allows a cell to respond to the hormone insulin by taking up glucose; the *adrenergic receptor* on heart muscle increases the rate of the heartbeat when it binds to adrenaline.

GENE REGULATORY PROTEINS

function: Bind to DNA to switch genes on or off.

examples: The *lactose repressor* in bacteria silences the genes for the enzymes that degrade the sugar lactose; many different *homeodomain proteins* act as genetic switches to control development in multicellular organisms, including humans.

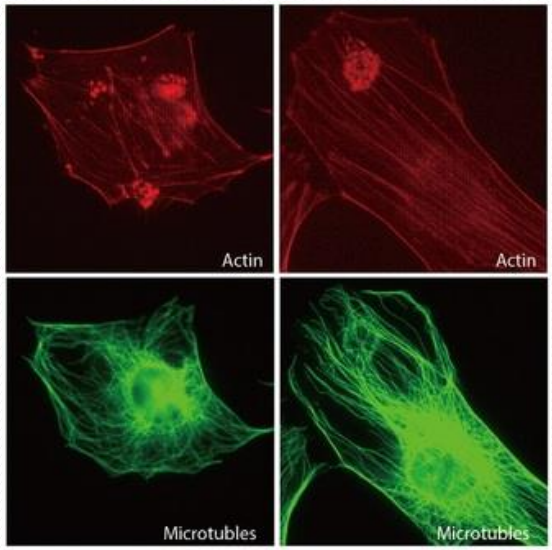
SPECIAL-PURPOSE PROTEINS

function: Highly variable.

examples: Organisms make many proteins with highly specialized properties. These molecules illustrate the amazing range of functions that proteins can perform. The *antifreeze proteins* of Arctic and Antarctic fishes protect their blood against freezing; *green fluorescent protein* from jellyfish emits a green light; *monellin*, a protein found in an African plant, has an intensely sweet taste; mussels and other marine organisms secrete *glue proteins* that attach them firmly to rocks, even when immersed in seawater.

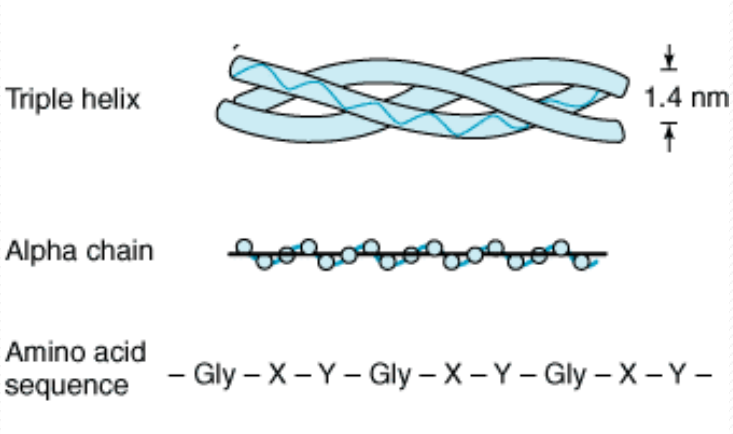
STRUCTURAL PROTEINS

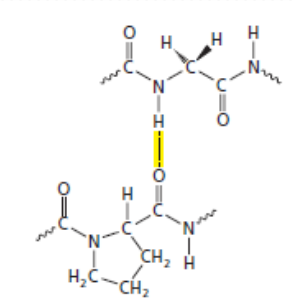
- Components of cytosekelton and extracellular matrix
  - ECM: Collagens, keratin, elastin
  - Cytoskeleton: Actin, microtubules
- They provide structural support to the cells and the tissues.

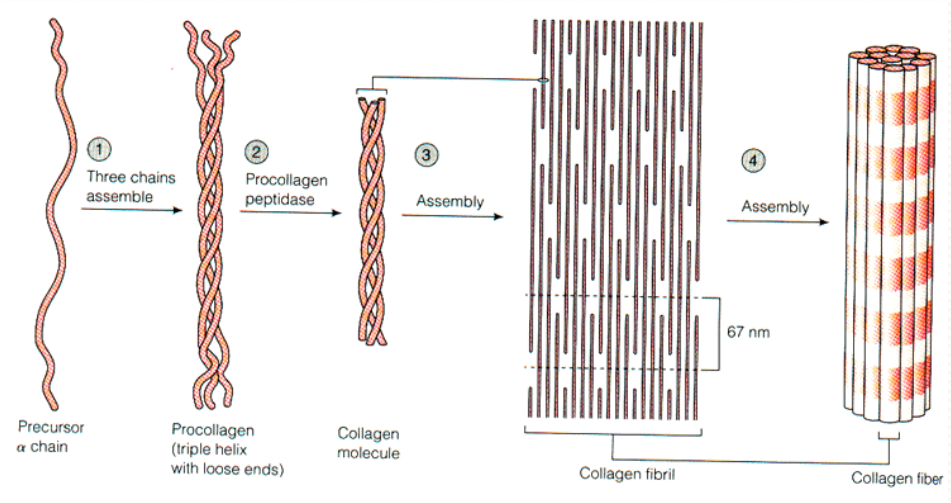


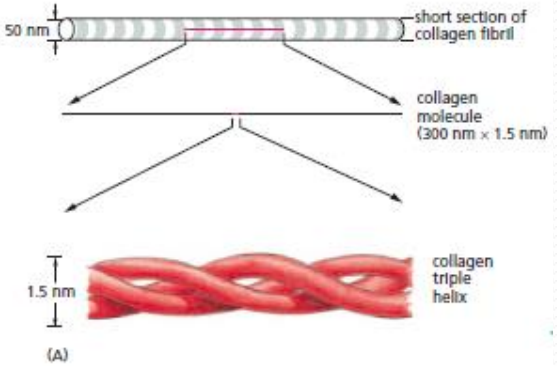
COLLAGEN

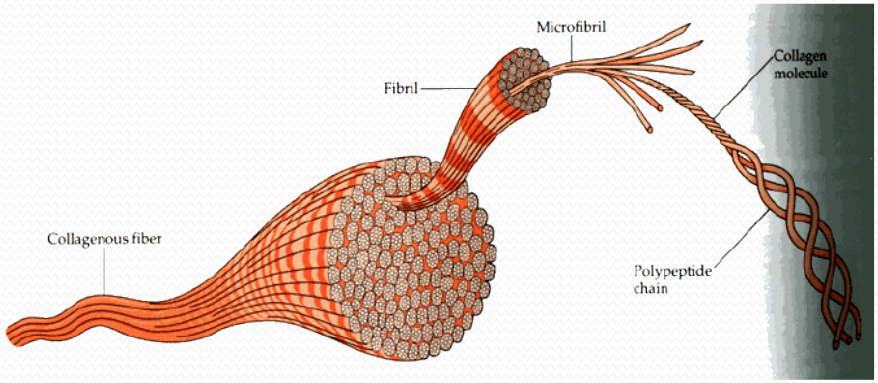
- Collagen is the major protein component of the connective tissue of vertebrates. It makes up about 30% of the total protein in mammals.
- The molecule consists of three left-handed helical chains coiled around each other to form a right-handed supercoil. Typically it contains about 35% Gly, 11% Ala, and 21% Pro and 4-Hydroxyproline.
- The collagen triple helix is stabilized by interchain hydrogen bonds. The sequence of the protein in the helical region consists of multiple repeats of the form –Gly–X–Y–, where X is often proline and Y is often 4-hydroxyproline.
- The glycine residues are located along the central axis of the triple helix, where tight packing of the protein strands can accommodate no other residue.
- For each –Gly–X–Y– triplet, one hydrogen bond forms between the amide hydrogen atom of glycine in one chain and the carbonyl oxygen atom of residue X in an adjacent chain. Hydrogen bonds involving the hydroxyl group of hydroxyproline may also stabilize the collagen triple helix.
- Unlike the more common α helix, the collagen helix has no intrachain hydrogen bonds.
- The tight wrapping of the chains in the collagen triple helix provides tensile strength greater than that of a steel wire of equal cross section.

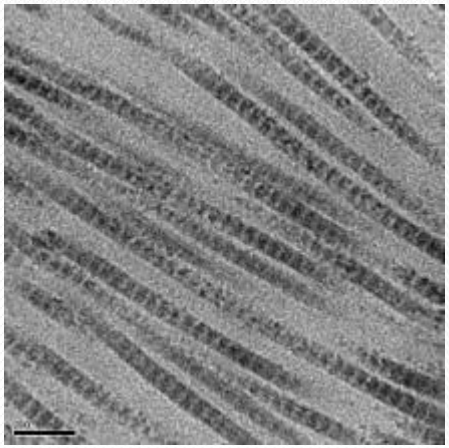





1
Three chains assemble
2
Procollagen peptidase
3
Assembly
4
Assembly
67 nm
Precursor α chain
Procollagen (triple helix with loose ends)
Collagen molecule
Collagen fibril
Collagen fiber


50 nm
short section of collagen fibril
collagen molecule (300 nm × 1.5 nm)
1.5 nm
collagen triple helix
(A)


Microfibril
Fibril
Collagen molecule
Collagenous fiber
Polypeptide chain



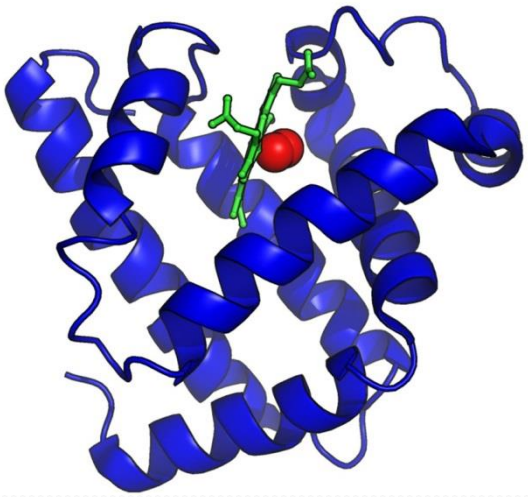
- In addition to hydroxyproline, collagen contains an additional modified amino acid residue called 5-hydroxylysine.
- Hydroxyproline and hydroxylysine residues are formed when specific proline and lysine residues are hydroxylated after incorporation into the polypeptide chains of collagen.
- The hydroxylation reactions are catalyzed by enzymes and require ascorbic acid (vitamin C). Hydroxylation is impaired in the absence of vitamin C, and the triple helix of collagen is not assembled properly.
- The limited conformational flexibility of proline and hydroxyproline residues prevents the formation of α helices in collagen chains and also makes collagen rigid.
- Crosslinking between the individual strands of collagen triple helix also contribute its rigidity.

