

Protein structure and function

Functions of proteins in humans:

Proteins are the most abundant and functionally diverse molecules in living systems. Virtually every life process depends on this class of molecules, for example:

- 1- Enzymes catalyze chemical reactions.
- 2- Contractile proteins in muscle permit movement.
- 3- In bone, the protein collagen forms a framework for the deposition of calcium phosphate crystals, acting like the steel cables in reinforced concrete.
- 4- In the bloodstream, proteins, such as hemoglobin and plasma albumin, shuttle molecules essential to life, whereas immunoglobulins fight infectious bacteria and viruses.
- 5- Many hormones are proteins or peptides. Protein hormones include insulin, prolactin and LH. Important peptide hormones include glucagon, ADH and calcitonin.

I-AMINO ACIDS:

Proteins are polymers of amino acids:

Although more than 300 different amino acids have been described in nature, only 20 are commonly found as constituents of mammalian proteins. These common amino acids are defined as those for which at least one codon exists in the genetic code. Proteins may also contain derived amino acids, which are usually formed by an enzymatic reaction on a common amino acid has been incorporated into a protein structure. Examples of derived amino acids are cystine, hydroxyproline and hydroxylysine.

Structure of amino acid:

Each amino acid (except for proline, which has a secondary amino group) has a carboxyl group, a primary amino group, and a distinctive side chain ("R-group") bonded to the α -carbon atom (Figure 1.1A).

At physiologic pH (approximately pH 7.4), the carboxyl group is dissociated, forming the negatively charged carboxylate ion ($-\text{COO}^-$), and the amino group is protonated ($-\text{NH}_3^+$). In proteins, almost all of these carboxyl and amino groups are combined through peptide linkage and, in general, are not available for chemical reaction except for hydrogen bond formation (Figure 1.1B).

Thus, it is the nature of the side chains that ultimately dictates the role an amino acid plays in a protein. It is, therefore, useful to classify the amino acids according to the properties of their side chains, that is, whether they are nonpolar (have an even distribution of electrons) or polar (have an uneven distribution of electrons, such as acids and bases)

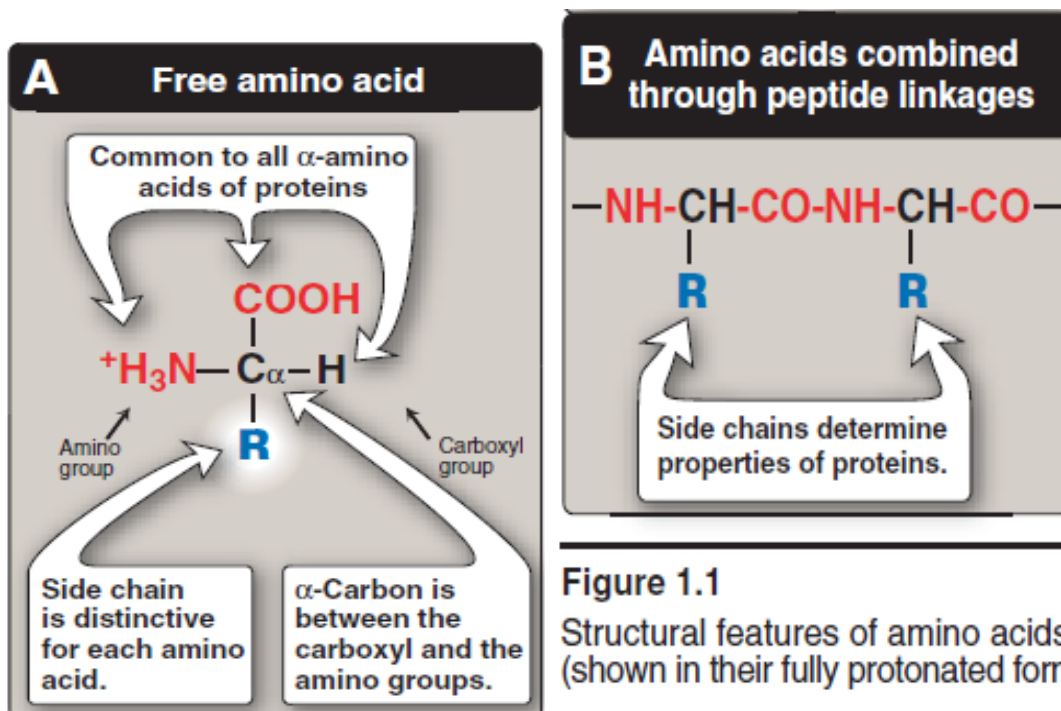
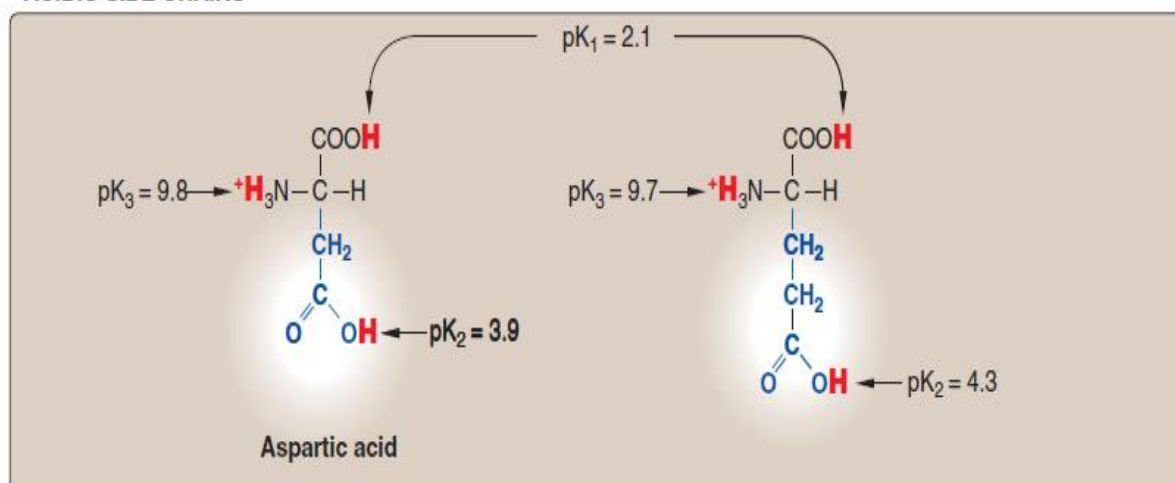
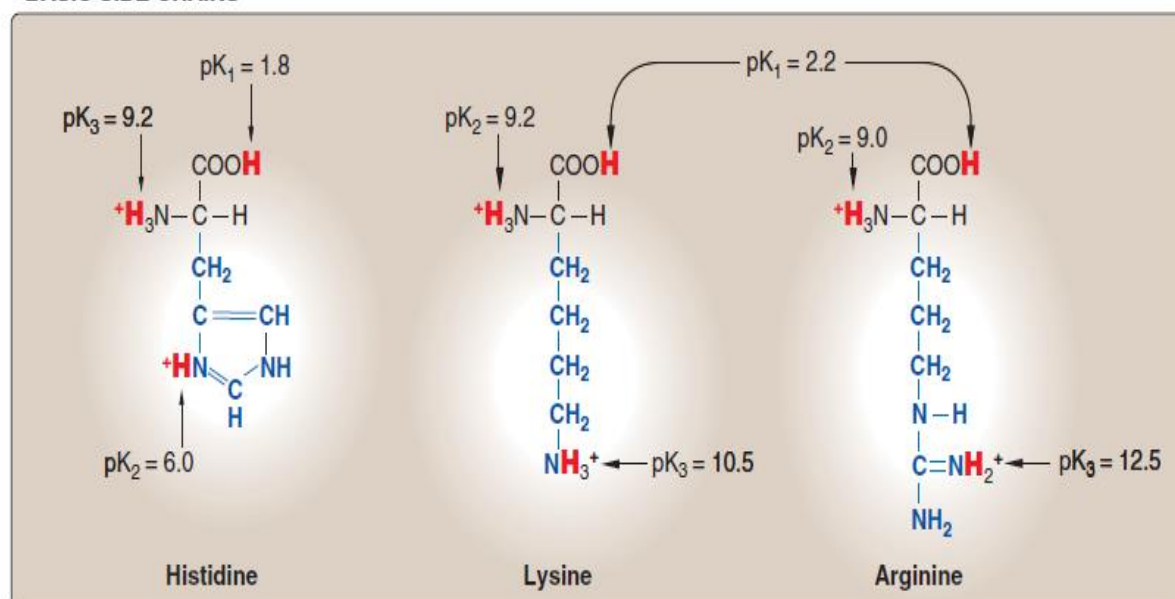


Figure 1.1
Structural features of amino acids (shown in their fully protonated form).

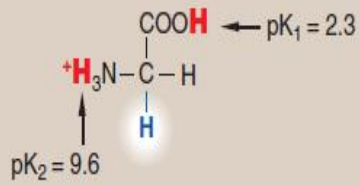
ACIDIC SIDE CHAINS



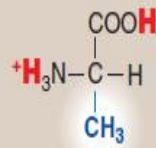
BASIC SIDE CHAINS



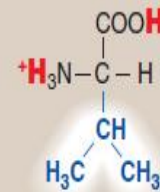
NONPOLAR SIDE CHAINS



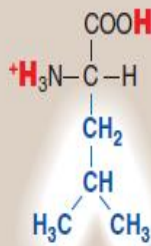
Glycine



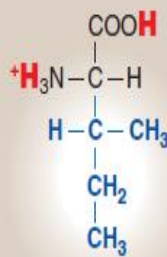
Alanine



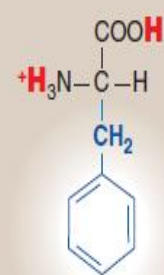
Valine



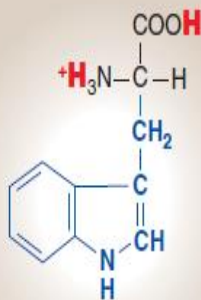
Leucine



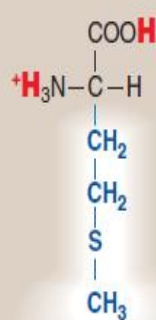
Isoleucine



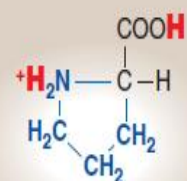
Phenylalanine



Tryptophan

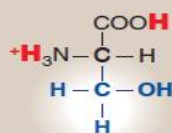


Methionine

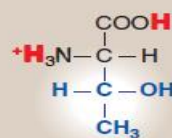


Proline

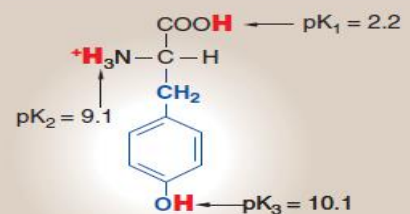
UNCHARGED POLAR SIDE CHAINS



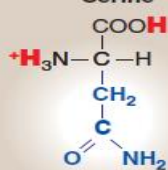
Serine



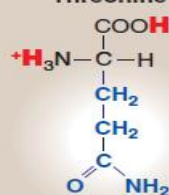
Threonine



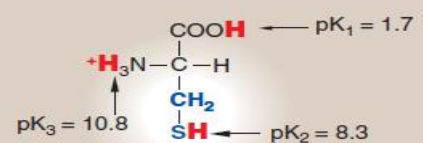
Tyrosine



Asparagine



Glutamine



Cysteine

Side chains define chemical nature and structures of different amino acids:

- **Alkyl amino acids** have alkyl group side chains and include: glycine, alanine, valine, leucine, and isoleucine. Glycine has a simplest structure with $R=H$.
- **The aromatic amino acids** are phenylalanine, tyrosine, and tryptophan.
- **Sulfur-containing amino acids** are cysteine and methionine.
- **Cysteine** contain a sulfhydryl group ($-SH$), in proteins the $-SH$ groups of two proteins are oxidized to form a dimer cystine which contains a covalent cross-link called a disulfide bond.
- **The hydroxyl-containing amino acids** are serine, tyrosin and threonine that can participate in hydrogen bond formation.
- **asparagine and glutamine** each contain a carbonyl group and amide group both of which can also participate in hydrogen bonds.
- **Proline** is unique in structure that is incorporates the alpha-amino group in its side chain. Then it has a secondary amino group.
- **The dicarboxylic monoamino acids** contain a carboxylic group in their side chain, aspartate and glutamate at physiological PH negatively charged.
- **The dibasic monocarboxylic acids** include lysine, arginine, and histidine at physiological PH they are positively charged.
- **Amino acids with nonpolar side chains** promotes hydrophobic interaction

Optical properties of amino acids

The α -carbon of an amino acid is attached to four different chemical groups and is, therefore, a chiral or optically active carbon atom. Glycine is the exception because its α -carbon has two hydrogen substituents and, therefore, is optically inactive.

Amino acids that have an asymmetric center at the α -carbon can exist in two forms, designated D and L, that are mirror images of each other (Figure 1.8). The two forms in each pair are termed stereoisomers, optical isomers, or enantiomers. All amino acids found in proteins are of the L-configuration. However, D-amino acids are found in some antibiotics and in plant and bacterial cell walls.

