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UNIT I

Biomolecules

Foundational Concept: Biomolecules have unique properties that determine how they contribute to the structure and function of cells and how they participate in the processes necessary to maintain life.

CHAPTER 1 Structure and Function of Proteins and Their Constituent Amino Acids

CHAPTER 2 Transmission of Genetic Information from the Gene to the Protein

CHAPTER 3 Transmission of Heritable Information from Generation to Generation and Processes That Increase Genetic Diversity

CHAPTER 4 Principles of Bioenergetics and Fuel Molecule Metabolism

Unit I MINITEST

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CHAPTER 1

Structure and Function of Proteins and Their Constituent Amino Acids

Read This Chapter to Learn About

- Amino Acids
- Protein Structure
- Functions of Proteins
- Enzyme Structure and Function
- Enzyme Kinetics

AMINO ACIDS

Proteins are constructed from an ensemble of 20 or so naturally-occurring amino acids. In contrast with the saccharides, which possess only carbonyl and hydroxyl functionality, the amino acids boast a wide array of functional groups, as shown in the following table. The properties of each amino acid residue are governed by the characteristics of the side chain. Thus the various amino acids can be classified as polar, nonpolar, neutral, acidic, or basic, as shown in Figure 1-1. As monomers at physiological pH, all amino acids exist as ionized species, as shown in Figure 1-2. The carboxylic acid is entirely deprotonated, and the amino group is completely protonated—so even though the molecule is ionized, there is a net coulombic charge of zero. Such a species is known as a **zwitterion**.

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TABLE 1-1 The Naturally-occurring Amino Acids

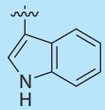
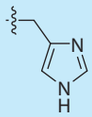
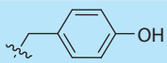
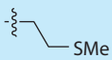
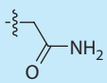
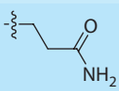
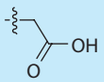
Name	Abbreviation 3-Letter	Abbreviation 1-Letter	Side Chain Structure	Side Chain Functionality	Side Chain pK_a
glycine	Gly	G		none	
alanine	Ala	A		alkane	
valine	Val	V		branched alkane	
leucine	Leu	L		branched alkane	
isoleucine	Ile	I		branched alkane	
phenylalanine	Phe	F		phenyl ring	
tryptophan	Trp	W		indole	
histidine	His	H		imidazole	6.1
tyrosine	Tyr	Y		phenol	10.1
serine	Ser	S		1° alcohol	
threonine	Thr	T		2° alcohol	
methionine	Met	M		dialkyl sulfide	
cysteine	Cys	C		mercaptan	8.2
asparagine	Asn	N		amide	
glutamine	Gln	Q		amide	
aspartic acid	Asp	D		carboxylic acid	3.7

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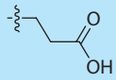
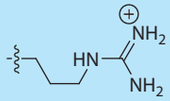
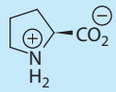
TABLE 1-1 The Naturally-occurring Amino Acids (cont.)

Name	Abbreviation 3-Letter	Abbreviation 1-Letter	Side Chain Structure	Side Chain Functionality	Side Chain pK_a
glutamic acid	Glu	E		carboxylic acid	4.3
lysine	Lys	K		1° amine	10.5
arginine	Arg	R		guanidine	12.5
proline	Pro	P		none	