TRANSPORT PROTEINS

- **Myoglobin** (Mb) and the related protein **hemoglobin** (Hb) carry out their biological functions by selectively and reversibly binding molecular oxygen (O_2).
- Hemoglobin binds oxygen efficiently in the lungs and release oxygen in the tissues.
- A molecule of hemoglobin carries four noncovalently bound *heme* groups, ring-shaped molecules each with a single central iron atom.
- Heme gives hemoglobin (and blood) its red color. By binding reversibly to dissolved oxygen gas through its iron atom, heme enables hemoglobin to pick up oxygen in the lungs and release it in tissues that need it.

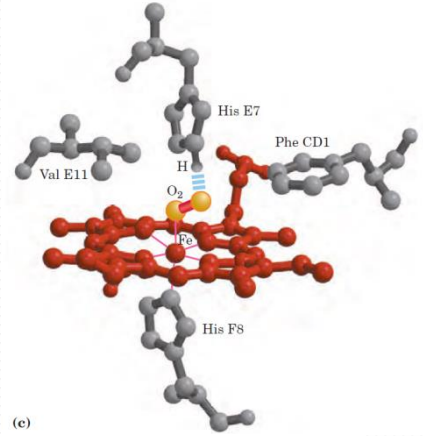
Myglobin

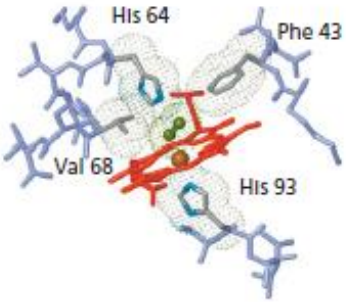
- Myoglobin is a relatively simple oxygen-binding protein found in almost all mammals, primarily in muscle tissue. As a transport protein, it facilitates oxygen diffusion in muscle.
- Myoglobin is a single polypeptide of 153 amino acid residues with one molecule of heme. Aminoacids are mostly contained in 8 α helices that constitute myoglobin.

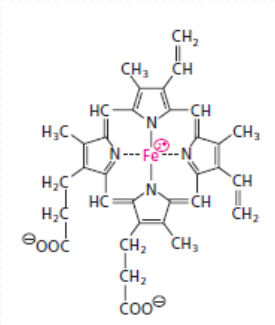


Myglobin

- The interior of myoglobin is made up almost exclusively of hydrophobic amino acid residues, particularly those that are highly hydrophobic—valine, leucine, isoleucine, phenylalanine, and methionine.
- The surface of the protein contains both hydrophilic and hydrophobic residues.
- As is the case with most proteins, the tertiary structure of myoglobin is stabilized by hydrophobic interactions within the core. Folding of the polypeptide chain is driven by the energy minimization that results from formation of this hydrophobic core.
- The binding of heme prosthetic group of myoglobin occurs in the hydrophobic cleft and the binding is due to a number of weak interactions including hydrophobic interactions, van der Waals contacts, and hydrogen bonds.
- Two histidine residues interact with the iron atom and the bound oxygen. Accessibility of the heme group to molecular oxygen depends on slight movement of nearby amino acid side chains



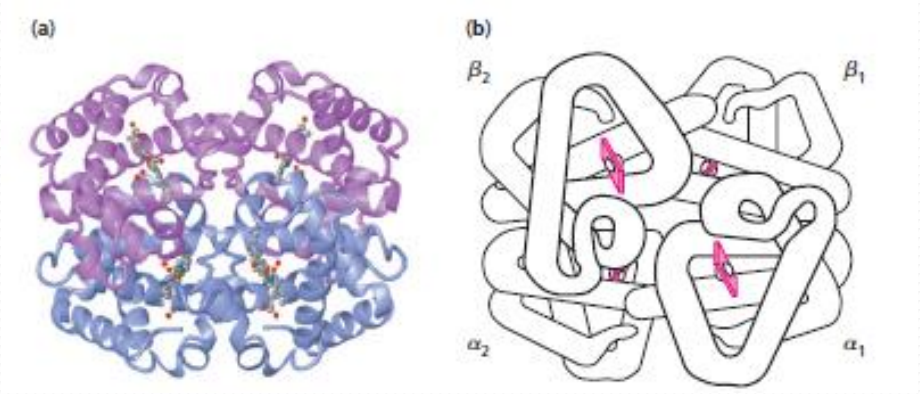




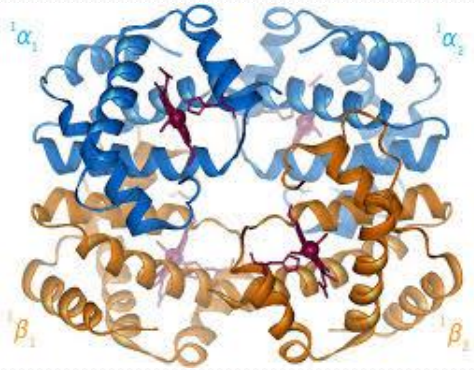
Heme group

Hemoglobin

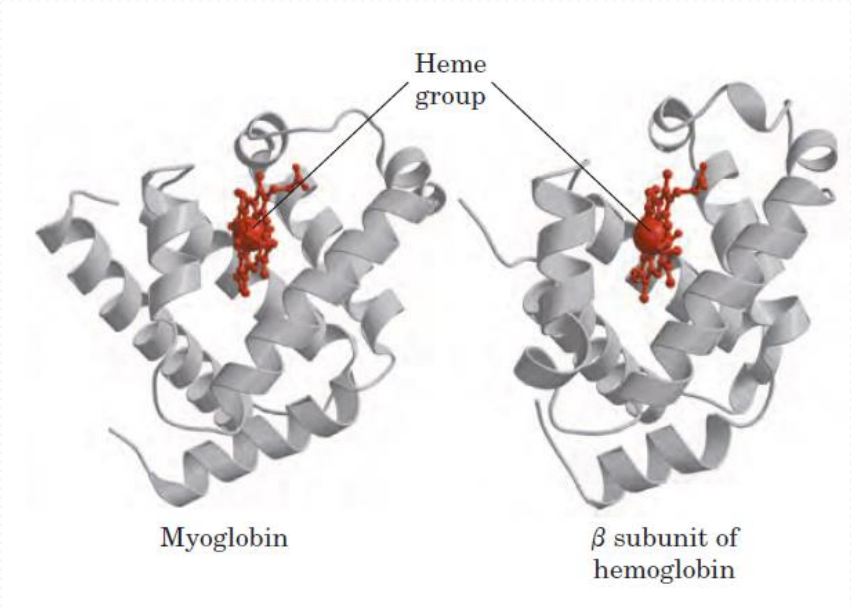
- **Oxygen Is Transported in Blood by Hemoglobin.** Erythrocytes function to carry hemoglobin.
- Hemoglobin is more complex than myoglobin because it is a multisubunit protein.
- In adult mammals, hemoglobin contains two different globin subunits called α-*globin* and β-*globin*. Hemoglobin is an $\alpha_2\beta_2$ tetramer—it contains two α chains and two β chains.



- Each of the four globin subunits contains a heme prosthetic group identical to that found in myoglobin.
- The α and β subunits face each other across a central cavity.
- The tertiary structure of each of the four chains is almost identical to that of myoglobin.
- Hemoglobin, however, is not simply a tetramer of myoglobin molecules. Each α chain interacts extensively with β chain so hemoglobin is actually a dimer of α β subunits.



- Each of these globin subunits is similar in structure and sequence to myoglobin reflecting their evolution from a common ancestor.



- Interactions between the subunits of a multimeric protein can permit a highly sensitive response to small changes in ligand concentration.
- Interactions among the subunits in hemoglobin cause conformational changes that alter the affinity of the protein for oxygen.
- The modulation of oxygen binding allows the O_2-transport protein to respond to changes in oxygen demand by tissues.
  - In arterial blood passing from the lungs through the heart to the peripheral tissues, hemoglobin is about 96% saturated with oxygen. In the venous blood returning to the heart, hemoglobin is only about 64% saturated.
  - When oxygen binds, the electronic properties of heme iron change; this also accounts for the change in color from the dark purple of oxygen-depleted venous blood to the bright red of oxygen-rich arterial blood.
- Myoglobin is relatively insensitive to small changes in the concentration of dissolved oxygen and so functions well as an oxygen-storage protein.

- Some small molecules, such as carbon monoxide (CO) and nitric oxide (NO), coordinate to heme iron with greater affinity than does O2.
- When a molecule of CO is bound to heme, O2 is excluded, which is why CO is highly toxic to aerobic organisms. By surrounding and sequestering heme, oxygen binding proteins regulate the access of CO and other small molecules to the heme iron.
- Carbon monoxide binds to free heme molecules more than 20,000 times better than does O2, but it binds only about 200 times better when the heme is bound in myoglobin.
- The difference may be partly explained by steric hindrance.

O2 binding to hemoglobin

- The binding of O2 to the heme in myoglobin or hemoglobin also depends on molecular motions, or “breathing,” in the protein structure.
- The heme molecule is deeply buried in the folded polypeptide, with no direct path for oxygen to move from the surrounding solution to the ligand binding site.
- If the protein were rigid, O2 could not enter or leave the heme pocket at a measurable rate. However, rapid molecular flexing of the amino acid side chains produces transient cavities in the protein structure, and O2 evidently makes its way in and out by moving through these cavities.