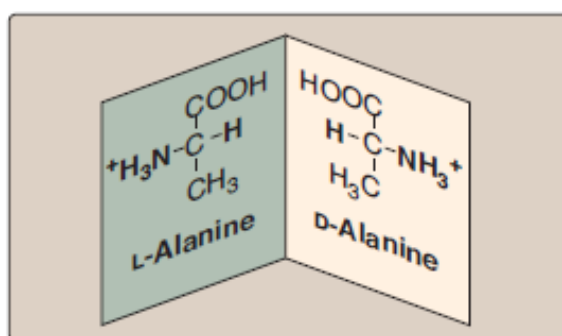


Figure 1.8
D and L forms of alanine
are mirror images.

Acidic and basic properties of amino acids:

At physiologic pH, amino acids have a negatively charged group (--COO^-) and a positively charged group (--NH_3^+), both attached to the α -carbon.

[Note: the side chains of Glutamic acid and aspartic acid contain a negatively charged carboxylate group --COO^- . The side chains of Histidine, Arginine, and Lysine are positively charged] **Substances, such as amino acids, that can act either as an acid or a base are defined as amphoteric.**

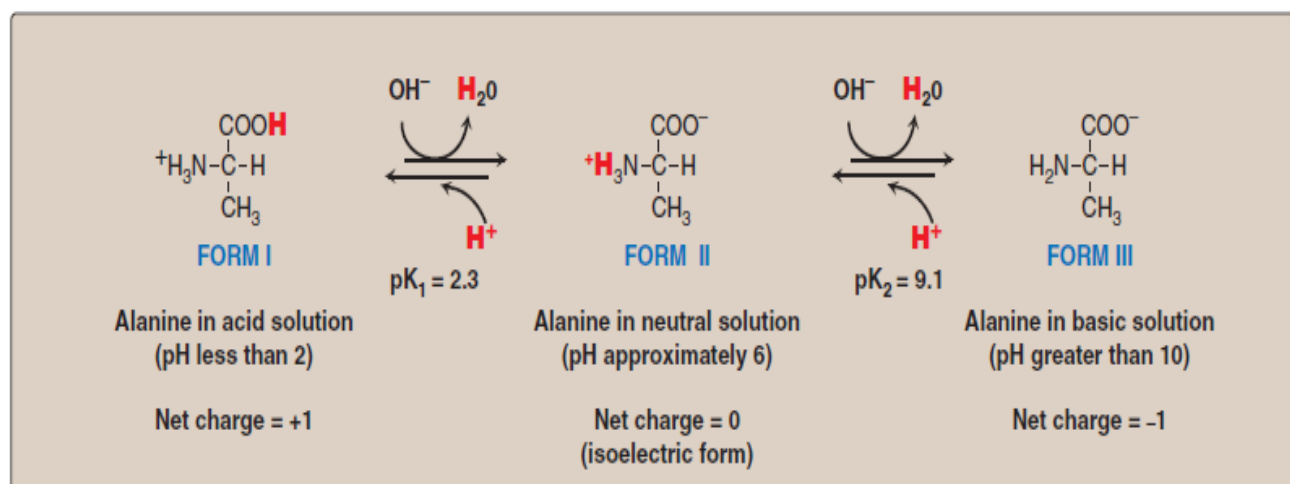


Figure 1.10
Ionic forms of alanine in acidic, neutral, and basic solutions.

II-STRUCTURE OF PROTEINS

The complexity of protein structure is best analyzed by considering the molecule in terms of four organizational levels, namely, primary, secondary, tertiary, and quaternary.

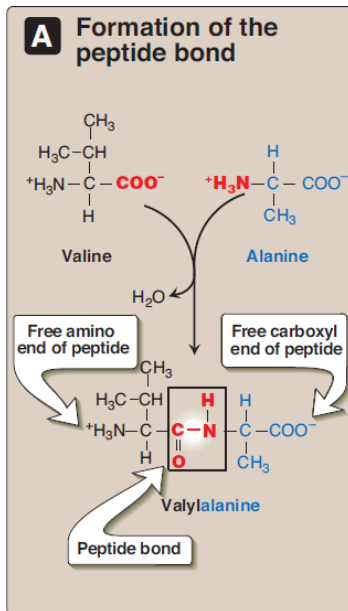
1- Primary structure of proteins:

The sequence of amino acids in a protein is called the primary structure of the protein. Understanding the primary structure of proteins is important because many genetic diseases result in proteins with abnormal amino acid sequences, which cause improper folding and loss or impairment of normal function. If the primary structures of the normal and the mutated proteins are known, this information may be used to diagnose or study the disease.

Peptide bond:

In proteins, amino acids are joined covalently by peptide bonds, which are amide linkages between the α -carboxyl group of one amino acid and the α -amino group of another. For example, valine and alanine can form the dipeptide valylalanine through the formation of a peptide bond (Figure below).

Peptide bonds are not broken by conditions that denature proteins, such as heating or high concentrations of urea. Prolonged exposure to a strong acid or base at elevated temperatures is required to hydrolyze these bonds nonenzymically.



Naming the peptide: By convention, the free amino end (N-terminal) of the peptide chain is written to the left and the free carboxyl end (C-terminal) to the right. Therefore, all amino acid sequences are read from the N- to the C-terminal end of the peptide.

Linkage of many amino acids through peptide bonds results in an unbranched chain called a polypeptide. Each component amino acid in a polypeptide is called a “residue” because it is the portion of the amino acid remaining after the atoms of water are lost in the formation of the peptide bond.

Example: The primary structure of insulin illustrates the value of knowledge for understanding a protein's biosynthesis and physiological forms. Insulin is produced in pancreatic islet cells as a single-chain, inactive precursor, proinsulin. Insulin consists of two polypeptide chains A and B covalently joined

by disulfide bonds

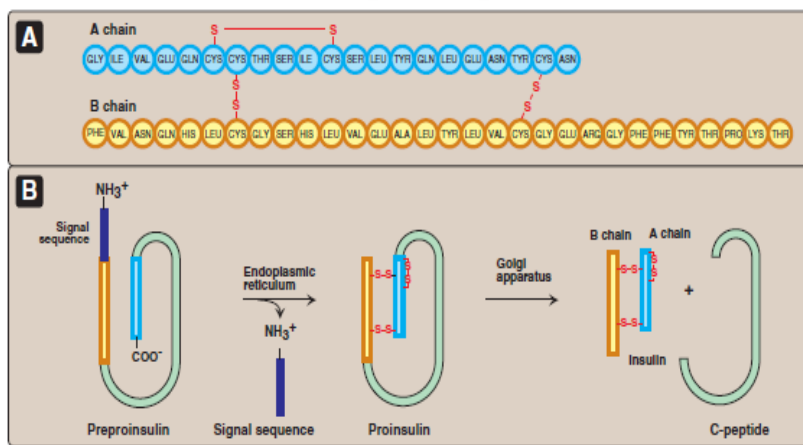


Figure 23.3
A. Structure of insulin. B. Formation of human insulin from preproinsulin.

2- Secondary structure of proteins:

The polypeptide backbone does not assume a random three-dimensional structure, but instead generally forms regular arrangements of amino acids that are located near to each other in the linear sequence. These arrangements are termed the secondary structure of the polypeptide. The α -helix, and β -sheet, they are examples of secondary structures frequently encountered in proteins. Side chains not considered at this level.

A. α -Helix

Several different polypeptide helices are found in nature, but the α -helix is the most common. It is a spiral structure, consisting of a tightly packed, coiled polypeptide backbone core, with the side chains of the component amino acids extending outward from the central axis to avoid interfering sterically with each other (Figure 2.6). A very diverse group of proteins contains α -helices. For example, the **keratins** they are a major component of tissues such as hair and skin, **and myoglobin**, whose structure also highly α -helical, is a globular, flexible molecule.

1. Hydrogen bonds: An α -helix is stabilized by extensive hydrogen bonding between the peptide-bond carbonyl oxygens and amide hydrogens that are part of the polypeptide backbone (see Figure 2.6). Hydrogen bonds are individually weak, but they collectively serve to stabilize the helix.

2. Amino acids per turn: Each turn of an α -helix contains 3.6 amino acids. Thus, amino acid residues spaced three or four residues apart in the primary sequence are spatially close together when folded in the α -helix.

3. Amino acids that disrupt an α -helix:

- **Proline** disrupts an α -helix because its secondary amino group is not geometrically compatible with the right-handed spiral of the α -helix. Instead, it inserts a kink in the chain, which interferes with the smooth, helical structure.
- **Large numbers of charged amino acids** (for example, glutamate, aspartate, histidine, lysine, or arginine) also disrupt the helix by forming ionic bonds, or by electrostatically repelling each other.
- **Finally, amino acids with bulky side chains**, such as tryptophan, or amino acids, such as valine or isoleucine, that branch at the β -carbon (the first carbon in the R-group, next to the α -carbon) can interfere with formation of the α -helix if they are present in large numbers.

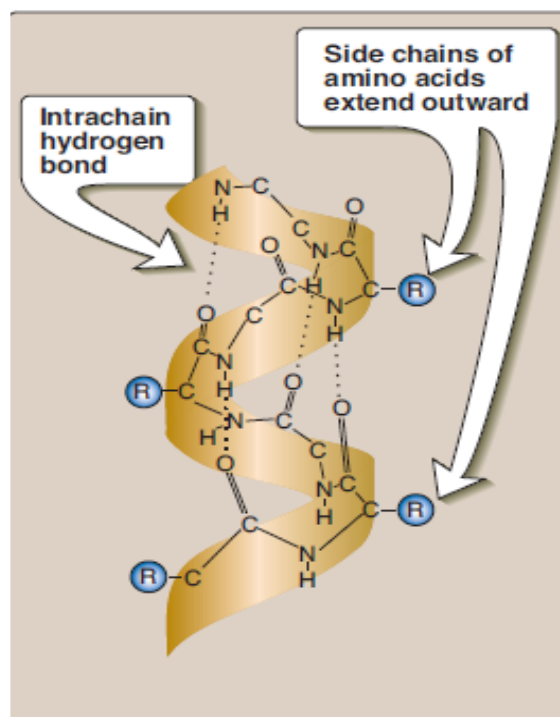


Figure 2.6
 α -Helix showing peptide backbone.

B. β -Sheet

The β -sheet is another form of secondary structure in which all of the peptide bond components are involved in hydrogen bonding (Figure 2.7A). The surfaces of β -sheets appear “pleated,” and these structures are, therefore, often called “ β -pleated sheets.” When illustrations are made of protein structure, β -strands are often visualized as broad arrows (Figure 2.7B).

1. Comparison of a beta-sheet and an α -helix: Unlike the α -helix, β -sheets are composed of two or more peptide chains (β -strands), or segments of polypeptide chains, which are almost fully extended. Note also that in β -sheets the hydrogen bonds are perpendicular to the polypeptide backbone (see Figure 2.7A).

2. Parallel and antiparallel sheets: A β -sheet can be formed from two or more separate polypeptide chains or segments of polypeptide chains that are arranged either **antiparallel** to each other (**with the N-terminal and C-terminal ends of the β -strands alternating as shown in Figure 2.7B**), or **parallel** to each other (**with all the N-termini of the β -strands together as shown in Figure 2.7C**).

C. β -bends (reverse turns)

β -bends reverse the direction of a polypeptide chain, helping it form a compact, globular shape. They are usually found on the surface of protein molecules, and often include charged residues. **β -bends** are stabilized by the formation of hydrogen and ionic bonds.

D. Supersecondary structures.

The α -helix, β -pleated sheet, and other secondary structures are combined in many ways as the polypeptide chain folds back on itself in a protein. The combination of α - and β -strands produces various kinds of supersecondary structures in proteins.