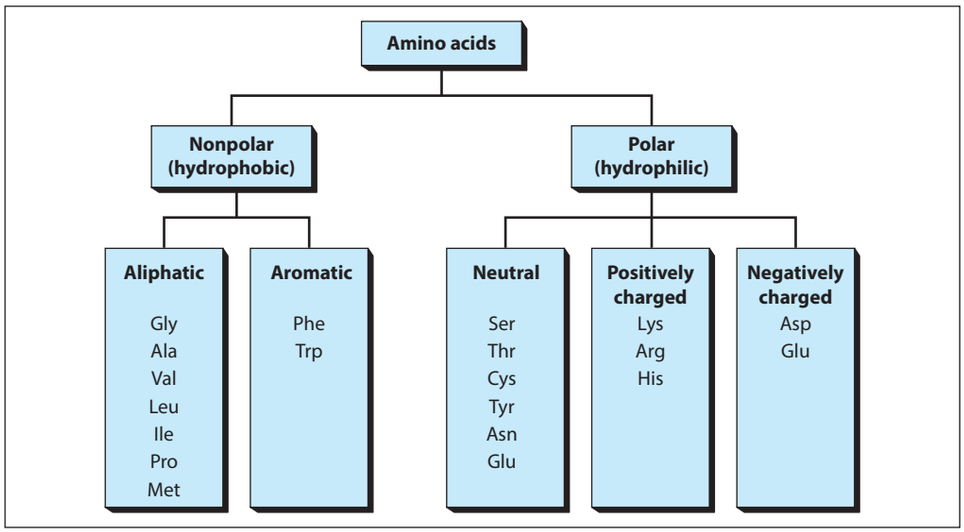


FIGURE 1-1 Classification of amino acids.

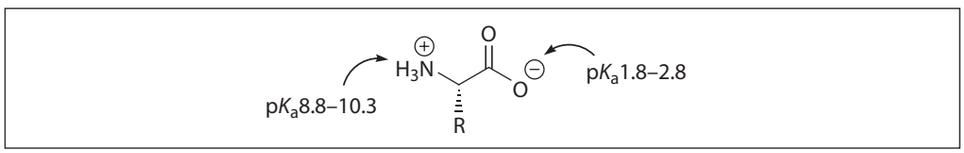


FIGURE 1-2 Zwitterionic nature of amino acids.

Nature takes advantage of these diverse amino acids, particularly in the realm of enzyme catalysis, by assembling them together into synergistic arrangements. In contrast to the polysaccharides, which are connected by acetal linkages, amino acids are bound together by a relatively robust amide linkage. Thus the primary structure of proteins can be described as a polyamide backbone embellished with functionalized

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side chains at regular intervals (see Figure 1-3). While there is hindered rotation about the amide C—N bond, there is relatively free rotation about the C—C bonds in the backbone. Thus the polymer can adopt a variety of conformations, the energetics of which are determined by many complex factors, including intermolecular hydrogen bonding, hydrophobic interactions, and solvation effects.

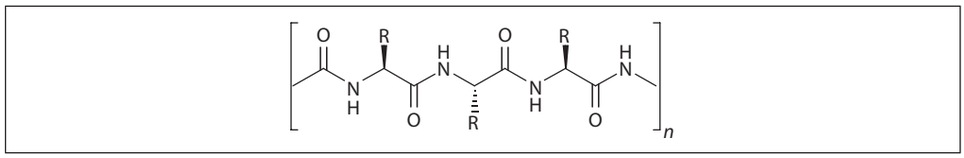

FIGURE 1-3 A polypeptide primary structure.

PROTEIN STRUCTURE

The overall structure of a given protein is governed by an array of parameters, and a hierarchical taxonomy has been developed to describe and analyze these factors.

Primary Structure

The **primary structure** of a protein is the simple connectivity of amino acid to amino acid along the peptide chain. The primary structure includes any disulfide bridges that exist in the protein, as shown in Figure 1-4.

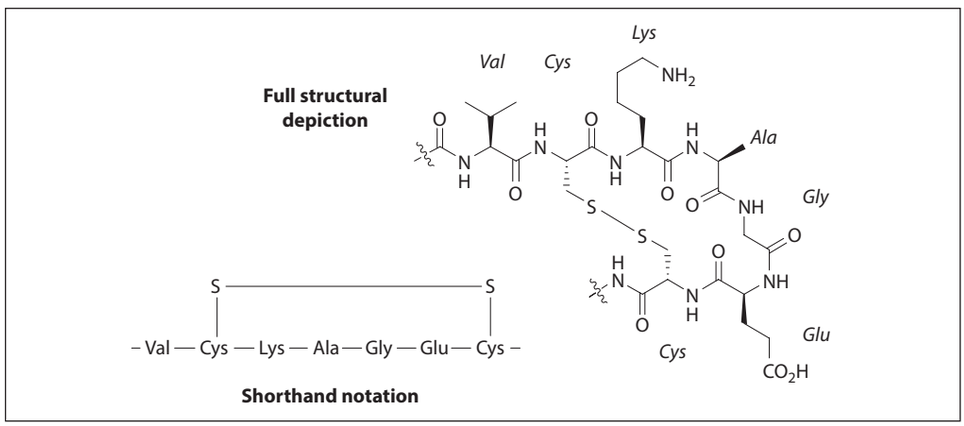

FIGURE 1-4 Primary structure of a peptide fragment, showing a disulfide bridge.