O2 binding to hemoglobin

- X-ray analysis has revealed two major conformations of hemoglobin: the **R state** and the **T state.** Although oxygen binds to hemoglobin in either state, it has a significantly higher affinity for hemoglobin in the R state.
- Oxygen binding stabilizes the R state. When oxygen is absent experimentally, the T state is more stable and is thus the predominant conformation of **deoxyhemoglobin.**
- T and R originally denoted “tense” and “relaxed,” respectively, because the T state is stabilized by a greater number of ion pairs, many of which lie at the$\alpha_1\beta_2$ (and $\alpha_2\beta_1$) interface.
- The binding of O_2 to a hemoglobin subunit in the T state triggers a change in conformation to the R state.
- When the entire protein undergoes this transition, the structures of the individual subunits change little, but the subunit pairs slide past each other and rotate, narrowing the pocket between the subunits.
- In this process, some O_2 with high affinity would bind it efficiently in the lungs but would not release much of it in the tissues. If the protein bound oxygen with a sufficiently low affinity to release it in the tissues, it would not pick up much oxygen in the lungs.

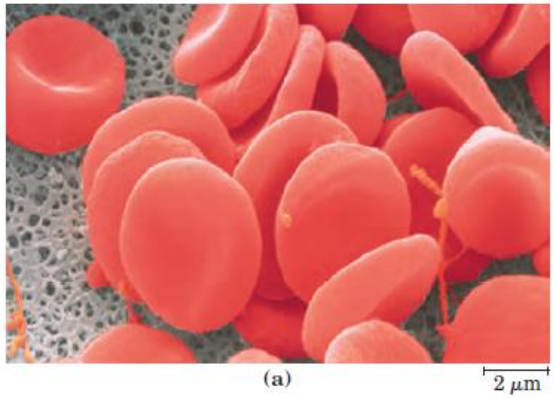
O2 binding to hemoglobin

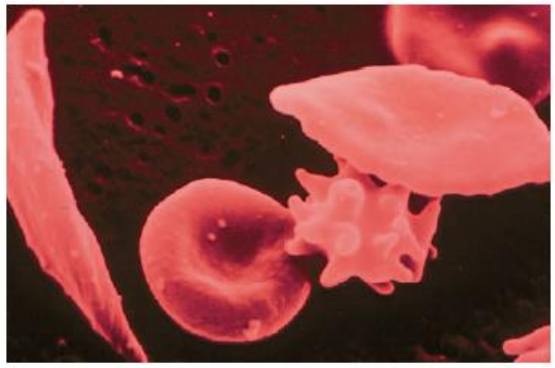
- Hemoglobin solves the problem by undergoing a transition from a low-affinity state (the T state) to a high-affinity state (the R state) as more O2 molecules are bound.
- O2 binding to individual subunits of hemoglobin can alter the affinity for O2 in adjacent subunits.
- The first molecule of O2 that interacts with deoxyhemoglobin binds weakly, because it binds to a subunit in the T state.
- Its binding, however, leads to conformational changes that are communicated to adjacent subunits, making it easier for additional molecules of O2 to bind.

- An **allosteric protein** is one in which the binding of a ligand to one site affects the binding properties of another site on the same protein.
- Cooperative binding of a ligand to a multimeric protein, such as we observe with the binding of O_2 to hemoglobin, is a form of allosteric binding often observed in multimeric proteins.

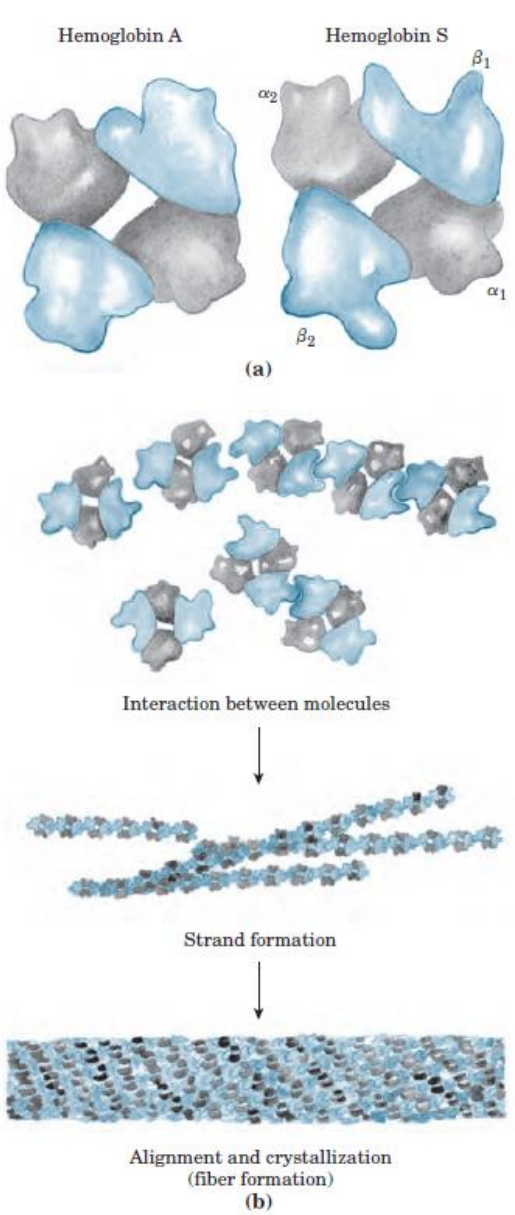
Sickle Cell Anemia

- Sickle-cell anemia is a genetic disease in which an individual has inherited the allele for sickle-cell hemoglobin from both parents. The erythrocytes of these individuals are fewer and also abnormal.
- In addition to an unusually large number of immature cells, the blood contains many long, thin, crescent-shaped erythrocytes that look like the blade of a sickle.
- When hemoglobin from sickle cells (called hemoglobin S) is deoxygenated, it becomes insoluble and forms polymers that aggregate into tubular fibers. Normal hemoglobin (hemoglobin A) remains soluble on deoxygenation.
- The insoluble fibers of deoxygenated hemoglobin S are responsible for the deformed sickle shape of the erythrocytes, and the proportion of sickled cells increases greatly as blood is deoxygenated.





- The altered properties of hemoglobin S result from a single amino acid substitution, a Val instead of a Glu residue at position 6 in the two chains.
- Replacement of the Glu residue by Val creates a “sticky” hydrophobic contact point at position 6 of the chain, which is on the outer surface of the molecule. These sticky spots cause deoxyhemoglobin S molecules to associate abnormally with each other, forming the long, fibrous aggregates characteristic of this disorder.
- Sickle-cell anemia, asoccurs in individuals homozygous for the sickle-cell allele of the gene encoding the subunit of hemoglobin.
- Individuals who receive the sickle-cell allele from only one parent and are thus heterozygous experience a milder condition called sickle-cell trait; only about 1% of their erythrocytes become sickled on deoxygenation. These individuals may live completely normal lives if they avoid vigorous exercise or other stresses on the circulatory system.
- Sickle-cell anemia is a life-threatening and painful disease. They become weak, dizzy, and short of breath, and they alsomexperience heart murmurs and an increased pulse rate.
- Because sickled cells are very fragile and rupture easily; this results in anemia (“lack of blood”).
- An even more serious consequence is that capillaries become blocked by the long, abnormally shaped cells, causing severe pain and interfering with normal organ function—a major factor in the early death of many people with the disease.

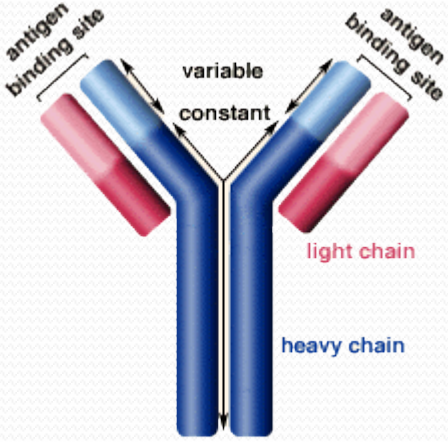


Antibodies

- All vertebrates have an immune system capable of distinguishing molecular “self” from “nonself” and then destroying those entities identified as nonself.
- In this way, the immune system eliminates viruses, bacteria, and other pathogens and molecules that may pose a threat to the organism.
- On a physiological level, the response of the immune system to an invader is an intricate and coordinated set of interactions among many classes of proteins, molecules, and cell types.

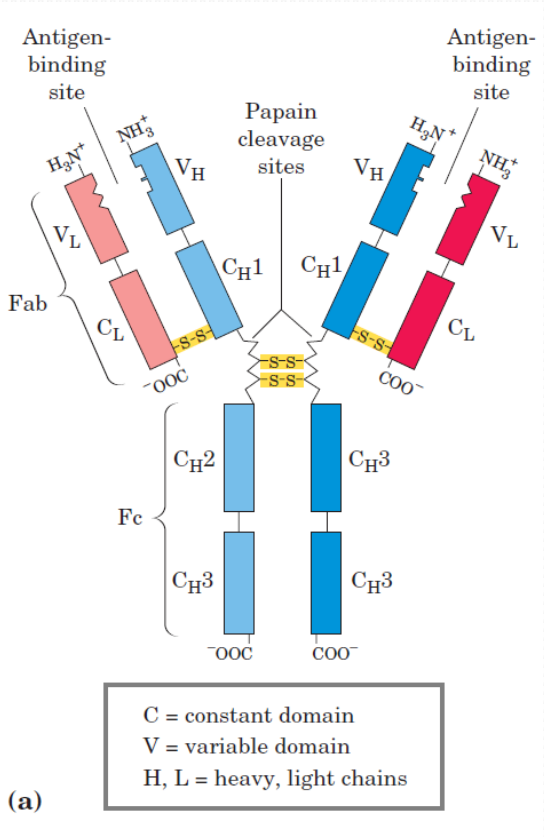
Antibodies

- Humans are capable of producing more than 10^8 different antibodies with distinct binding specificities.
- This extraordinary diversity makes it likely that any chemical structure on the surface of a virus or invading cell will be recognized and bound by one or more antibodies.
- Antibody diversity is derived from random reassembly of a set of immunoglobulin gene segments through genetic recombination mechanisms.
- Antibodies are produced by B cells of the immune system.