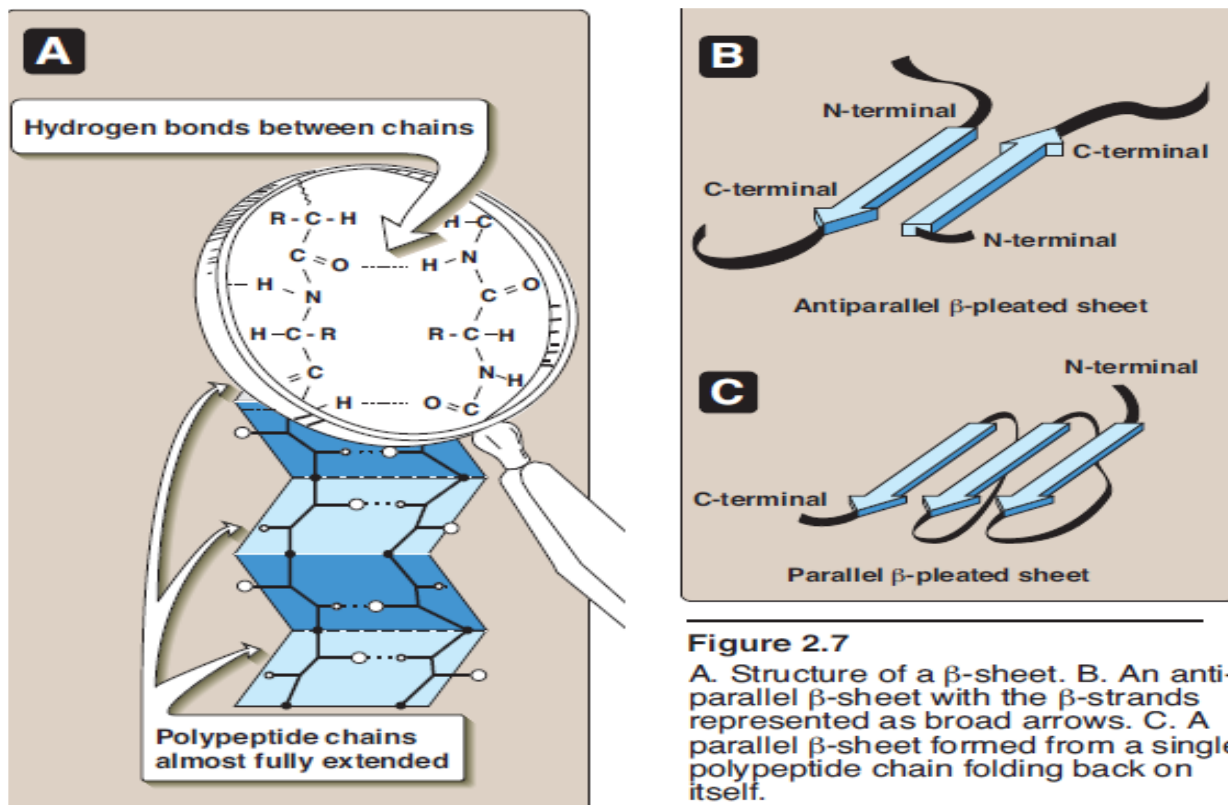


Figure 2.7
A. Structure of a β -sheet. B. An antiparallel β -sheet with the β -strands represented as broad arrows. C. A parallel β -sheet formed from a single polypeptide chain folding back on itself.

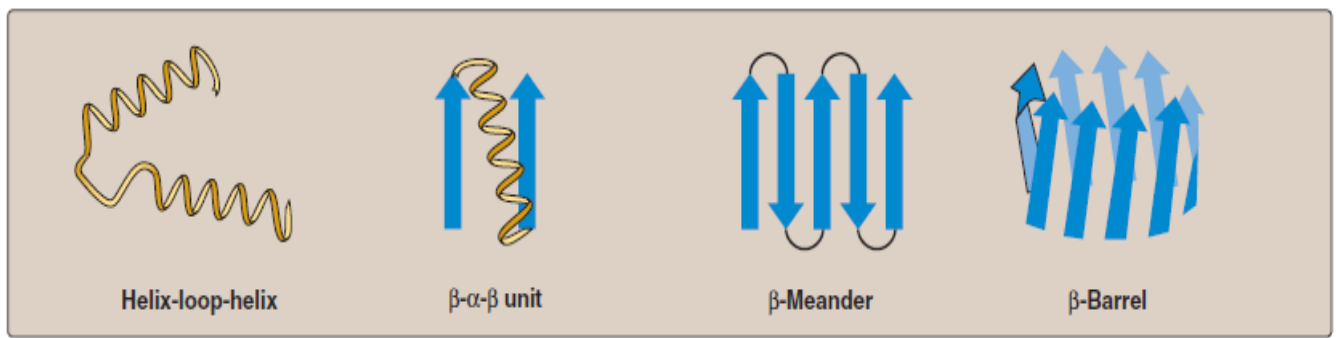


Figure 2.8

Some common structural motifs combining α -helices and/or β -sheets. The names describe their schematic appearance.

3- Tertiary structure of proteins:

It refers to the three-dimensional structure of polypeptide. It includes the relationships between side chains in the space. “Tertiary” refers both to the folding of domains (the basic units of structure and function), and to the final arrangement of domains in the polypeptide.

A. Interactions stabilizing tertiary structure

The unique three-dimensional structure of each polypeptide is determined by its amino acid sequence. Interactions between the amino acid side chains guide the folding of the polypeptide to form a compact structure. The following **four types of interactions cooperate in stabilizing the tertiary structures of globular proteins.**

- 1. Disulfide bonds:** A disulfide bond is a covalent linkage formed from the sulfhydryl group ($-SH$) of each of two cysteine residues, to produce a cystine residue (Figure 2.9).. A disulfide bond contributes to the stability of the three-dimensional shape of the protein molecule, and prevents it from becoming denatured in the extracellular environment. For example, many disulfide bonds are found in proteins such as immunoglobulins that are secreted by cells.
- 2. Hydrophobic interactions:** Amino acids with nonpolar side chains tend to be located in the interior of the polypeptide molecule, where they associate with other hydrophobic amino acids (Figure 2.10).
- 3. Hydrogen bonds:** Amino acid side chains containing oxygen- or nitrogen-bound hydrogen, such as the alcohol groups of serine and threonine, can form hydrogen bonds with electron-rich atoms, such as the oxygen of a carboxyl group or carbonyl group of a peptide bond. Formation of hydrogen bonds between polar groups on the surface of proteins and the aqueous solvent enhances the solubility of the protein.
- 4. Ionic interactions:** Negatively charged groups, such as the carboxylate group ($-COO^-$) in the side chain of aspartate or glutamate, can interact with positively charged groups, such as the amino group ($-NH_3^+$) in the side chain of lysine (see Figure 2.11).

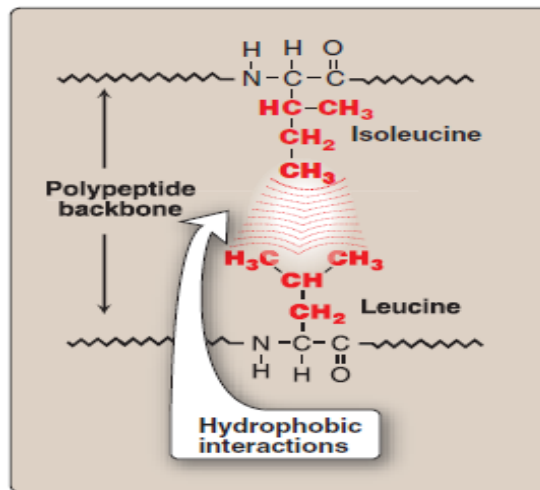


Figure 2.10
Hydrophobic interactions between amino acids with nonpolar side chains.

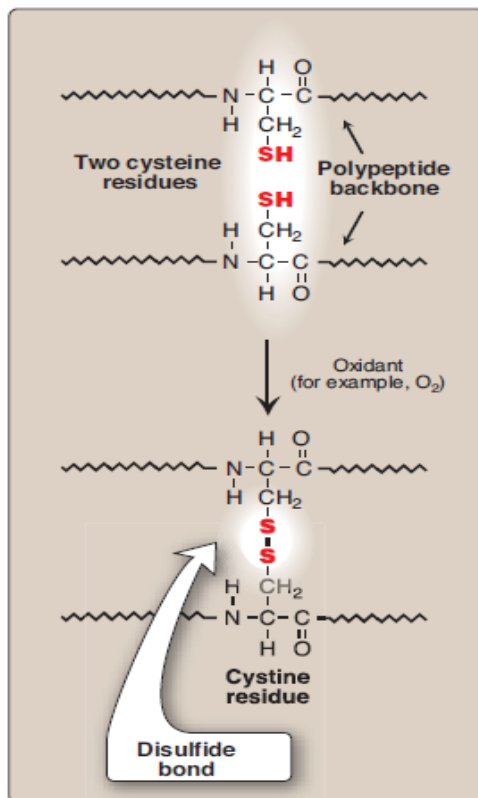


Figure 2.9
Formation of a disulfide bond by the oxidation of two cysteine residues, producing one cystine residue.

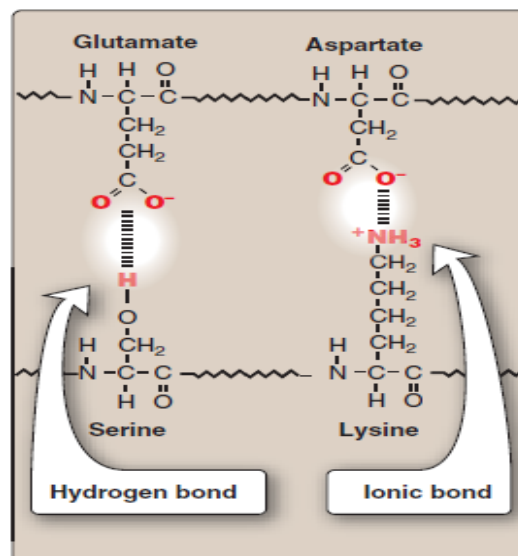


Figure 2.11
Interactions of side chains of amino acids through hydrogen bonds and ionic bonds (salt bridges).

V. QUATERNARY STRUCTURE OF PROTEINS

Many proteins consist of a single polypeptide chain, and are defined as monomeric proteins. However, others may consist of two or more polypeptide chains that may be structurally identical or totally unrelated.

The arrangement of these polypeptide subunits is called the quaternary structure of the protein. Subunits are held together by noncovalent interactions (for example, hydrogen bonds, ionic bonds, and hydrophobic interactions). Subunits may either function independently of each other, or may work cooperatively, as in **hemoglobin**, in which the binding of oxygen to one subunit of the tetramer increases the affinity of the other subunits for oxygen.

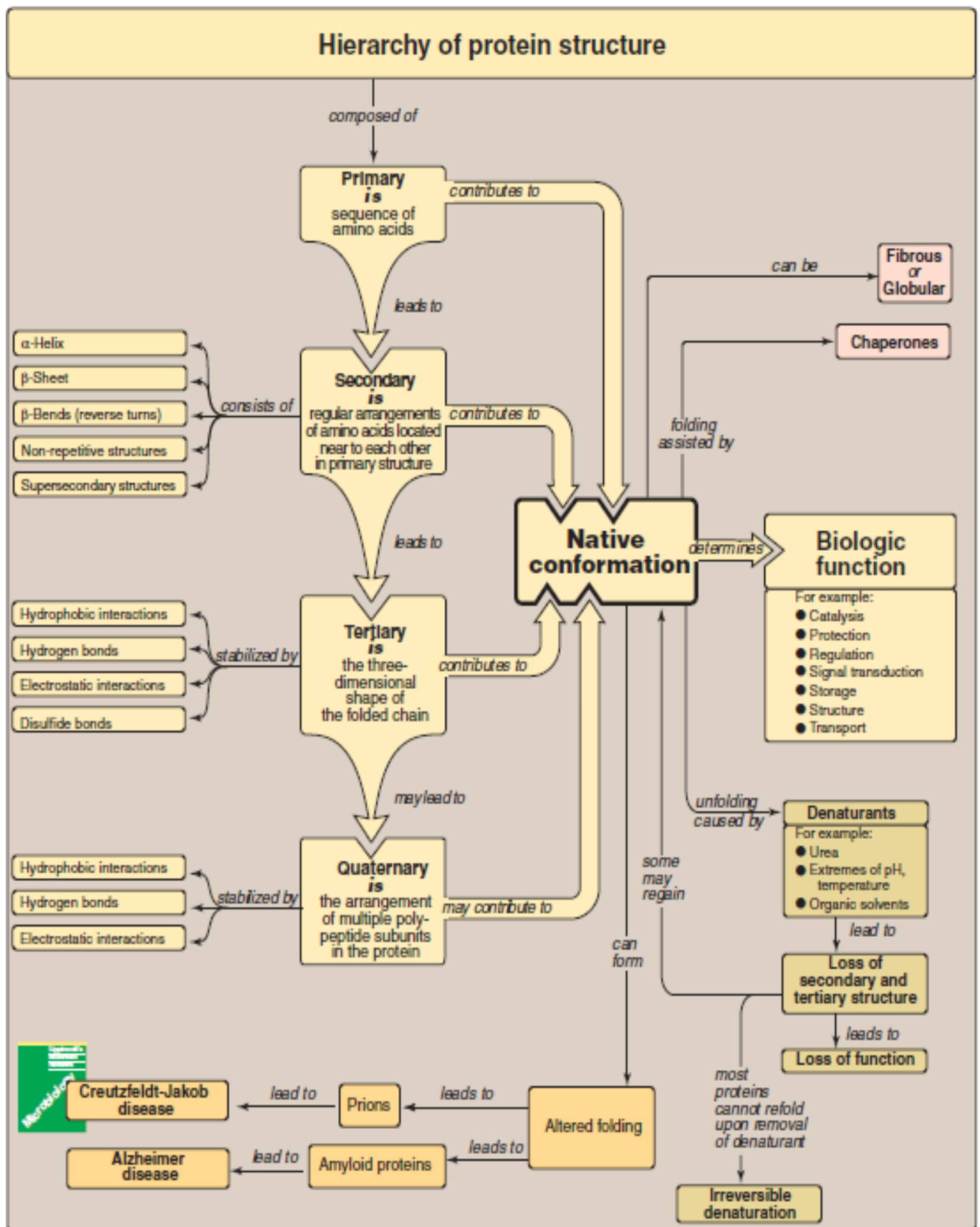


Figure 2.15
Key concept map for protein structure.