Secondary Structure

The polypeptide strands tend to form well-defined local motifs that constitute the **secondary structure** of proteins. Four of the more common patterns are shown in Figure 1-5. Two types of β-sheets are encountered—an **antiparallel sheet**, in which the two adjacent strands run in opposite directions, and a **parallel sheet** constituted of

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adjacent strands oriented in the same direction; the ***β*-turn motif** is seen at the ends of β-sheets. The ***α*-helical motif** has a very well-defined pattern, with hydrogen bonding occurring between every fourth amino acid residue, and each turn consisting of 3.6 amino acid residues.

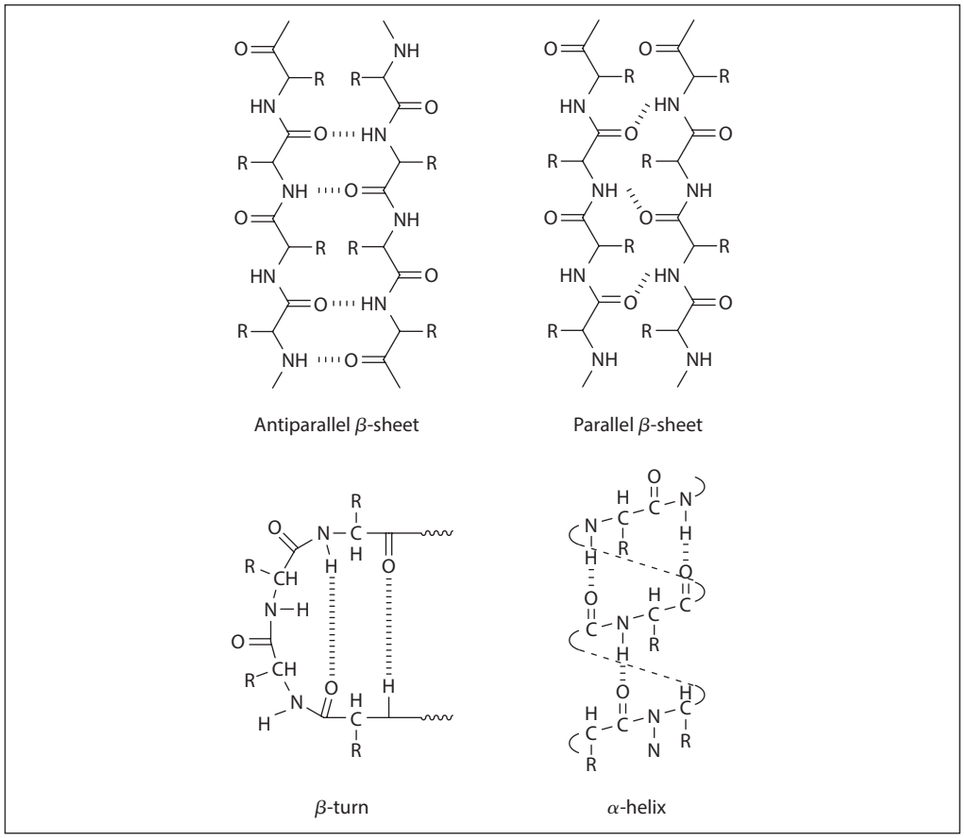

FIGURE 1-5 Four structural motifs in the secondary structure of peptides.

Given the fact that a typical protein is about 300 amino acids long (and can number in the tens of thousands), it is noteworthy that such a small number of secondary motifs constitute such a large proportion of the overall structure of proteins. This is due to the particular conformational constraints along the peptide chain. The peptide bond (i.e., amide bond) itself is planar and not prone to rotation, due to the resonance of the nitrogen lone pair with the carbonyl group (see Figure 1-6, left). The other two

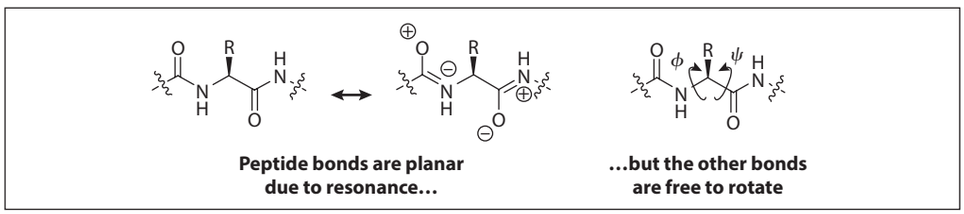

FIGURE 1-6 Conformational constraints in peptides.