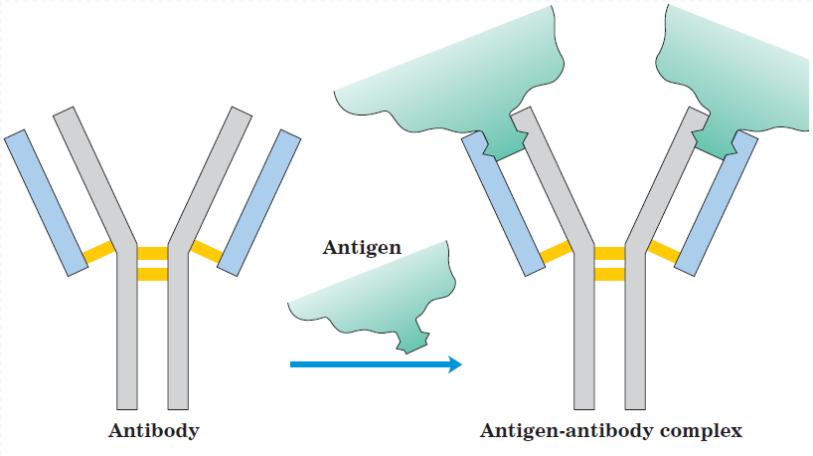
Antibody Structure

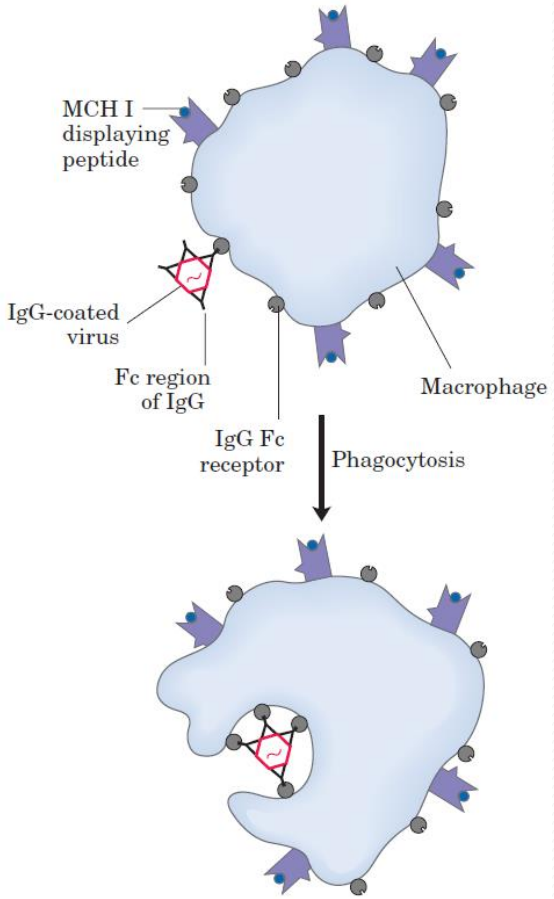
- **Immunoglobulin G (IgG)** is the major class of antibody molecule and one of the most abundant proteins in the blood serum.
- IgG has four polypeptide chains: two large ones, called heavy chains, and two light chains, linked by noncovalent and disulfide bonds.
- The heavy chains of an IgG molecule interact at one end, then branch to interact separately with the light chains, forming a Y-shaped molecule.



Antibody Mechanism of Action

- IgG is produced by memory B cells of the immune system.
- IgG binds to an invading bacterium or virus, it activates certain leukocytes such as macrophages to engulf and destroy the invader.
- A class of receptors on the cell surface of macrophages recognizes and binds the Fc region of IgG. When these Fc receptors bind an antibody-pathogen complex, the macrophage engulfs the complex by phagocytosis.

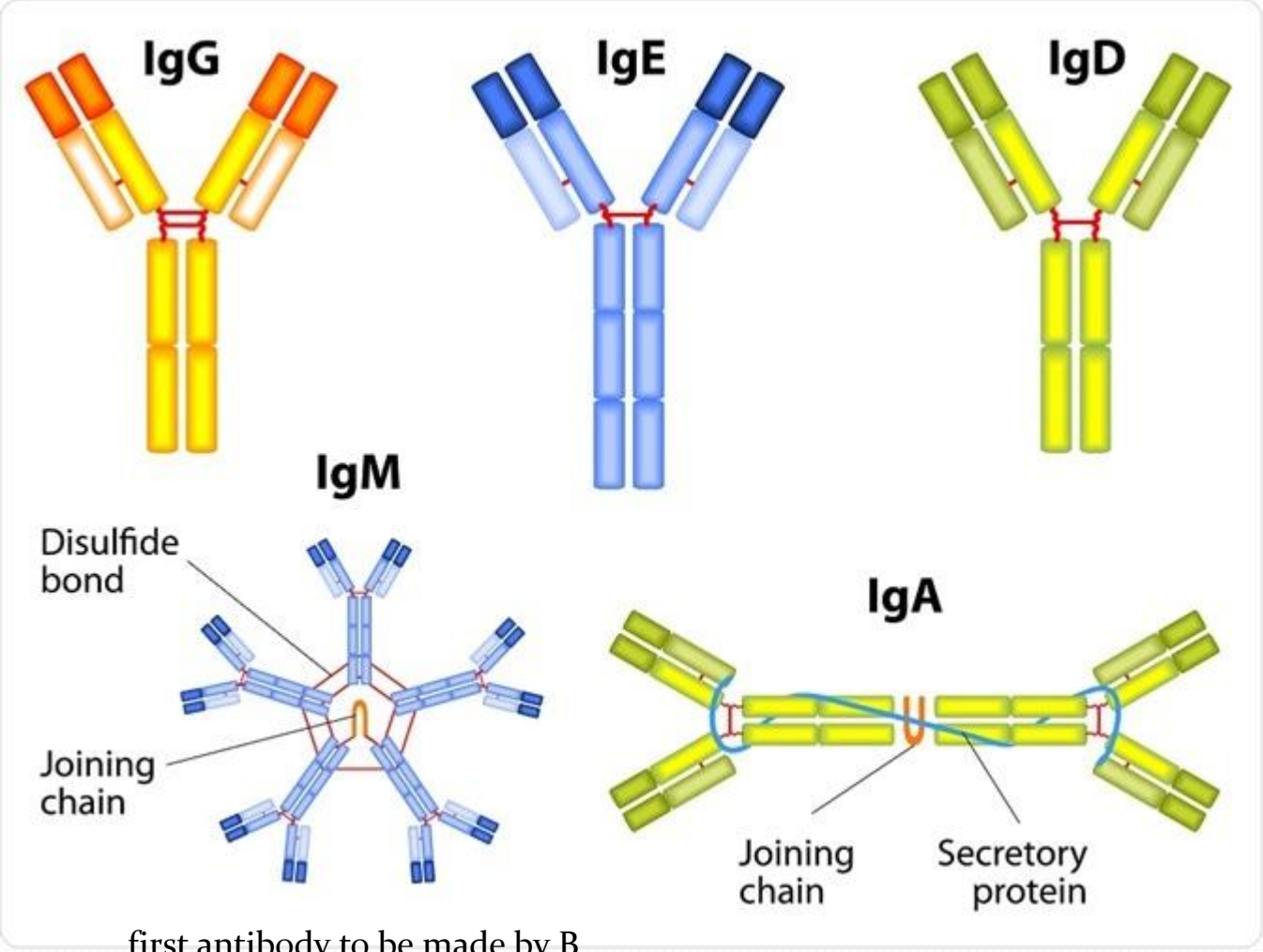




major antibody in secondary immune responses

allergic response

unique function is unclear.



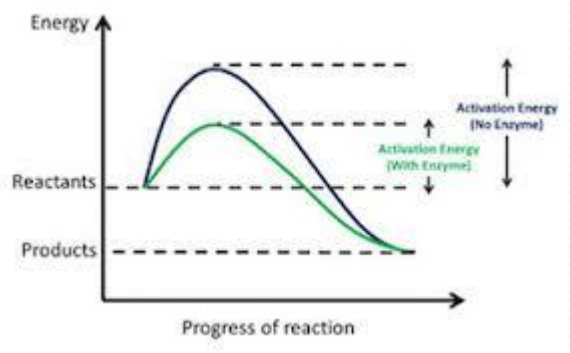
first antibody to be made by B lymphocytes and is the major antibody in the early stages of a primary immune response.

Found principally in secretions such as saliva, tears, and milk

ENZYMES

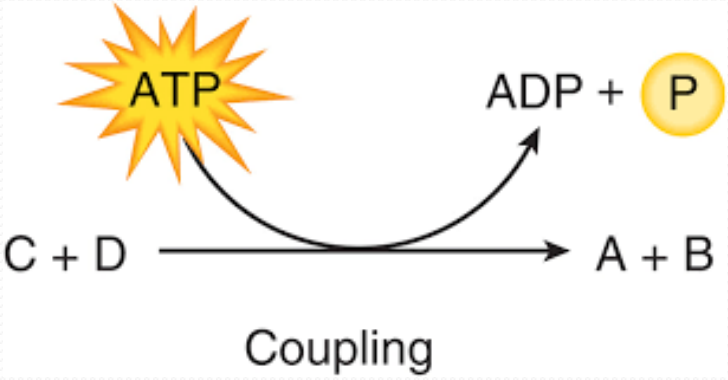
- Enzymes are extraordinarily efficient, selective, biological catalysts (biocatalysts).
- Every living cell has hundreds of different enzymes catalyzing the reactions essential for life—even the simplest living organisms contain hundreds of different enzymes.
- In multicellular organisms, the complement of enzymes differentiates one cell type from another but most of the enzymes are common to all cells. These enzymes catalyze the reactions of the central metabolic pathways necessary for the maintenance of life.

- In the absence of the enzymes, metabolic reactions will not proceed at significant rates under physiological conditions.
- The primary role of enzymes is to enhance the rates of these reactions to make life possible.
- Enzyme-catalyzed reactions are 10^3 to 10^{20} times faster than the corresponding uncatalyzed reactions.
- A catalyst is defined as a substance that speeds up the attainment of equilibrium. It may be temporarily changed during the reaction but it is unchanged in the overall process since it recycles to participate in multiple reactions.
- Reactants bind to a catalyst and products dissociate from it.
- It lowers the amount of energy needed in order for the reaction to proceed.