III- CLASSIFICATION OF PROTEINS ON OVERALL SHAPE:

1- Globular proteins: hemoglobin and myoglobin.

2- Fibrous proteins: collagen, and elastin.

We described the types of secondary and tertiary structures. By arranging these fundamental structural elements in different combinations, widely diverse proteins can be constructed that are capable of various specialized functions. We will examine the relationship between structure and function for the clinically important globular hemeproteins and fibrous structural proteins.

1- Fibrous proteins:

A. Collagen

Collagen is present in all tissues and organs where it provides the framework that gives the tissues their form and structural strength. The collagen superfamily of proteins includes more than 25 collagen types, and these types distributed in the tissues of the body according to their function. The most common collagen type is type I.

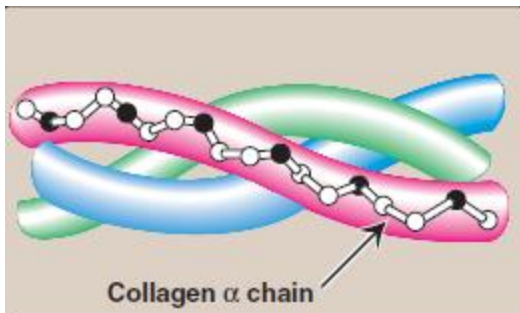


Figure 4.1
Triple-stranded helix of collagen.

Collagen is the most abundant protein in the human body. A typical collagen molecule is a long, rigid structure in which three polypeptides (referred to as “α chains”) are wound around one another in a rope-like triple helix (Figure 4.1). Although these molecules are found throughout the body, their types and organization are dictated by the structural role collagen plays in a particular organ.

- In some tissues, collagen may be dispersed as a gel that gives support to the structure, as in the extracellular matrix or the vitreous humor of the eye.
- In other tissues, collagen may be bundled in tight, parallel fibers that provide great strength, as in tendons.
- In the cornea of the eye, collagen is stacked so as to transmit light with a minimum of scattering.
- Collagen of bone occurs as fibers arranged at an angle to each other so as to resist mechanical shear from any direction.

Structure of collagen:

1. Amino acid sequence: Collagen is rich in proline and glycine, both of which are important in the formation of the triple-stranded helix. Proline facilitates the formation of the helical conformation of each α-chain because its ring structure causes “kinks” in the peptide chain. Glycine, the smallest amino acid, is found in every third position of the polypeptide chain. It fits into the restricted spaces where the three chains of the helix come together.

The glycine residues are part of a repeating sequence, –Gly–X–Y–, where X is frequently proline and Y is often hydroxyproline (but can be hydroxylysine, Figure 4.5). Thus, most of the alpha chain can be regarded as a polytripeptide whose sequence can be represented as (–Gly–Pro–Hyp–)

2. Triple-helical structure:

collagen, a fibrous protein, has an elongated, triple-helical structure that places many of its amino acid side chains on the surface of the triple-helical molecule. [Note: This allows bond formation between the exposed R-groups of neighboring collagen monomers, resulting in their aggregation into long fibers.]

3. Hydroxyproline and hydroxylysine: Collagen contains hydroxyproline (hyp) and hydroxylysine (hyl), which are not present in most other proteins. These residues result from the hydroxylation of some of the proline and lysine residues after their incorporation into polypeptide chains (Figure 4.6). Hydroxyproline is important in stabilizing the triple-helical structure of collagen because it maximizes interchain hydrogen bond formation.

The enzyme-catalyzed hydroxylation of proline requires ascorbic acid (vitamin C); thus in vitamin C deficiency (scurvy) there is a poor synthesis of new collagen. Patients with vitamin C deficiency have bleeding gum and also often show bruises on the limbs as a result of subcutaneous extravasation of blood due to capillary fragility.

4. Glycosylation: The hydroxyl group of the hydroxylysine residues of collagen may be enzymatically glycosylated. Most commonly, glucose and galactose are sequentially attached to the polypeptide chain prior to triple-helix formation.

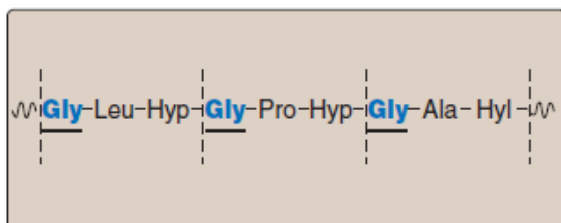


Figure 4.5

Amino acid sequence of a portion of the α_1 chain of collagen. [Note: Hyp is hydroxyproline and Hyl is hydroxylysine.]

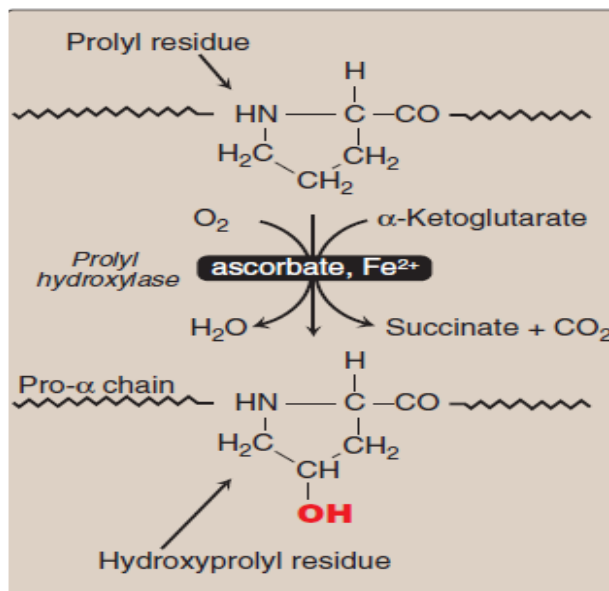


Figure 4.6

Hydroxylation of prolyl residues of pro- α chains of collagen by *prolyl hydroxylase*.

B. ELASTIN

In contrast to collagen, which forms fibers that are tough and have high tensile strength, elastin is a connective tissue protein with rubber-like properties. Elastic fibers composed of elastin and glycoprotein microfibrils are found in the lungs, the walls of large arteries, and elastic ligaments. They can be stretched to several times their normal length, but recoil to their original shape when the stretching force is relaxed.

2- Globular proteins:

Globular hemeproteins

Hemeproteins are a group of proteins that contain a heme group a tightly bound prosthetic group. The role of the heme group is dictated by the environment created by the three-dimensional structure of the protein. In hemoglobin and myoglobin, the two most abundant hemeproteins in humans, the heme group serves to reversibly bind oxygen.

Structure of heme:

Heme is a complex of protoporphyrin IX and ferrous iron (Fe^{2+}) (Figure 3.1). The iron is held in the center of the heme molecule by bonds to the four nitrogens of the porphyrin ring. The heme Fe^{2+} can form two additional bonds, one on each side of the planar porphyrin ring. In myoglobin and hemoglobin, one of these positions is coordinated to the side chain of a histidine residue of the globin molecule, whereas the other position is available to bind oxygen (Figure 3.2).

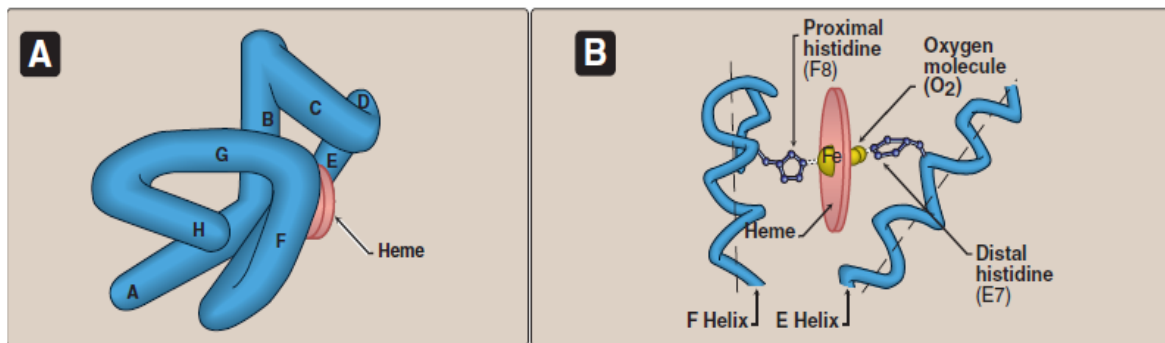
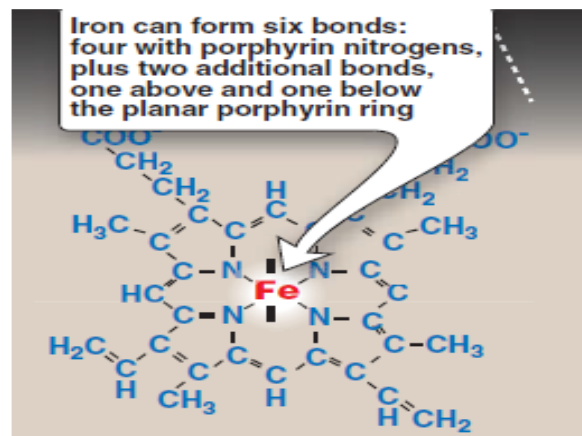


Figure 3.2

A. Model of myoglobin showing helices A to H. B. Schematic diagram of the oxygen-binding site of myoglobin.

Structure and function of myoglobin

Myoglobin, a hemeprotein present in heart and skeletal muscle, functions both as a reservoir for oxygen, and as an oxygen carrier that increases the rate of transport of oxygen within the muscle cell. Myoglobin consists of a single polypeptide chain that is structurally similar to the individual subunit polypeptide chains of the hemoglobin molecule. This homology makes myoglobin a useful model for interpreting some of the more complex properties of hemoglobin.

Binding of the heme group: The heme group of myoglobin sits in a crevice in the molecule, which is lined with nonpolar amino acids. Notable exceptions are two histidine residues (Figure 3.2B). One, the proximal histidine (F8), binds directly to the iron of heme. The second, or distal histidine (E7), does not directly interact with the heme group, but helps stabilize the binding of oxygen to the ferrous iron. The protein, or globin, portion of myoglobin thus creates a special microenvironment for the heme that permits the reversible binding of one oxygen molecule (oxygenation). The simultaneous loss of electrons by the ferrous iron (oxidation) occurs only rarely.

Structure and function of hemoglobin

Hemoglobin is found exclusively in red blood cells (RBCs), where its main function is to transport oxygen (O₂) from the lungs to the capillaries of the tissues. Hemoglobin A, the major hemoglobin in adults, is composed of four polypeptide chains—two α chains and two β chains—held together by noncovalent interactions (Figure 3.3).

Each subunit has stretches of α -helical structure, and a heme-binding pocket similar to that described for myoglobin. However, the tetrameric hemoglobin molecule is structurally and functionally more complex than myoglobin. For example, hemoglobin can transport H⁺ and CO₂ from the tissues to the lungs, and can carry four molecules of O₂ from the lungs to the cells of the body. Furthermore, the oxygen-binding properties of hemoglobin are regulated by interaction with allosteric effectors.

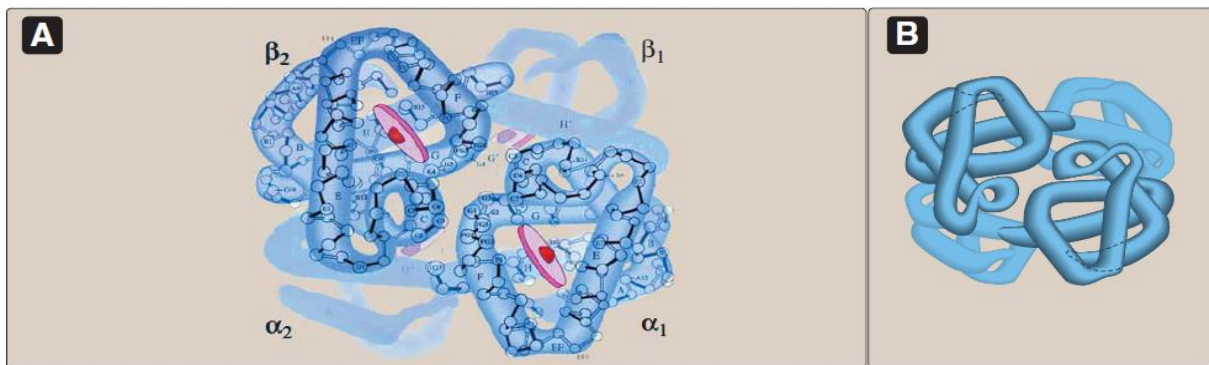


Figure 3.3

A. Structure of hemoglobin showing the polypeptide backbone. B. Simplified drawing showing the helices.

D. Binding of oxygen to myoglobin and hemoglobin

Myoglobin can bind only one molecule of oxygen, because it contains only one heme group. In contrast, hemoglobin can bind four oxygen molecules—one at each of its four heme groups. The degree of saturation (Y) of these oxygen-binding sites on all myoglobin or hemoglobin molecules can vary between zero (all sites are empty) and 100% (all sites are full, Figure 3.5).