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bonds (one C—N and one C—C bond) do have some conformational flexibility, and the dihedral (rotational) angles are defined as ϕ for the C—N bond and ψ for the C—C bond (see Figure 1-6, right). However, even these bonds do not enjoy unfettered rotational freedom. Due to steric interactions, ϕ and ψ have only certain ranges of values that lead to stable conformations overall. Each secondary motif (e.g., α-helix and β-sheet) is associated with a unique range of ϕ and ψ values.

In this context, two amino acids deserve particular attention. With no α-substituent, glycine exhibits a high degree of rotational freedom (see Figure 1-7); consequently, this amino acid is frequently found at hinge sites in a protein. Conversely, proline's cyclic nature essentially shuts down ψ rotational freedom and forces the protein chain to pucker, forming what is known as a **hairpin turn**.

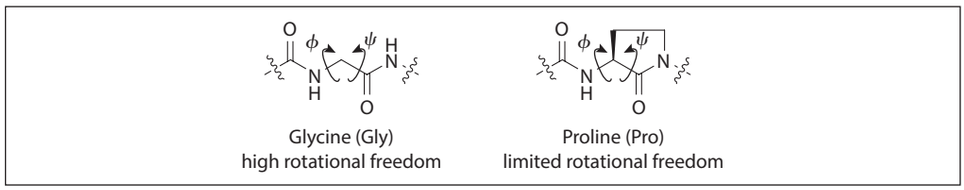

FIGURE 1-7 Two conformationally defining amino acids.

Tertiary Structure

All of the various secondary motifs are assembled together into a global three-dimensional **tertiary structure**, which is the actual shape of the molecule that would be revealed in an X-ray crystallographic analysis. Because of the many convolutions in protein folding, amino acid residues that are quite far apart in the primary sense can be very close to each other in the final folded (or native) protein. The tertiary structure of proteins is often shown in **ribbon diagrams** (see Figure 1-8), in which β-sheets are shown as flat arrows and α-helices are represented as coils, with the less-structured nonrepetitive loops being depicted as ropes connecting the other secondary structures.

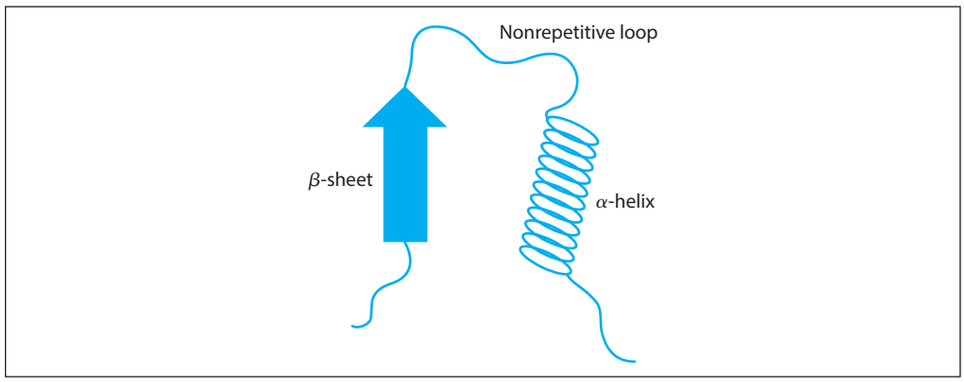

FIGURE 1-8 Ribbon diagram for representing tertiary structure of peptides.

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Quaternary Structure

Finally, two or more separately folded protein strands may associate with each other to form the active form of a protein, which falls under the category of **quaternary structure**. The conventions and depictional devices for tertiary and quaternary structures are identical—the only difference is that tertiary structure describes the global conformation of a single molecule, whereas quaternary structure describes a supramolecular array of multiple protein molecules.