- Enzymes are highly specific for the reactants, or **substrates**, they act on, but the degree of substrate specificity varies.
- Some enzymes act on a group of related substrates, and others on only a single compound.
- Perhaps the most important aspect of enzyme specificity is **reaction specificity**—that is, the lack of formation of wasteful by-products. Reaction specificity is reflected in the exceptional purity of product (essentially 100%).
- The specificity of enzymes not only saves energy for cells but also precludes the buildup of potentially toxic metabolic by-products.

- Enzymes can do more than simply increase the rate of a single, highly specific reaction.
- Some can also combine, or couple, two reactions that would normally occur separately.
- This property allows the energy gained from one reaction to be used in a second reaction.
- Coupled reactions are a common feature of many enzymes—the hydrolysis of ATP, for example, is often coupled to less favorable metabolic reactions.



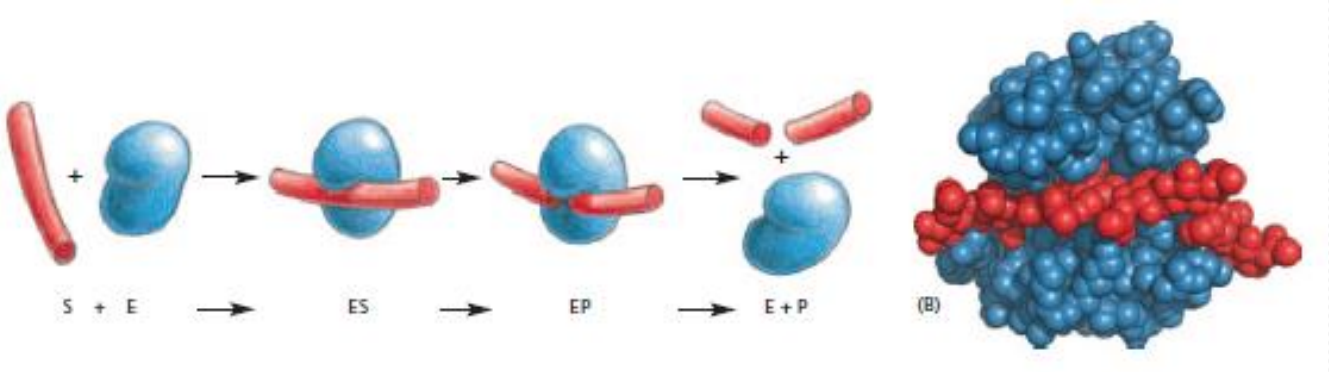
- Enzymes:

(1) they function as catalysts,

(2) they catalyze highly specific reactions,

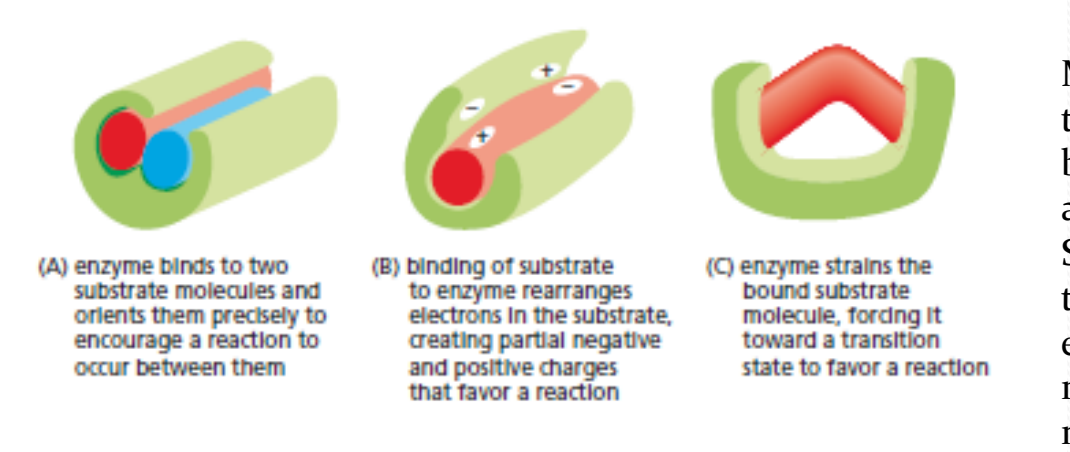
(3) they can couple reactions,

(4) their activity can be regulated.



Enzyme Mehanism of Action

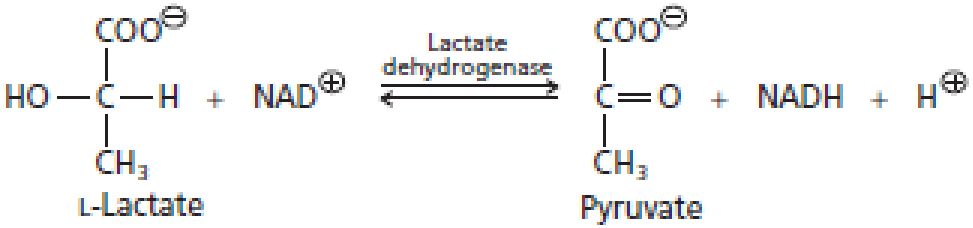
- In reactions involving two or more substrates, the active site acts like a template or mold that brings the reactants together in the proper orientation for the reaction to occur.
- The active site of an enzyme contains precisely positioned chemical groups that speed up the reaction by altering the distribution of electrons in the substrates.
- Binding to the enzyme also changes the shape of the substrate, bending bonds so as to drive the bound molecule toward a particular transition state.



Many enzymes participate intimately in the reaction by briefly forming a covalent bond between the substrate and an amino acid side chain in the active site. Subsequent steps in the reaction restore the side chain to its original state, so the enzyme remains unchanged after the reaction and can go on to catalyze many more reactions.

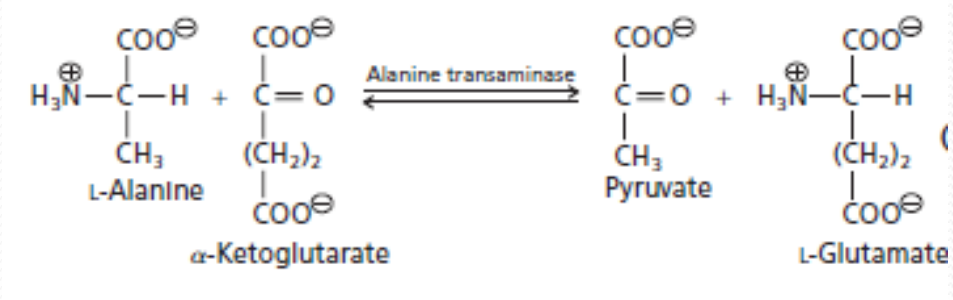
Oxidoreductases

- **Oxidoreductases** catalyze oxidation–reduction reactions.



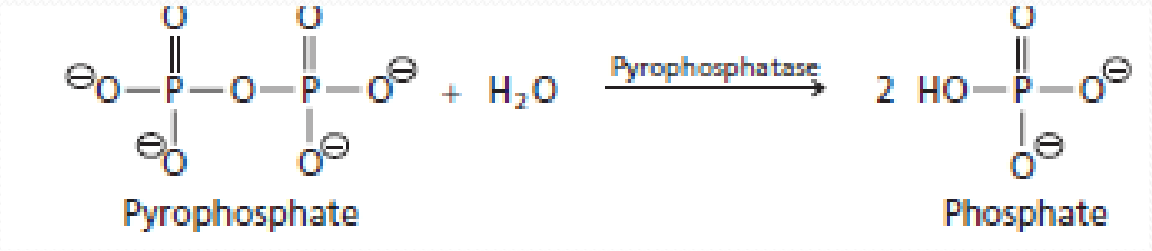
Transferases

- **Transferases** catalyze group transfer reactions and many require the presence of coenzymes.
- In group transfer reactions a portion of the substrate molecule usually binds covalently to the enzyme or its coenzyme.
- This group includes kinases, enzymes that catalyze the transfer of a phosphoryl group from ATP.



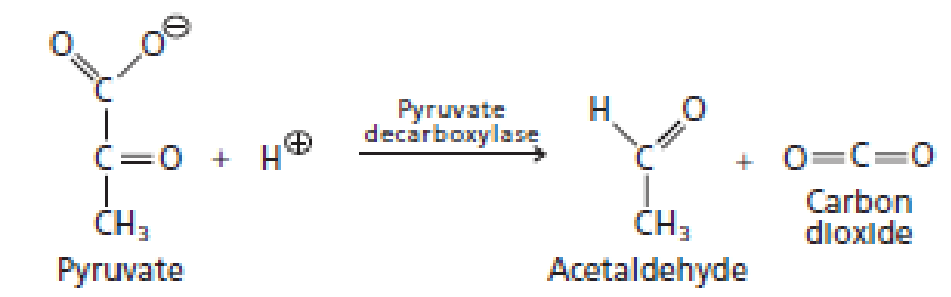
Hydrolases

- **Hydrolases** catalyze hydrolysis. They are a special class of transferases with water serving as the acceptor of the group transferred.



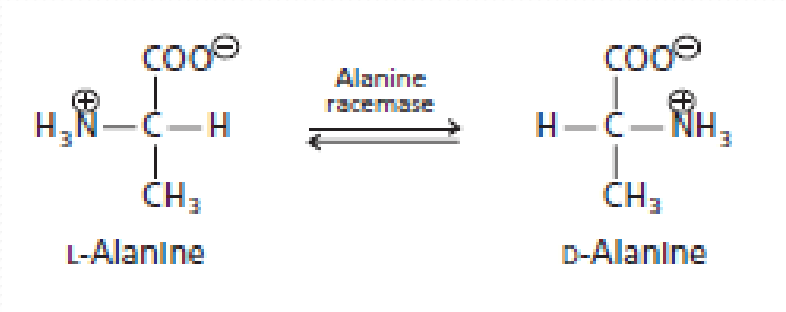
Lyases

- **Lyases** catalyze lysis of a substrate generating a double bond in nonhydrolytic, nonoxidative, elimination reactions.
- In the reverse direction, lyases catalyze the addition of one substrate to the double bond of a second substrate.