1. Oxygen dissociation curve:

A plot of Y measured at different partial pressures of oxygen (pO₂) is called the oxygen **dissociation curve**. The curves for myoglobin and hemoglobin show important differences (see Figure 3.5). This graph illustrates that myoglobin has a higher oxygen affinity at all pO₂ values than does hemoglobin. The partial pressure of oxygen needed to achieve half-saturation of the binding sites (P₅₀) is approximately 1 mm Hg for myoglobin and 26 mm Hg for hemoglobin. The higher the oxygen affinity (that is, the more tightly oxygen binds), the lower the P₅₀. [Note: pO₂ may also be represented as PO₂.]

a. Myoglobin (Mb): The oxygen dissociation curve for myoglobin has a hyperbolic shape (see Figure 3.5). This reflects the fact that myoglobin reversibly binds a single molecule of oxygen. Thus, oxygenated (MbO₂) and deoxygenated (Mb) myoglobin exist in a simple equilibrium:



The equilibrium is shifted to the right or to the left as oxygen is added to or removed from the system. [Note: Myoglobin is designed to bind oxygen released by hemoglobin at the low pO₂ found in muscle. Myoglobin, in turn, releases oxygen within the muscle cell in response to oxygen demand.]

b. Hemoglobin (Hb): The oxygen dissociation curve for hemoglobin is sigmoidal in shape (see Figure 3.5), indicating that the subunits cooperate in binding oxygen. Cooperative binding of oxygen by the four subunits of hemoglobin means that the binding of an oxygen molecule at one heme group increases the oxygen affinity of the remaining heme groups in the same hemoglobin molecule (Figure 3.6). This effect is referred to as heme-heme interaction. Although it is more difficult for the first oxygen molecule to bind to hemoglobin, the subsequent binding of oxygen occurs with high affinity, as shown by the steep upward curve in the region near 20–30 mm Hg (see Figure 3.5).

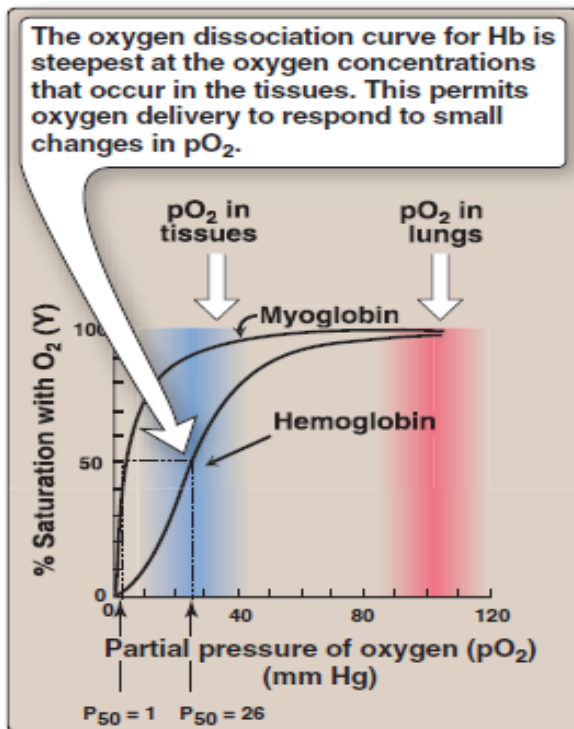


Figure 3.5
Oxygen dissociation curves for myoglobin and hemoglobin (Hb).

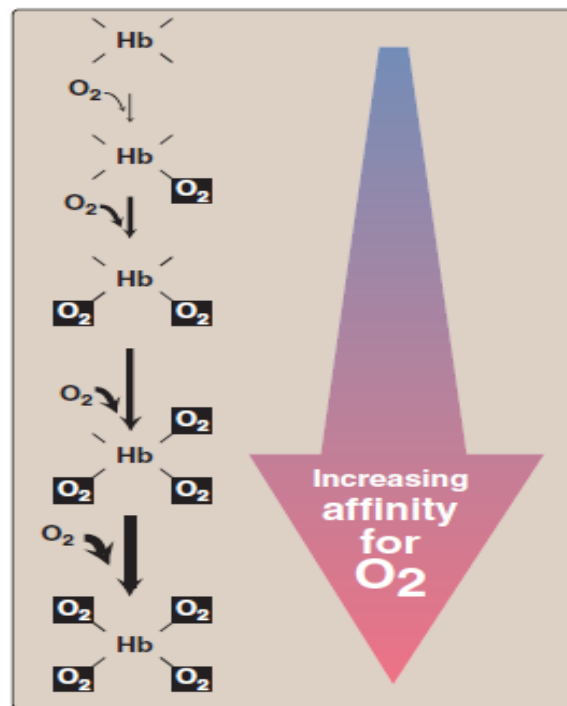


Figure 3.6
Hemoglobin (Hb) binds oxygen with increasing affinity.

E. Allosteric effects

The ability of hemoglobin to reversibly bind oxygen is affected by:

- 1- The pO_2 (through heme-heme interactions as described above).
- 2- The pH of the environment.
- 3- The partial pressure of carbon dioxide, pCO_2 .
- 4- The availability of 2,3-bisphosphoglycerate.

These are collectively called allosteric (“other site”) effectors, because their interaction at one site on the hemoglobin molecule affects the binding of oxygen to heme groups at other locations on the molecule. [Note: The binding of oxygen to myoglobin is not influenced by allosteric effectors.]

1. Heme-heme interactions: The sigmoidal oxygen dissociation curve reflects specific structural changes that are initiated at one heme group and transmitted to other heme groups in the hemoglobin tetramer. The net effect is that the affinity of hemoglobin for the last oxygen bound is approximately 300 times greater than its affinity for the first oxygen bound.

a. Loading and unloading oxygen: The cooperative binding of oxygen allows hemoglobin to deliver more oxygen to the tissues in response to relatively small changes in the partial pressure of oxygen. This can be seen in Figure 3.5, which indicates pO_2 in the alveoli of the lung and the capillaries of the tissues. For example, in the lung, the concentration of oxygen is high and hemoglobin becomes virtually saturated (or “loaded”) with oxygen. In contrast, in the peripheral tissues, oxyhemoglobin releases (or “unloads”) much of its oxygen for use in the oxidative metabolism of the tissues (Figure 3.7).

b. Significance of the sigmoidal oxygen dissociation curve:

The steep slope of the oxygen dissociation curve over the range of oxygen concentrations that occur between the lungs and the tissues permits hemoglobin to carry and deliver oxygen efficiently from sites of high to sites of low pO_2 . A molecule with a hyperbolic oxygen dissociation curve, such as myoglobin, could not achieve the same degree of oxygen release within this range of partial pressures of oxygen. Instead, it would have maximum affinity for oxygen throughout this oxygen pressure range and, therefore, would deliver no oxygen to the tissues.

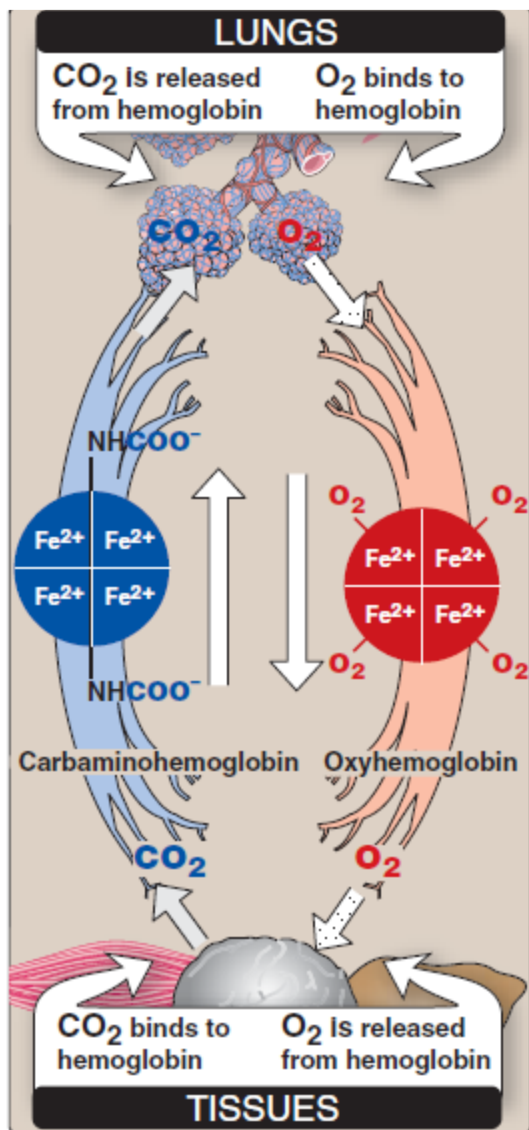


Figure 3.7
Transport of oxygen and carbon dioxide by hemoglobin.

Adult Hb (HbA)

Contains two types of globin two α - chains and two β - chains. The amino acid sequences of the two type of subunits are identical at 27 positions.

1. Fetal Hb (HbF)

Is a tetramer consisting of two α -chains identical to those found in HbA, plus two γ -chains. Under physiologic conditions, Hb F has a higher affinity for oxygen than does Hb A. The higher oxygen affinity of Hb F facilitates the transfer of oxygen from the maternal circulation across the placenta to the RBCs of the fetus.

2. Hemoglobin A2 (Hb A2): Hb A2 is a minor component of normal adult hemoglobin, first appearing shortly before birth and, ultimately, constituting about 2% of the total hemoglobin. It is composed of two α -globin chains and two δ -globin chains ($\alpha_2\delta_2$)

3. Hemoglobin A1c (HbA1c): Under physiologic conditions, Hb A is slowly and nonenzymically glycosylated, the extent of glycosylation being dependent on the plasma concentration of a particular hexose. The most abundant form of glycosylated hemoglobin is HbA1c. It has glucose residues attached predominantly to the NH₂ groups of the N-terminal valines of the β -globin chains. Increased amounts of Hb A1c are found in RBCs of patients with diabetes mellitus, because their Hb A has contact with higher glucose concentrations during the 120-day lifetime of these cells.

2. Bohr effect: The release of oxygen from hemoglobin is enhanced when the pH is lowered or when the hemoglobin is in the presence of an increased pCO₂.

3. Effect of 2,3-bisphosphoglycerate on oxygen affinity: 2,3-Bisphospho glycerate (2,3-BPG) is an important regulator of the binding of oxygen to hemoglobin. 2,3-BPG decreases the oxygen affinity of hemoglobin

4. Binding of CO₂: Most of the CO₂ produced in metabolism is hydrated and transported as bicarbonate ion. However, some CO₂ is carried as carbamate bound to the N-terminal amino groups of hemoglobin (forming carbaminohemoglobin). The binding of CO₂ stabilizes the deoxy form of hemoglobin, resulting in a decrease in its affinity for oxygen.

5. Binding of CO: Carbon monoxide (CO) binds tightly (but reversibly) to the hemoglobin iron, forming carbon monoxy hemoglobin (or carboxyhemoglobin). When CO binds to one or more of the four heme sites, hemoglobin shifts to the relaxed conformation, causing the remaining heme sites to bind oxygen with high affinity. This shifts the oxygen dissociation curve to the left, and changes the normal sigmoidal shape toward a hyperbola. As a result, the affected hemoglobin is unable to release oxygen to the tissues.

[Note: The affinity of hemoglobin for CO is 220 times greater than for oxygen. Consequently, even minute concentrations of CO in the environment can produce toxic concentrations of carbon monoxyhemoglobin in the blood].

Minor hemoglobins

It is important to remember that human hemoglobin A (Hb A) is just one member of a functionally and structurally related family of proteins, the hemoglobins.

Hemoglobinopathies:

Hemoglobinopathies have traditionally been defined as a family of genetic disorders caused by production of structurally abnormal hemoglobin molecule, synthesis of insufficient amount of normal hemoglobin, or, rarely both. Sickle cell anemia (Hb S), hemoglobin C disease (Hb C), hemoglobin SC disease (Hb S + Hb C), and the thalassemia syndromes are representative hemoglobinopathies that have severe clinical consequences.

IV-ENZYMES.

I. OVERVIEW

Virtually all reactions in the body are mediated by enzymes, which are protein catalysts that increase the rate of reactions without being changed in the overall process. Enzymes direct all metabolic events. This chapter examines the nature of these catalytic molecules and their mechanism of action.

II. Classification of enzymes:

The international union of biochemistry and molecular biology has established a system whereby all enzymes are classified into six major classes, each with numerous sub groups. **Enzymes are classified based on the reactions they catalyze.**

Class I: Oxidoreductase: Enzymes catalyzing oxidation reduction reactions.(Example: Lactate-dehydrogenase)

Class II. Transferases: Enzymes catalyzing a transfer of a group other than hydrogen (methyl, acyl, amino or phosphate groups).