Protein structures are stabilized by a variety of factors, including covalent bonding (e.g., disulfide bridges) and a host of noncovalent forces, such as hydrogen bonding, pi-pi interactions, and dipole-ion interactions). Regions containing a large number of nonpolar amino acids tend to aggregate together in what is called the **hydrophobic effect**. The origin of this effect lies in the fact that nonpolar side chains cannot form hydrogen bonds with the surrounding water molecules. Consequently, the solvation shell (or cage) around a nonpolar group consists of water molecules with limited mobility, incurring an entropic cost. Having nonpolar groups self-associate therefore minimizes the surface area of the solvent cage.

Any number of environmental factors can disrupt the stabilizing forces and lead to the unfolding (or denaturing) of proteins. These include changes in temperature, ionic strength of the solution, the addition of cosolvents (such as ethanol), and even mechanical agitation.

FUNCTIONS OF PROTEINS

The three-dimensional shape of a protein determines the function of that protein. Proteins have the most diverse functions of any of the biological molecules. Some of those functions include protection, contraction, binding, transport, structural support, acting as hormones, and catalyzing chemical reactions. Many of these functions will be elaborated on in subsequent chapters of this book.

- **Protective proteins** have a critical role in the immune system, serving as antibodies. These antibodies come in several different varieties, but they generally work by binding to and inactivating cells displaying molecules that are recognized by the antibody.
- **Contractile proteins** are responsible for motor function or movement. In prokaryotic cells these proteins are part of structures such as flagella and cilia. In eukaryotic cells, specialized proteins such as actin and myosin are used for muscle contraction.
- **Binding proteins** are highly variable in their function. DNA-binding proteins have critical roles in the regulation of protein synthesis and regulation. Some binding

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proteins are critical for transportation. Examples include the transport of oxygen by hemoglobin and the transport of electrons by cytochromes.

- **Structural proteins** function as their name implies. They provide support within cells and tissues. Structural proteins within cells form microtubules, actin filaments, and intermediate filaments—all critical elements of the cytoskeleton. Proteins critical to support within tissues include collagen and keratin, whose shapes are particularly well-suited to providing strength and support.
- Many **hormones** have peptide structures. These hormones play a critical role in maintaining homeostasis within the organism. An example of a human peptide hormone is insulin, which regulates blood glucose levels.
- Proteins that catalyze chemical reactions are **enzymes**. These will be considered in the following sections.

ENZYME STRUCTURE AND FUNCTION

Enzymes are a special category of proteins that serve as biological catalysts speeding up chemical reactions. The enzymes, often with names ending in the suffix *-ase*, function generally to maintain homeostasis within a cell by determining which metabolic pathways occur in that cell. The maintenance of a stable cellular environment and the functioning of the cell are essential to life.

Enzymes function more specifically by lowering the activation energy (see Figure 1-9) required to initiate a chemical reaction, thereby increasing the rate at which the reaction occurs. Most enzymatic reactions are reversible. Enzymes are unchanged during a reaction and are recycled and reused. Enzymes can be involved in catabolic reactions that break down molecules or anabolic reactions that are involved in biosynthesis. The classification of enzymes is based on their reaction type.

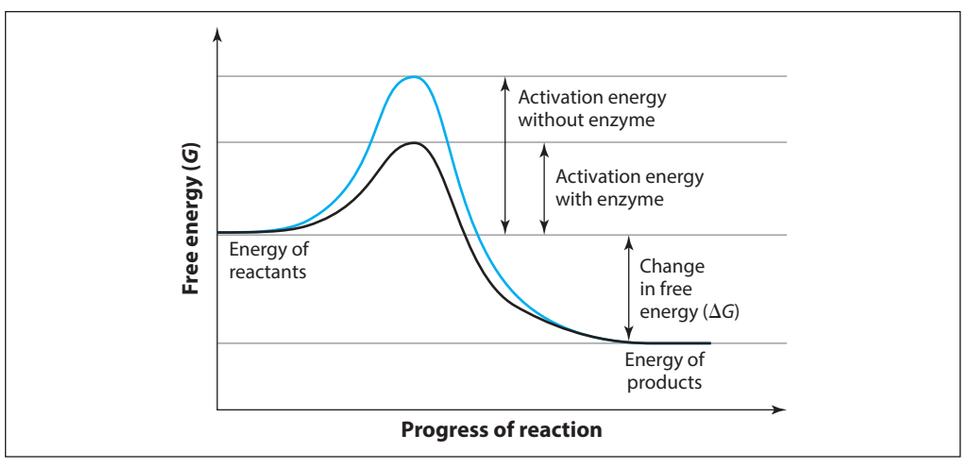

FIGURE 1-9 Increasing rate by lowering the activation energy.