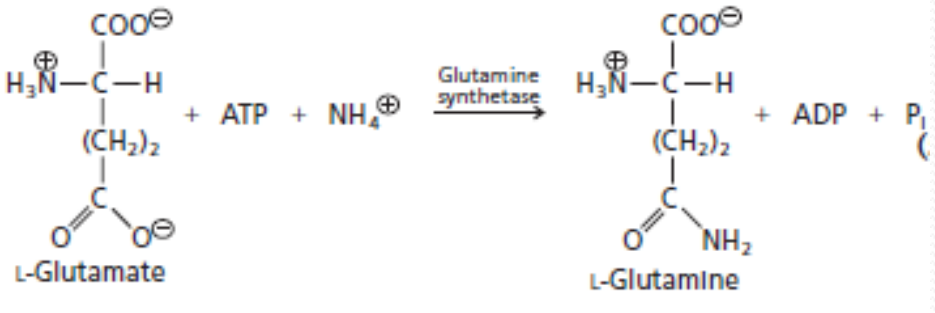
Isomerases

- **Isomerases** catalyze structural change within a single molecule (isomerization reactions).
- Because these reactions have only one substrate and one product, they are among the simplest enzymatic reactions.

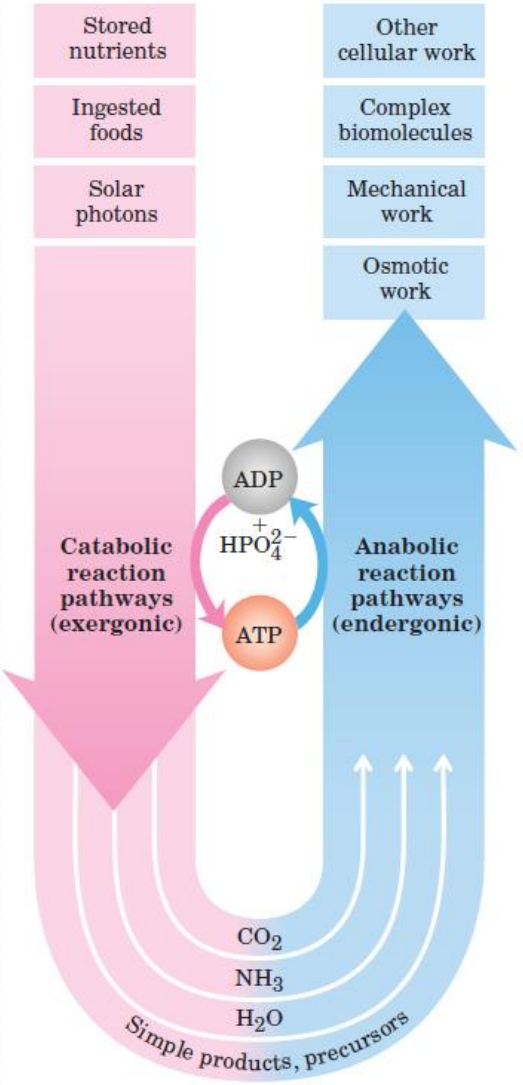


Ligases

- **Ligases** catalyze ligation, or joining, of two substrates. These reactions require the input of chemical potential energy in the form of a nucleoside triphosphate such as ATP.

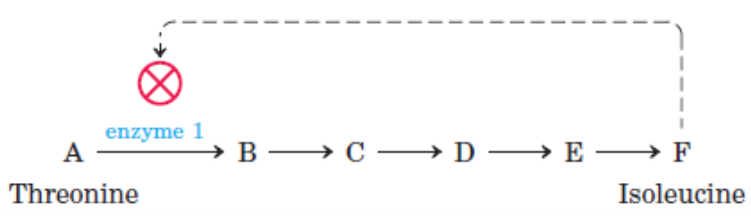


- The thousands of enzyme-catalyzed chemical reactions in cells are functionally organized into many sequences of consecutive reactions, called **pathways,** in which the product of one reaction becomes the reactant in the next.
- Some pathways degrade organic nutrients into simple end products in order to extract chemical energy and convert it into a form useful to the cell; together these degradative, free-energy-yielding reactions are designated **catabolism.**
- Other pathways start with small precursor molecules and convert them to progressively larger and more complex molecules, including proteins and nucleic acids. Such synthetic pathways, which invariably require the input of energy, are collectively designated **anabolism.**
- The overall network of enzyme-catalyzed pathways constitutes cellular **metabolism.**
- ATP is the major connecting link (the shared intermediate) between the catabolic and anabolic components of this network.

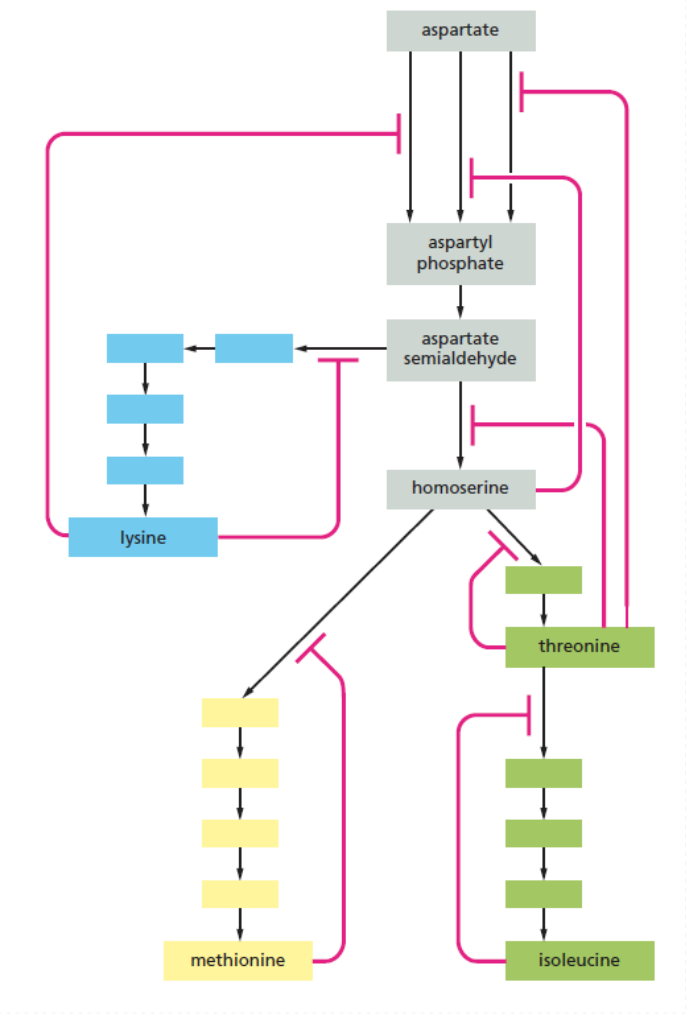


Metabolism Is Regulated to Achieve Balance and Economy

- Key enzymes in each metabolic pathway are regulated so that each type of precursor molecule is produced in a quantity appropriate to the current requirements of the cell.
- A common type of control occurs when a molecule other than a substrate specifically binds to an enzyme at a special *regulatory site*, altering the rate at which the enzyme converts its substrate to product.
- In feedback inhibition, for example, an enzyme acting early in a reaction pathway is inhibited by a late product of that pathway. Thus, whenever large quantities of the final product begin to accumulate, the product binds to an earlier enzyme and slows down its catalytic action, limiting further entry of substrates into that reaction pathway.
- Where pathways branch or intersect, there are usually multiple points of control by different final products, each of which works to regulate its own synthesis.
- Feedback inhibition can work almost instantaneously and is rapidly reversed when product levels fall.



Consider the pathway in *E. coli* that leads to the synthesis of the amino acid isoleucine, a constituent of proteins. The pathway has five steps catalyzed by five different enzymes. If a cell begins to produce more isoleucine than is needed for protein synthesis, the unused isoleucine accumulates and the increased concentration inhibits the catalytic activity of the first enzyme in the pathway, immediately slowing the production of isoleucine. Such **feedback inhibition** keeps the production and utilization of each metabolic intermediate in balance.

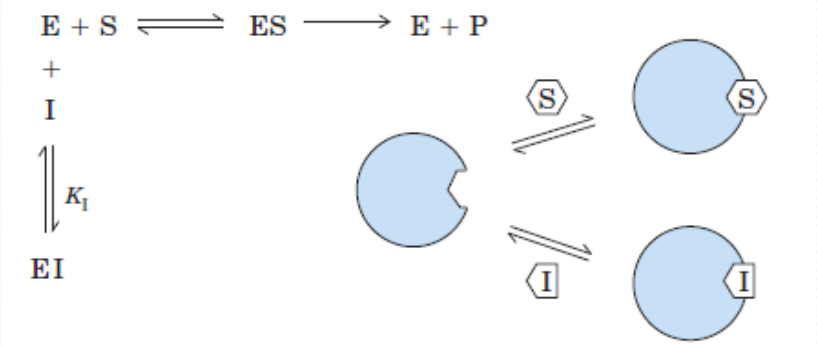


Feedback inhibition at multiple points regulates connected metabolic pathways.

- During feedback inhibition, for example, the binding of an inhibitor at a **regulatory site** on the protein causes the protein to shift to a conformation in which its **active site**—located elsewhere in the protein—becomes less accommodating to the substrate molecule.