Class III. Hydrolases: Enzymes catalyzing hydrolysis of ester, ether, peptido, glycosyl, acid-anhydride, C-C, C-halide, or P-N-bonds by utilizing water.

Class IV. Lyases: Enzymes that catalyze removal of groups from substances by mechanisms other than hydrolysis, leaving double bonds.

Class V. Isomerases: Includes all enzymes catalyzing interconversion of optical, geometric, or positional isomers.

Class VI. Ligases or synthetases: Enzymes catalyzing the linking together of 2 compounds coupled to the breaking of a pyrophosphate bond in ATP or similar trinucleotides: GTP, UTP etc. included are enzymes catalyzing reactions forming C-O, C-S, C-N, and C-C bonds.

Most commonly used enzyme names have the suffix “-ase” attached to the substrate of the reaction (for example, glucosidase and urease), or to a description of the action performed (for example, lactate dehydrogenase and adenyl cyclase).

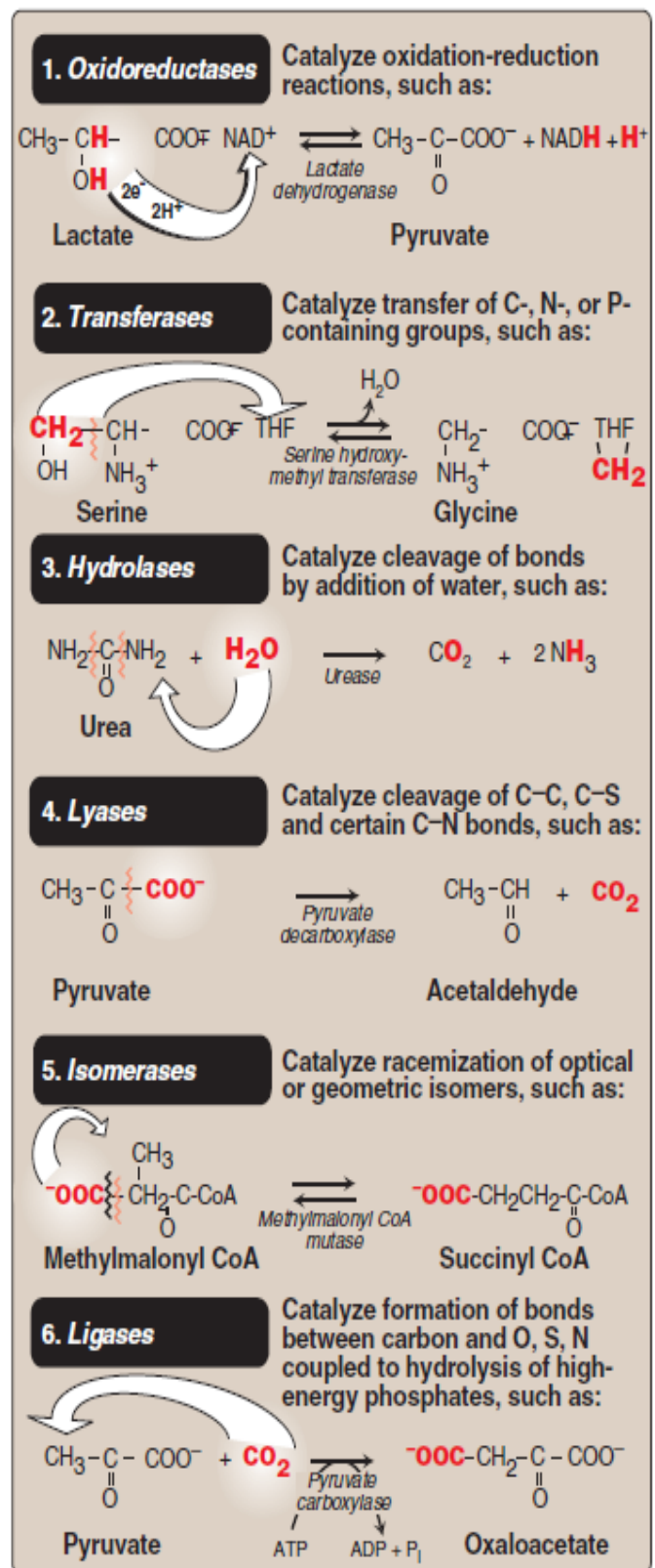


Figure 5.1

The six major classes of enzymes with examples. THF = tetrahydrofolate.

III. PROPERTIES OF ENZYMES

Enzymes are protein catalysts that increase the velocity of a chemical reaction, and are not consumed during the reaction.

A. Active sites

Enzyme molecules contain a special pocket or cleft called the active site. The active site contains amino acid side chains that participate in substrate binding and catalysis (Figure 5.2). The substrate binds the enzyme, forming an enzyme–substrate (ES) complex. ES is converted to an enzyme–product (EP) complex that subsequently dissociates to enzyme and product.

B. Catalytic efficiency

Enzyme-catalyzed reactions are highly efficient, proceeding from 10^3 – 10^8 times faster than uncatalyzed reactions.

C. Specificity

Enzymes are highly specific, interacting with one or a few substrates and catalyzing only one type of chemical reaction.

D. Regulation

Enzyme activity can be regulated, that is, increased or decreased, so that the rate of product formation responds to cellular need.

E. Location within the cell

Many enzymes are localized in specific organelles within the cell. Such compartmentalization serves to isolate the reaction substrate or product from other competing reactions. This provides a favorable environment for the reaction, and organizes the thousands of enzymes present in the cell into purposeful pathways.

F. Holoenzymes

Some enzymes require molecules other than proteins for enzymic activity. The term **holoenzyme** refers to the active enzyme with its nonprotein component, whereas the enzyme without its nonprotein moiety is termed an **apoenzyme and is inactive**.

If the **nonprotein moiety** is a metal ion such as Zn^{2+} or Fe^{2+} , it is called a **cofactor**. If it is a small organic molecule, it is termed a **coenzyme**. Coenzymes that only transiently associate with the enzyme are called **cosubstrates**. Cosubstrates dissociate from the enzyme in an altered state (NAD^+ is an example). If the **coenzyme is permanently** associated with the enzyme and returned to its original form, it is called a **prosthetic group** (FAD is an example). Coenzymes frequently are derived from vitamins. For example, NAD^+ contains niacin and FAD contains riboflavin.

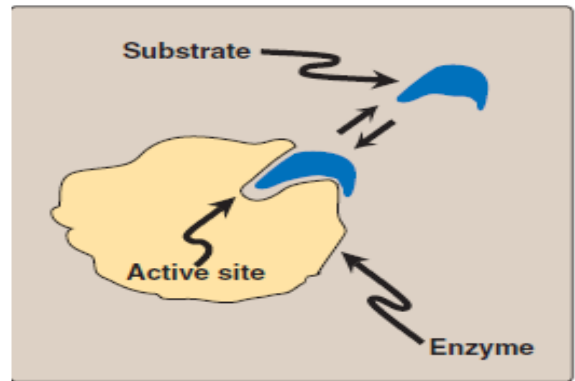


Figure 5.2
Schematic representation of an enzyme with one active site binding a substrate molecule.

IV. HOW ENZYME WORKS?

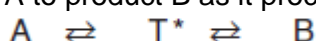
The mechanism of enzyme action can be viewed from two different perspectives.

- 1-The first treats catalysis in terms of energy changes that occur during the reaction, that is, enzymes provide an alternate, energetically favorable reaction pathway different from the uncatalyzed reaction.
- 2-The second perspective describes how the active site chemically facilitates catalysis.

A. Energy changes occurring during the reaction

Virtually all chemical reactions have an energy barrier separating the reactants and the products. This barrier, called the **free energy of activation**, is the energy difference between that of the reactants and a high-energy intermediate that occurs during the formation of product.

For example, Figure 5.4 shows the changes in energy during the conversion of a molecule of reactant A to product B as it proceeds through the transition state (high-energy intermediate), T^* :



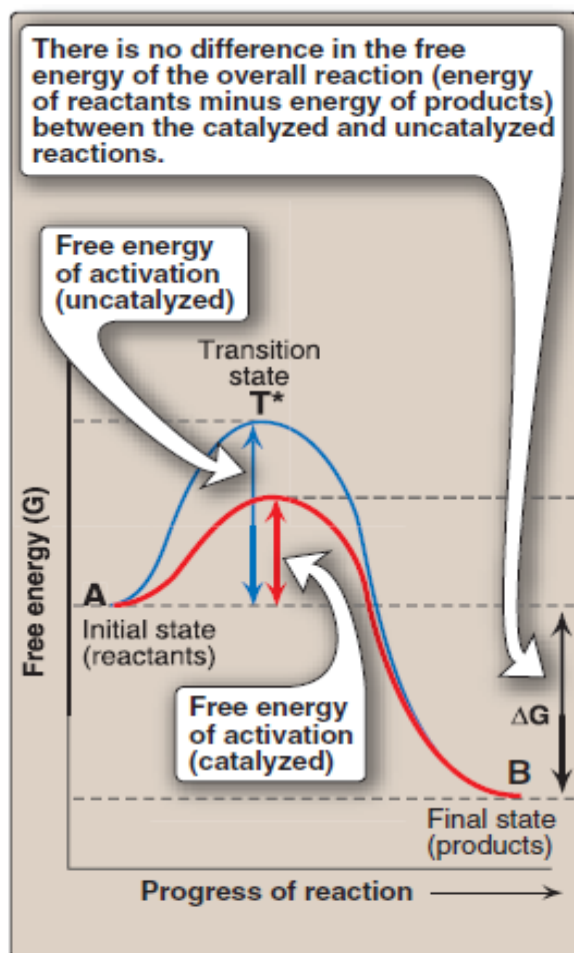


Figure 5.4
Effect of an enzyme on the activation energy of a reaction.

1. Free energy of activation:

The peak of energy in Figure 5.4 is the difference in free energy between the reactant and T^* , where the high-energy intermediate is formed during the conversion of reactant to product. Because of the high free energy of activation, the rates of uncatalyzed chemical reactions are often slow.

2. Rate of reaction:

For molecules to react, they must contain sufficient energy to overcome the energy barrier of the transition state. In the absence of an enzyme, only a small proportion of a population of molecules may possess enough energy to achieve the transition state between reactant and product. The rate of reaction is determined by the number of such energized molecules. **In general, the lower the free energy of activation, the more molecules have sufficient energy to pass through the transition state, and, thus, the faster the rate of the reaction.**

3. Alternate reaction pathway:

An enzyme allows a reaction to proceed rapidly under conditions prevailing in the cell by providing an alternate reaction pathway with a lower free energy of activation (Figure 5.4). The enzyme does not change the free energies of the reactants or products and, therefore, does not change the equilibrium of the reaction. It does, however, accelerate the rate with which equilibrium is reached.

B. Chemistry of the active site

The active site is not a passive receptacle for binding the substrate, but rather is a complex molecular machine employing a diversity of chemical mechanisms to facilitate the conversion of substrate to product. A number of factors are responsible for the catalytic efficiency of enzymes, including the following:

Transition-state stabilization: The active site often **acts as a flexible molecular template that binds the substrate and initiates its conversion to the transition state**, a structure in which the bonds are not like those in the substrate or the product (see T^* at the top of the curve in Figure 5.4). By stabilizing the transition state, the enzyme greatly increases the concentration of the reactive intermediate that can be converted to product and, thus, accelerates the reaction.

Enzymes, in general, provide speed, specificity, and regulatory control to reactions in the body. Enzymes are usually proteins that act as catalysts, compounds that increase the rate of chemical reactions. Enzyme-catalyzed reactions have three basic steps:

(1) Binding of substrate: $E + S \rightleftharpoons ES$

(2) Conversion of bound substrate to bound product: $ES \rightleftharpoons EP$

(3) Release of product: $EP \rightleftharpoons E + P$

An enzyme binds the substrates of the reaction it catalyzes and brings them together at the right orientation to react. The enzyme then participates in the making and breaking of bonds required for product formation, releases the products, and returns to its original state once the reaction is completed.

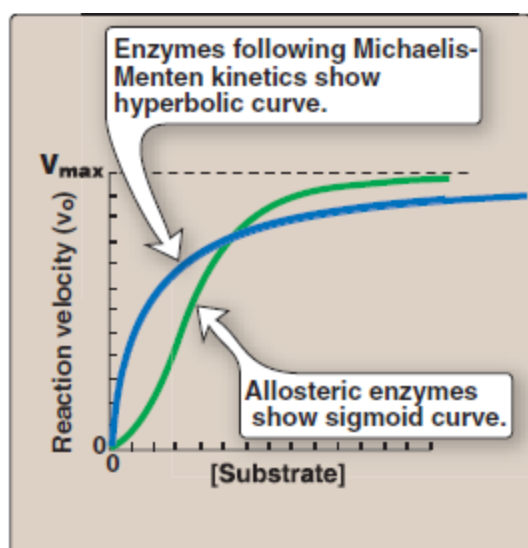
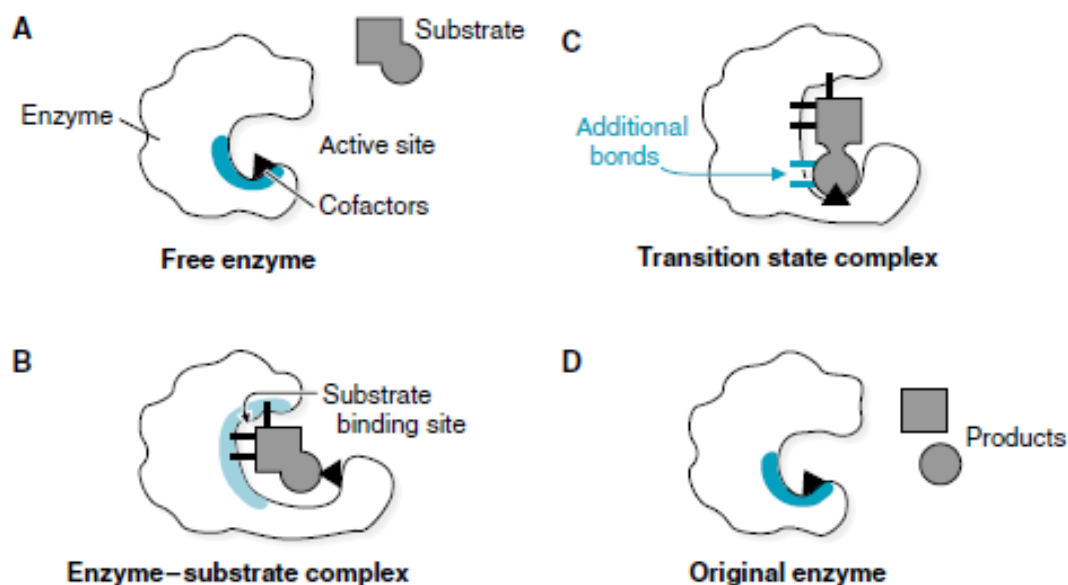


Figure 5.6
Effect of substrate concentration on reaction velocity.

V. FACTORS AFFECTING REACTION VELOCITY

A. Substrate concentration

1. Maximal velocity:

- The rate or velocity of a reaction (v) is the number of substrate molecules converted to product per unit time; velocity is usually expressed as μmol of product formed per minute.
- The rate of an enzyme-catalyzed reaction increases with substrate concentration until a maximal velocity (V_{max}) is reached (Figure 5.6).
- The leveling off of the reaction rate at high substrate concentrations reflects the saturation with substrate of all available binding sites on the enzyme molecules present.

2. Hyperbolic shape of the enzyme kinetics curve:

- Most enzymes show Michaelis-Menten kinetics, in which the plot of initial reaction velocity (v_0) against substrate concentration ($[S]$), is hyperbolic (similar in shape to that of the oxygen-dissociation curve of myoglobin).
- In contrast, allosteric enzymes do not follow Michaelis-Menton kinetics and show a sigmoidal curve that is similar in shape to the oxygen dissociation curve of hemoglobin.

B-Temperature

1. Increase of velocity with temperature:

The reaction velocity increases with temperature until a peak velocity is reached (Figure 5.7). This increase is the result of the increased number of molecules having sufficient energy to pass over the energy barrier and form the products of the reaction.

2. Decrease of velocity with higher temperature:

Further elevation of the temperature results in a decrease in reaction velocity as a result of temperature-induced denaturation of the enzyme (see Figure 5.7).

C-Effect of pH

1. Effect of pH on the ionization of the active site:

The concentration of H^+ affects reaction velocity in several ways. First, the catalytic process usually requires that the enzyme and substrate have specific chemical groups in either an ionized or un-ionized state in order to interact. For example, catalytic activity may require that an amino group of the enzyme be in the protonated form ($-NH_3^+$). At alkaline pH, this group is deprotonated, and the rate of the reaction, therefore, declines.

2. Effect of pH on enzyme denaturation: Extremes of pH can also lead to denaturation of the enzyme, because the structure of the catalytically active protein molecule depends on the ionic character of the amino acid side chains.

3. The pH optimum varies for different enzymes:

The pH at which maximal enzyme activity is achieved is different for different enzymes, and often reflects the $[H^+]$ at which the enzyme functions in the body. For example, pepsin, a digestive enzyme in the stomach, is maximally active at pH 2, whereas other enzymes, designed to work at neutral pH, are denatured by such an acidic environment (Figure 5.8).

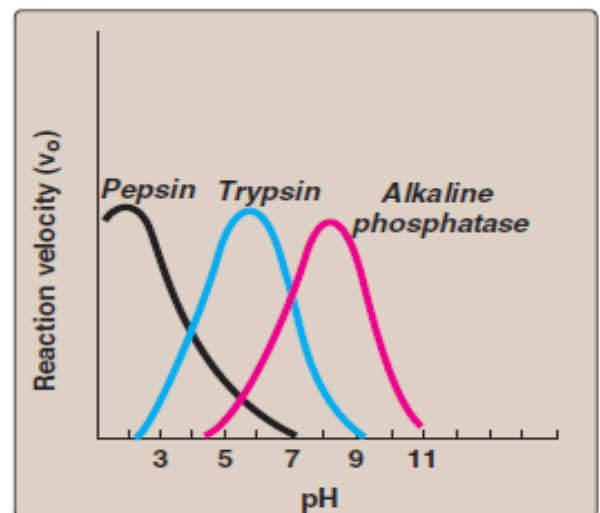
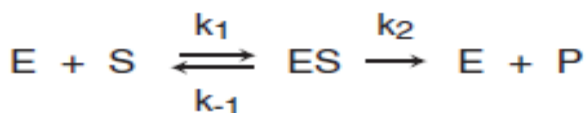


Figure 5.8

Effect of pH on enzyme-catalyzed reactions.

VI. MICHAELIS-MENTEN EQUATION

Leonor Michaelis and Maude Menten proposed a simple model that accounts for most of the features of enzyme-catalyzed reactions. In this model, the enzyme reversibly combines with its substrate to form an ES complex that subsequently yields product, regenerating the free enzyme. The model, involving one substrate molecule, is represented below:



Where **S** is the substrate **E** is the enzyme **ES** is the enzyme–substrate complex **P** is the product k_1 , k_{-1} , and k_2 are rate constants

A- Michaelis-Menten equation

The Michaelis-Menten equation describes how reaction velocity varies with substrate concentration:

Where

v_o = initial reaction velocity, **V_{max}** = maximal velocity,

K_m = Michaelis constant = $(k_{-1} + k_2)/k_1$

[S] = substrate concentration.

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

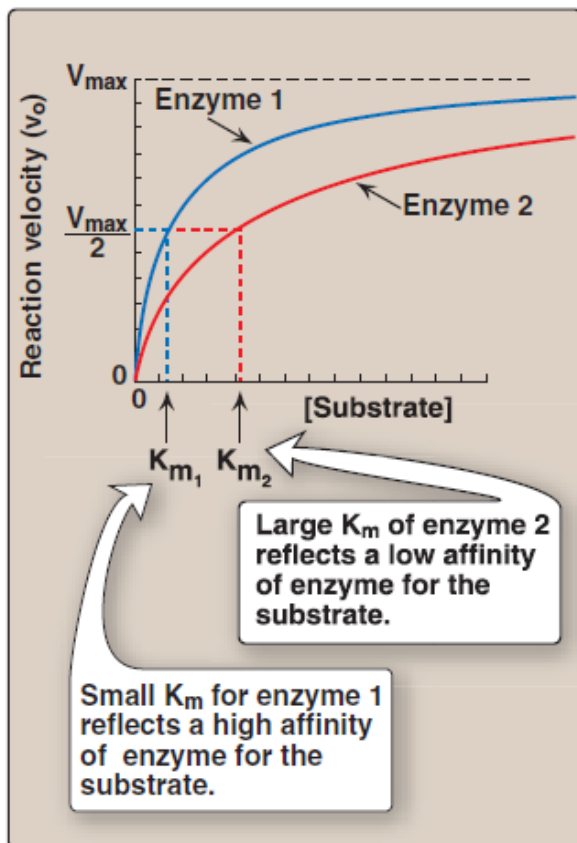


Figure 5.9

Effect of substrate concentration on reaction velocities for two enzymes: enzyme 1 with a small K_m , and enzyme 2 with a large K_m .

B- Important conclusions about Michaelis-Menten kinetics:

1. Characteristics of K_m : K_m —the Michaelis constant—is characteristic of an enzyme and its particular substrate, and reflects the affinity of the enzyme for that substrate. **K_m is numerically equal to the substrate concentration at which the reaction velocity is equal to $1/2V_{max}$.** K_m does not vary with the concentration of enzyme.

a. Small K_m : A numerically small (low) K_m reflects a high affinity of the enzyme for substrate, because a low concentration of substrate is needed to half-saturate the enzyme—that is, to reach a velocity that is $1/2V_{max}$ (Figure 5.9).

b. Large K_m : A numerically large (high) K_m reflects a low affinity of enzyme for substrate because a high concentration of substrate is needed to half-saturate the enzyme.

2. Relationship of velocity to enzyme

concentration: The rate of the reaction is directly proportional to the enzyme concentration at all substrate concentrations.

C- Lineweaver-Burk plot

This plot, the Lineweaver-Burk plot (also called a double-reciprocal plot) can be used to calculate

K_m and V_{max} , as well as to determine the mechanism of action of enzyme inhibitors.

The equation describing the Lineweaver-Burk plot is:

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

Where the intercept on the x-axis is equal to $-1/K_m$, and the intercept on the y-axis is equal to $1/V_{max}$.

VII. INHIBITION OF ENZYME ACTIVITY

Any substance that can diminish the velocity of an enzyme-catalyzed reaction is called an inhibitor.

In general, **irreversible inhibitors** bind to enzymes through covalent bonds.

Reversible inhibitors typically bind to enzymes through noncovalent bonds.

The two most commonly encountered types of reversible inhibition are competitive and noncompetitive.

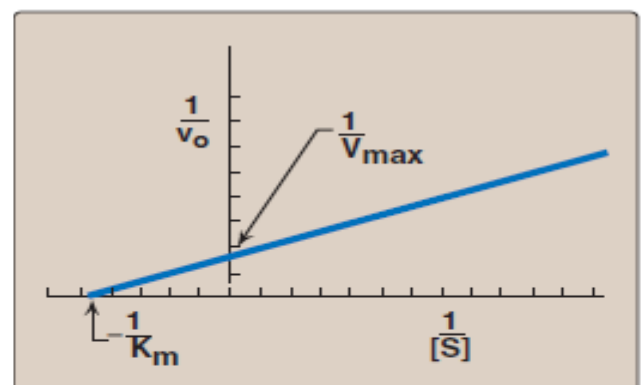
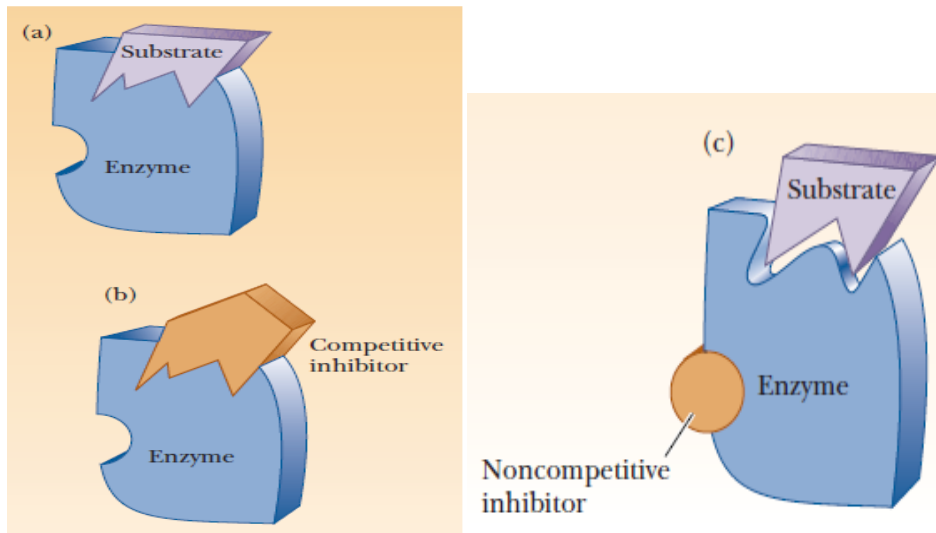


Figure 5.11
Lineweaver-Burk plot.



A. Competitive inhibition

This type of inhibition occurs when the inhibitor binds reversibly to the same site that the substrate would normally occupy and, therefore, competes with the substrate for that site.

1. Effect on V_{max} : The effect of a competitive inhibitor is reversed by increasing $[S]$. At a sufficiently high substrate concentration, the reaction velocity reaches the V_{max} observed in the absence of inhibitor (Figure 5.12).

2. Effect on K_m : A competitive inhibitor increases the apparent K_m for a given substrate. This means that, in the presence of a competitive inhibitor, more substrate is needed to achieve $1/2V_{max}$.