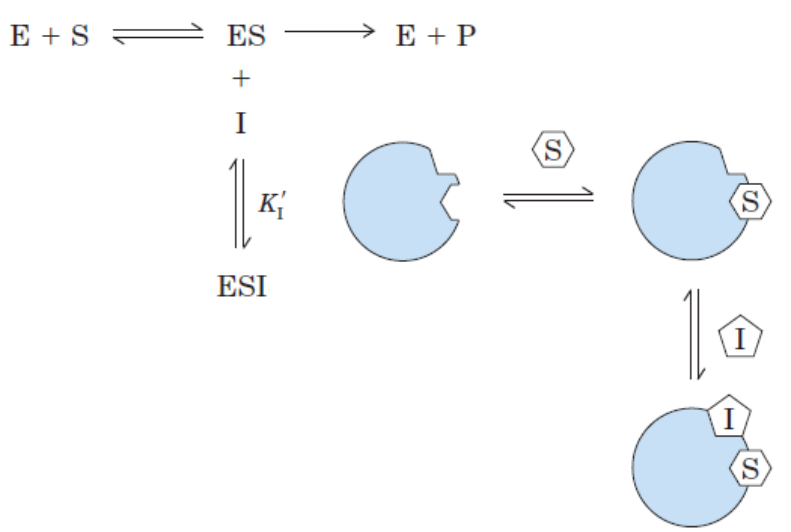
Competitive Inhibition

- **Competitive inhibitor** competes with the substrate for the active site of an enzyme. While the inhibitor (I) occupies the active site it prevents binding of the substrate to the enzyme.
- Many competitive inhibitors are compounds that resemble the substrate and combine with the enzyme to form an EI complex, but without leading to catalysis.



Uncompetitive inhibition

- An **uncompetitive inhibitor** binds at a site distinct from the substrate active site and, unlike a competitive inhibitor, binds only to the ES complex.



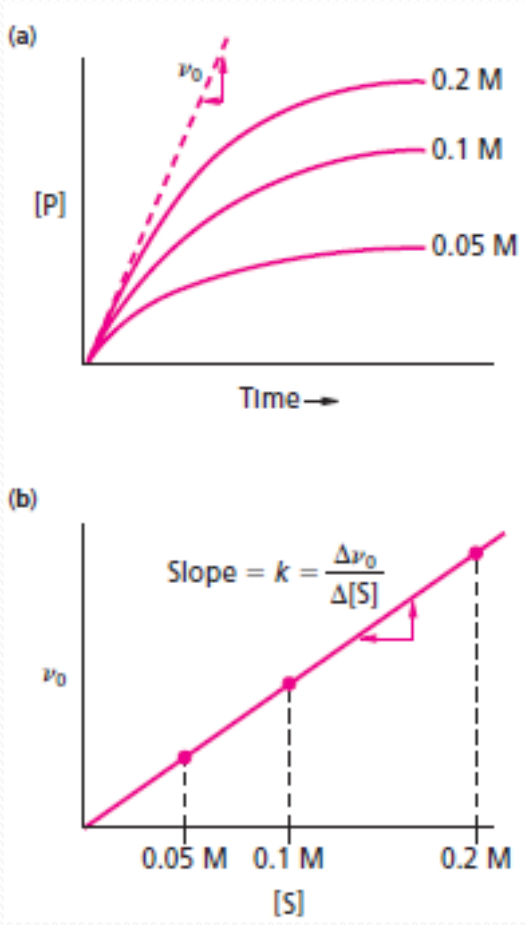
Enzyme Kinetics

- Kinetic experiments examine the relationship between the amount of product (P) formed in a unit of time ($\Delta[P]/\Delta t$) and the experimental conditions under which the reaction takes place.
- The basis of most kinetic measurements is the observation that the rate, or **velocity** (v), of a reaction varies directly with the concentration of each reactant.
- This observation is expressed in a **rate equation**. The rate equation for the nonenzymatic conversion of substrate (S) to product is written as:

$$\frac{\Delta[P]}{\Delta t} = v = k[S]$$

Enzyme Kinetics

- The rate equation reflects the fact that the velocity of a reaction depends on the concentration of the substrate ([S]). The symbol *k* is the rate constant and indicates the speed or efficiency of a reaction.
- As a reaction proceeds, the amount of product ([P]) increases and the amount of substrate ([S]) decreases. The velocity decreases over time as expected since the substrate is being depleted.
- The initial velocity (*v*o) can be determined from the slope of the progress curves (Figure a) or from the derivatives of the curves.
- A graph of initial velocity versus substrate concentration at the beginning of the experiment gives a straight line as shown in Figure b and the slope of this curve is the rate constant.

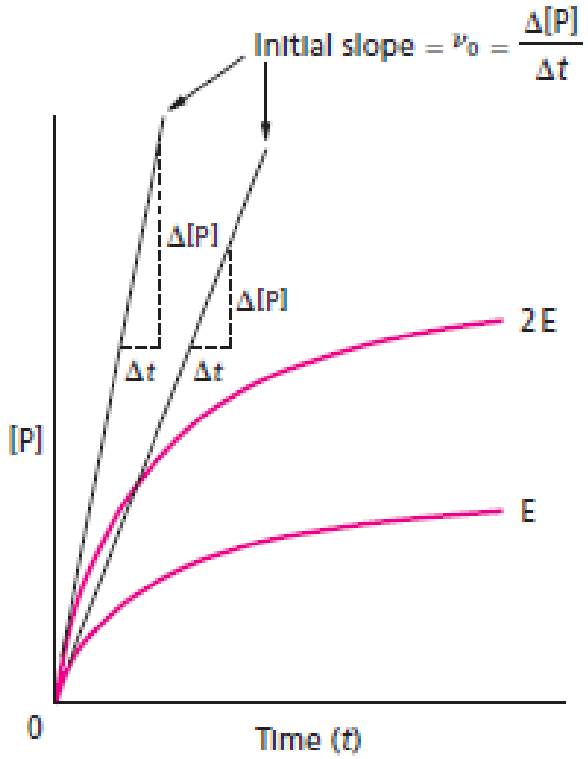


Enzyme Kinetics

- Enzymes bind substrates transiently. Think of an enzyme as lock, and the substrate as a matching key. Only specific substrates can fit into a given enzyme.
- Enzyme (E) binds a substrate to form an **enzyme–substrate complex (ES)**. ES complexes are formed when ligands bind noncovalently in their proper places in the active site.
- The substrate interacts transiently with the protein catalyst (and with other substrates in a multisubstrate reaction) on its way to forming the product of the reaction.

$$E + S \longrightarrow ES \longrightarrow E + P$$

- This reaction takes place in two distinct steps—**the formation of the enzyme–substrate complex** and the actual chemical reaction accompanied by **the dissociation of the enzyme and product**.
- Each step has a characteristic rate. The overall rate of an enzymatic reaction depends on the concentrations of both the substrate and the catalyst (enzyme).
- When the amount of enzyme is much less than the amount of substrate the reaction will depend on the amount of enzyme.
- The more enzyme present, the faster the reaction.



Michaelis-Menten Equation

The shape of the v_o vs. [S] curve is that of a rectangular hyperbola. The equation for a rectangular hyperbola is

$$y = \frac{ax}{b + x}$$

where *a* is the asymptote of the curve (the value of *y* at an infinite value of *x*) and *b* is the point on the *x* axis corresponding to a value of *a*/2.

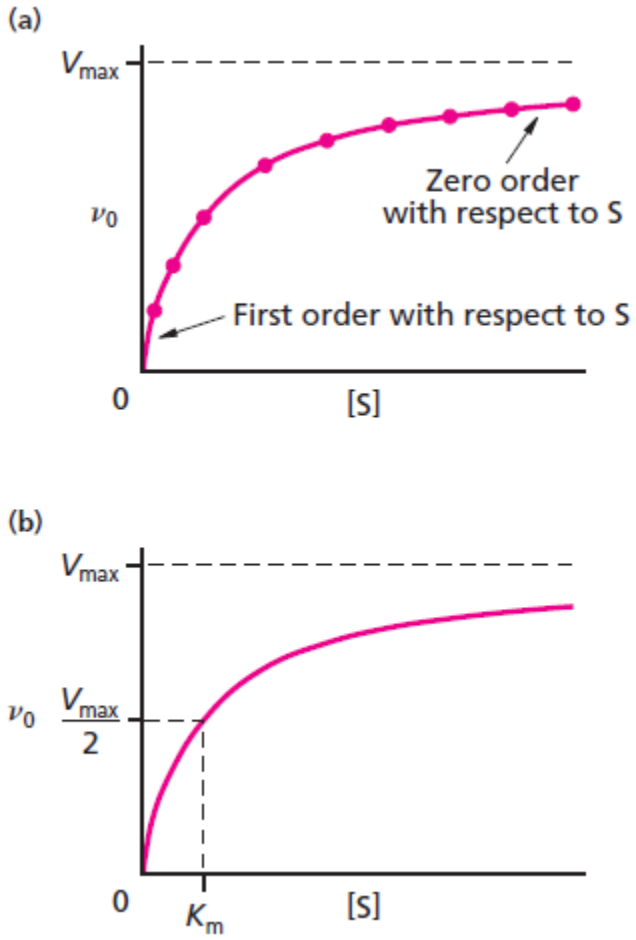
In enzyme kinetic experiments, $y = v_o$ and x = [S]. The asymptote value (*a*) is called $\mathbf{V_{max}}$. It's the maximum velocity of the reaction at infinitely large substrate concentrations.

Michaelis constant (*K*m) defined as the concentration of substrate when v_o is equal to one-half V_{max}

$$\text{Km} = [\text{S}] \text{ when } V_o = 1/2 V_{max}$$

The complete rate equation is called the **Michaelis-Menten Equation**:

$$v_0 = \frac{V_{max}[S]}{K_m + [S]}$$



Michaelis constant (Km)

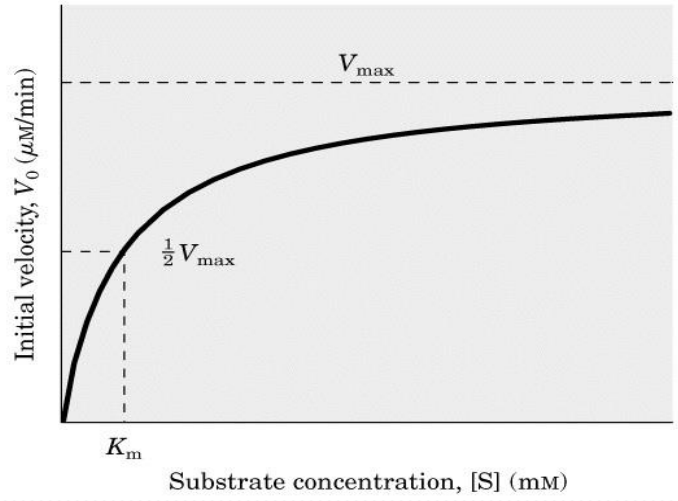
- *K*m is the substrate concentration when the initial velocity is one-half the *V*max value.
- *K*m is the same as the equilibrium constant for dissociation of the ES complex to E + S. Thus, *K*m becomes a measure of the affinity of E for S.
- The lower the value of *K*m, the more tightly the substrate is bound.
- *K*m values are sometimes used to distinguish between different enzymes that catalyze the same reaction. For example, mammals have several different forms of lactate dehydrogenase, each with a distinct *K*m value.
- *K*m is also a measure of the stability of the ES complex.