3. Effect on the Lineweaver-Burk plot: Competitive inhibition shows a characteristic Lineweaver-Burk plot in which the plots of the inhibited and uninhibited reactions intersect on the y-axis at $1/V_{max}$ (V_{max} is unchanged). The inhibited and uninhibited reactions show different x-axis intercepts, indicating that the apparent K_m is increased in the presence of the competitive inhibitor because $-1/K_m$ moves closer to zero from a negative value (see Figure 5.12).

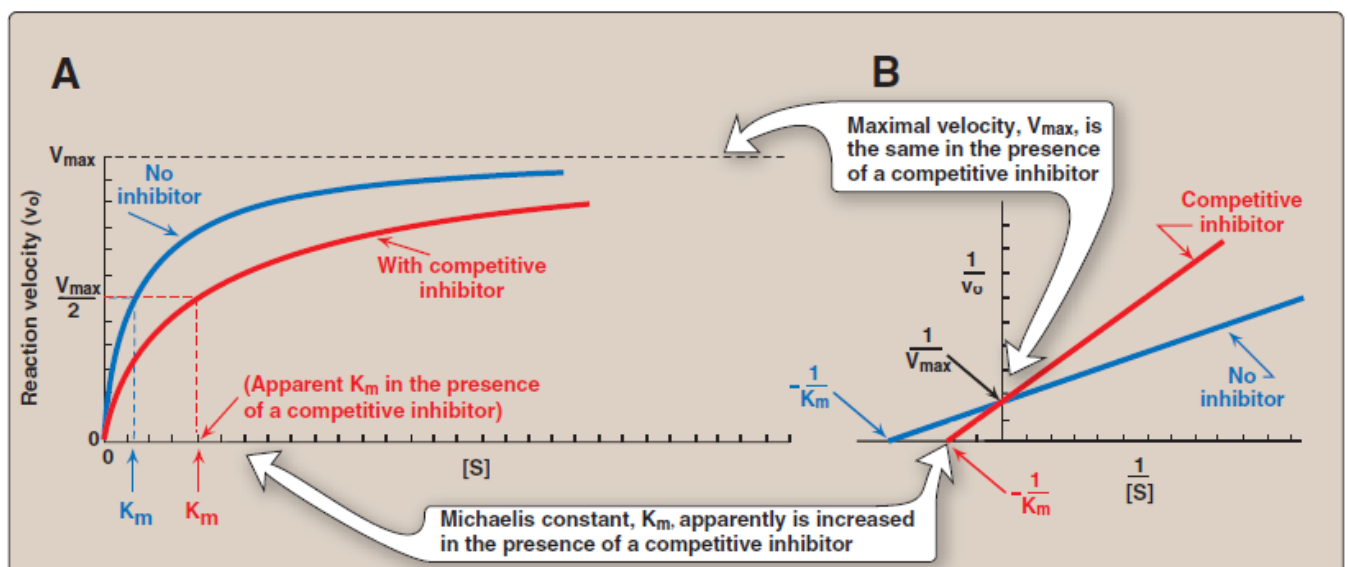


Figure 5.12

A. Effect of a competitive inhibitor on the reaction velocity (v_o) versus substrate ($[S]$) plot. B. Lineweaver-Burk plot of competitive inhibition of an enzyme.

B. Noncompetitive inhibition

This type of inhibition is recognized by its characteristic effect on V_{\max} (Figure 5.14). Noncompetitive inhibition occurs when the inhibitor and substrate bind at different sites on the enzyme. The noncompetitive inhibitor can bind either free enzyme or the ES complex, thereby preventing the reaction from occurring (Figure 5.15).

1. Effect on V_{\max} : Noncompetitive inhibition cannot be overcome by increasing the concentration of substrate. Thus, noncompetitive inhibitors decrease the apparent V_{\max} of the reaction.

2. Effect on K_m : Noncompetitive inhibitors do not interfere with the binding of substrate to enzyme. Thus, the enzyme shows the same K_m in the presence or absence of the noncompetitive inhibitor.

3. Effect on Lineweaver-Burk plot: Noncompetitive inhibition is readily differentiated from competitive inhibition by plotting $1/v_o$ versus $1/[S]$ and noting that the apparent V_{\max} decreases in the presence of a noncompetitive inhibitor, whereas K_m is unchanged (see Figure 5.14).

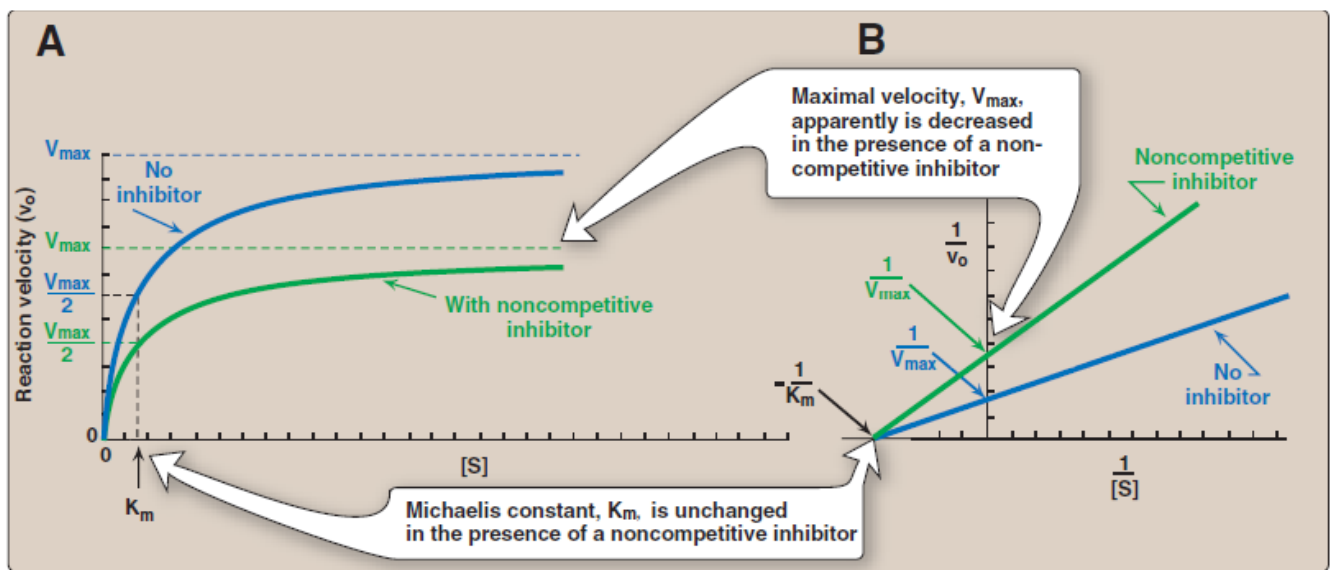


Figure 5.14

A. Effect of a noncompetitive inhibitor on the reaction velocity (v_o) versus substrate ($[S]$) plot. B. Lineweaver-Burk plot of noncompetitive inhibition of an enzyme.

VIII. REGULATION OF ENZYME ACTIVITY THROUGH CONFORMATIONAL CHANGES:

The regulation of the reaction velocity of enzymes is essential if an organism is to coordinate its numerous metabolic processes.

- 1- The rates of most enzymes are responsive to changes in substrate concentration. Thus, an increase in substrate concentration prompts an increase in reaction rate, which tends to return the concentration of substrate toward normal.
- 2- Some enzymes with specialized regulatory functions respond to allosteric effectors or covalent modification.
- 3- Some enzymes show altered rates of enzyme synthesis (or degradation) when physiologic conditions are changed.

A. Regulation of allosteric enzymes

Allosteric enzymes are regulated by molecules called effectors (also called modifiers) that bind noncovalently at a site other than the active site. These enzymes are usually composed of multiple subunits, and the regulatory (allosteric) site that binds the effector may be located on a subunit that is not itself catalytic. The presence of an allosteric effector can alter the affinity of the enzyme for its substrate, or modify the maximal catalytic activity of the enzyme, or both.

Effectors that inhibit enzyme activity are termed negative effectors, whereas those that increase enzyme activity are called positive effectors. Allosteric enzymes frequently catalyze the committed step early in a pathway.

1. Homotropic effectors: When the substrate itself serves as an effector, the effect is said to be homotropic. Most often, an allosteric substrate functions as a positive effector. In such a case, the presence of a substrate molecule at one site on the enzyme enhances the catalytic properties of the other substrate-binding sites—that is, their binding sites exhibit cooperativity

2. Heterotropic effectors: The effector may be different from the substrate, in which case the effect is said to be heterotropic. For example, consider the feedback inhibition shown in Figure 5.17. The enzyme that converts D to E has an allosteric site that binds the endproduct, G. If the concentration of G increases, the first irreversible step unique to the pathway is typically inhibited.

B. Regulation of enzymes by covalent modification

Many enzymes may be regulated by covalent modification, most frequently by the addition or removal of phosphate groups from specific serine, threonine, or tyrosine residues of the enzyme. Protein phosphorylation is recognized as one of the primary ways in which cellular processes are regulated.

C. Induction and repression of enzyme synthesis

The regulatory mechanisms described above modify the activity of existing enzyme molecules. However, cells can also regulate the amount of enzyme present by altering the rate of enzyme degradation or, more typically, the rate of enzyme synthesis. The increase (induction) or decrease (repression) of enzyme synthesis leads to an alteration in the total population of active sites.

Alterations in enzyme levels as a result of induction or repression of protein synthesis are slow (hours to days), compared with allosterically or covalently regulated changes in enzyme activity, which occur in seconds to minutes. Figure 5.19 summarizes the common ways that enzyme activity is regulated.

Zymogens:

Some enzymes are produced in nature in an inactive form which can be activated when they are required. Such type of enzymes is called Zymogens (Proenzymes). Many of the digestive enzymes and enzymes concerned with blood coagulation are in this group

Examples:

Pepsinogen - This zymogen is from gastric juice. When required Pepsinogen converts to Pepsin

Trypsinogen - This zymogen is found in the pancreatic juice, and when it is required gets converted to trypsin.

* The activation is brought about by specific ions or by other enzymes that are proteolytic.

Pepsinogen + H⁺ -----Pepsin

Trypsinogen converted to Trypsin by Enteropeptidase enzyme.

Zymogen forms of enzymes a protective mechanism to prevent auto digestion of tissue producing the digestive enzymes and to prevent intravascular coagulation of blood.

Enzymes in clinical diagnosis

Plasma enzymes can be classified into two major group:

- First, a relatively small group of enzymes are actively secreted into the blood by certain cell types.
- Second, a large number of enzyme species are released from cells during normal cell turnover. These enzymes almost always function intracellularly, and have no physiologic use in the plasma.

In healthy individuals, the levels of these enzymes are fairly constant, and represent a steady state in which the rate of release from damaged cells into the plasma is balanced by an equal rate of removal of the enzyme protein from the plasma. Increased plasma levels of these enzymes may indicate tissue damage.

Many diseases that cause tissue damage result in an increased release of intracellular enzymes into the plasma. The activities of many of these enzymes are routinely determined for diagnostic purposes in diseases of the heart, liver, skeletal muscle, and other tissues. The level of specific enzyme activity in the plasma frequently correlates with the extent of tissue damage. Thus, the degree of elevation of a particular enzyme activity in plasma is often useful in evaluating the diagnosis and prognosis for the patient.

Measurement of enzymes concentration of mostly the latter type in plasma gives valuable information about disease involving tissues of their origin.

- Isoenzymes and diseases of the heart

Most isoenzymes (also called isozymes) are enzymes that catalyze the same reaction. However, they do not necessarily have the same physical properties because of genetically determined differences in amino acid sequence.

Different organs frequently contain characteristic proportions of different isoenzymes. The pattern of isoenzymes found in the plasma may, therefore, serve as a means of identifying the site of tissue damage.

Creatine kinase:

Many isoenzymes contain different subunits in various combinations. For example, creatine kinase (CK) occurs as three isoenzymes. Each isoenzyme is a dimer composed of two polypeptides (called B and M subunits) associated in one of three combinations:

CK1 = BB, CK2 = MB, and CK3 = MM. [Note: Virtually all CK in the brain is the BB isoform, whereas in skeletal muscle it is MM. In cardiac muscle, about one-third is MB with the rest as MM.]

Diagnosis of myocardial infarction:

Measurement of blood levels of proteins with cardiac specificity is used in the diagnosis of myocardial infarction (MI) because myocardial muscle is the only tissue that contains more than 5% of the total CK activity as the CK2 (MB) isoenzyme. Appearance of this hybrid isoenzyme in plasma is virtually specific for infarction of the myocardium. Following an acute MI, this isoenzyme appears approximately 4–8 hours following onset of chest pain, reaches a peak of activity at approximately 24 hours, and returns to baseline after 48–72 hours.

Troponin T and troponin I are regulatory proteins involved in myocardial contractility. They are released into the plasma in response to cardiac damage. Cardiac troponin I (cTnI) is highly sensitive and specific for damage to cardiac tissue. cTnI appears in plasma within 4–6 hours after an MI, peaks in 8–28 hours, and remains elevated for 3–10 days. Elevated serum troponins then, are more predictive of adverse outcomes in unstable angina or myocardial infarction than the conventional assay of CK2.