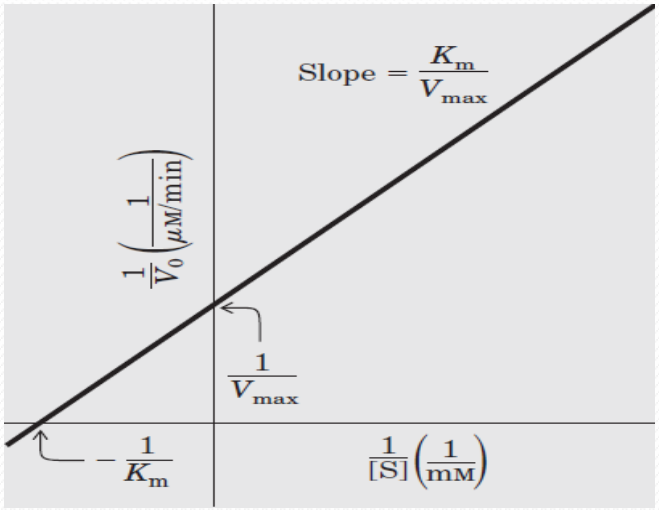
Lineweaver-Burk Equation

- It is not easy to determine V_{max} using the Michaelis Menten plot constructed with the experimental values obtained in an enzyme kinetics study. Only an approximation is possible.
- Lineweaver and Burk simplified the Michaelis-Menten equation to:

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

- A plot of $1/V_o$ versus $1/[S]$ (the "double reciprocal" of the Vo versus [S] plot we have been using to this point) yields a straight line.
- This line has a slope of K_m/V_{max}, an intercept of $1/V_{max}$ on the 1/Vo axis, and an intercept of $-1/K_m$ on the 1/[S] axis. The double-reciprocal presentation, also called a Lineweaver-Burk plot, has the great advantage of allowing a more accurate determination of V_{max}.

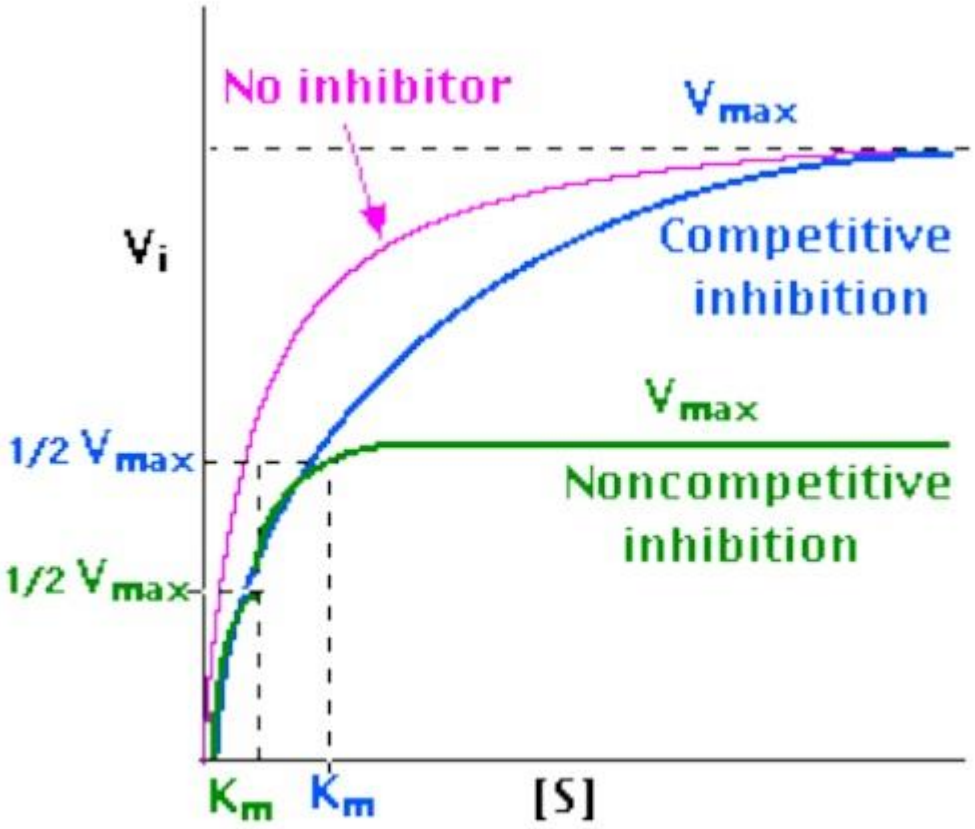




- k_{cat} is equivalent to the number of substrate molecules converted to product in a given unit of time on a single enzyme molecule when the enzyme is saturated with substrate.

$$k_{cat} = V_{max}/[E]$$

- k_{cat} is also called the **turnover number.**
- It shows:
  - how fast ES complex proceeds to E + P.
  - Number of catalytic cycles that each active site undergoes per unit time.
  - Rate constant of the reaction when enzyme is saturated with substrate.



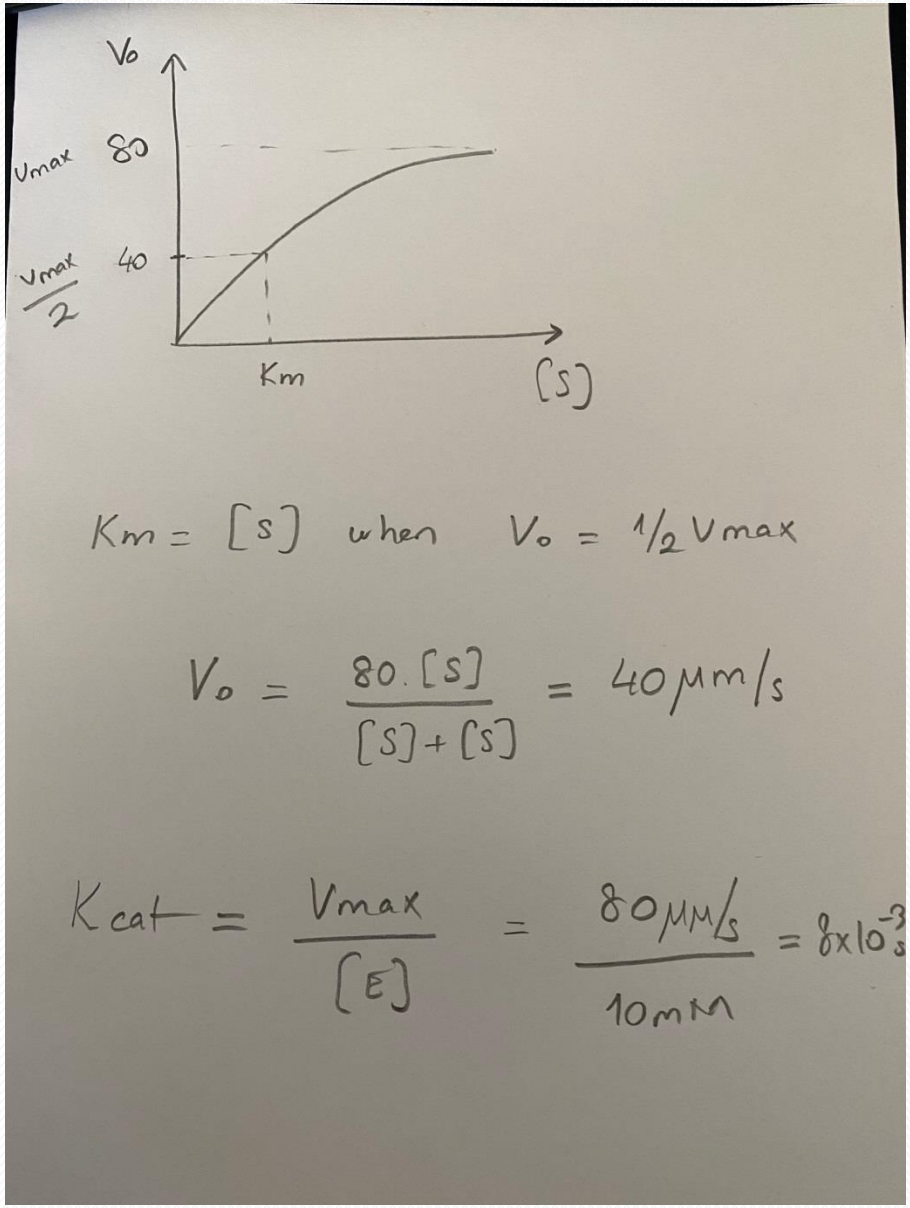
Michaelis–Menten curves for enzyme with or without inhibitor

Catalytic Efficiency

- Efficiency of the enzyme
- k_{cat} / K_m

Example

- Determine the initial velocity of an enzyme, E, when conditions are such that the substrate concentration used is enough to produce half of the enzyme's max rate which is 40 μM/s. If the [E] is 10 mM determine k_{cat}.


Vo
Vmax
80
Vmax/2
40
Km
[S]
Km = [S] when Vo = 1/2 Vmax
Vo = 80.[S] / [S]+[S] = 40 μm/s
Kcat = Vmax / [E] = 80 μM/s / 10 mM = 8x10^-3 s^-1

- Catalytic repertoire of an organism is not limited by the reactivity of amino acid side chains.
- **Cofactors** also participate in catalysis.
- Cofactors are required by inactive **apoenzymes** (proteins only) to convert them to active **holoenzymes**.
- There are two types of cofactors: **essential ions** (mostly metal ions) and organic compounds known as **coenzymes.**