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Enzyme Structure

As stated earlier, enzymes are proteins and, like all proteins, are made up of amino acids. Interactions between the component amino acids determine the overall shape of an enzyme, and it is this shape that is critical to an enzyme's ability to catalyze a reaction.

The area on an enzyme where it interacts with another substance, called a **substrate**, is the enzyme's active site. Based on its shape, a single enzyme typically only interacts with a single substrate (or single class of substrates); this is known as the **enzyme's specificity**. Any changes to the shape of the active site, termed **denaturation**, render the enzyme unable to function. Other sites on the enzyme can be used to bind cofactors and other items needed to regulate the enzyme's activity.

Enzyme Function

The **induced fit model** is used to explain the mechanism of action for enzyme function seen in Figure 1-10. Once a substrate binds loosely to the active site of an enzyme, a conformational change in shape occurs to cause tight binding between the enzyme and the substrate. This tight binding allows the enzyme to facilitate the reaction. A substrate with the wrong shape cannot initiate the conformational change in the enzyme necessary to catalyze the reaction.

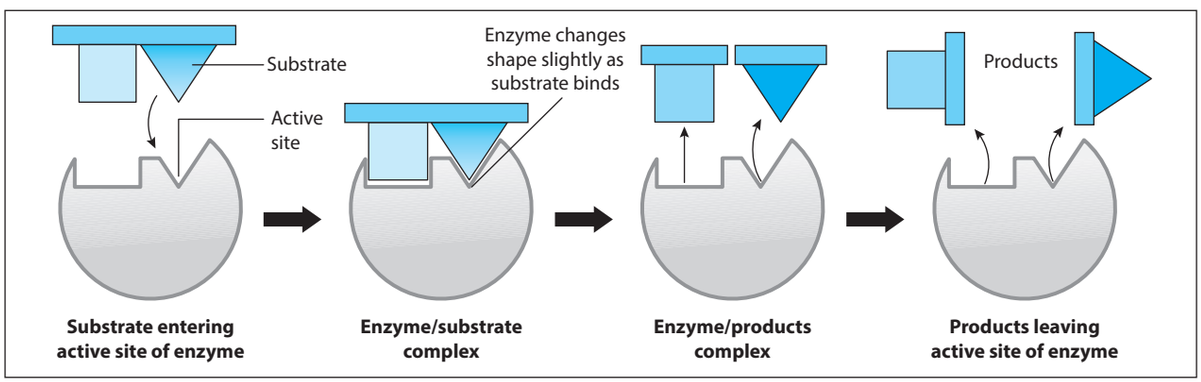

FIGURE 1-10 The induced fit model.

Some enzymes require assistance from other substances to work properly. If assistance is needed, the enzyme has binding sites for cofactors or coenzymes. **Cofactors** are various types of ions such as iron and zinc (Fe^{2+} and Zn^{2+}). **Coenzymes** are organic molecules usually derived from water-soluble vitamins obtained in the diet. For this reason, mineral and vitamin deficiencies can have serious consequences on enzymatic functions.

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FACTORS THAT AFFECT ENZYME FUNCTION

There are several factors that can influence the activity of a particular enzyme. The first is the **concentration** of the substrate and the concentration of the enzyme. Reaction rates stay low when the concentration of the substrate is low, whereas the rates increase when the concentration of the substrate increases. **Temperature** is also a factor that can alter enzyme activity. Each enzyme has an optimal temperature for functioning. In humans this is typically body temperature (37° C). At lower temperatures, the enzyme is less efficient. Increasing the temperature beyond the optimal point can lead to enzyme denaturation, which renders the enzyme useless. Enzymes also have an optimal pH in which they function best, typically around 7 in humans, although there are exceptions. Additionally, extreme changes in pH, ionic strength of the solution, and the addition of cosolvents can also lead to enzyme denaturation. The denaturation of an enzyme is not always reversible.

ENZYME KINETICS

The study of enzyme kinetics involves investigating the effects of various conditions on the reaction rate of enzymes. Most enzymes show an increased reaction rate with increasing substrate concentration until saturation is reached, meaning that increasing substrate concentration no longer increases reaction rate. This relationship can be seen in Figure 1-11.

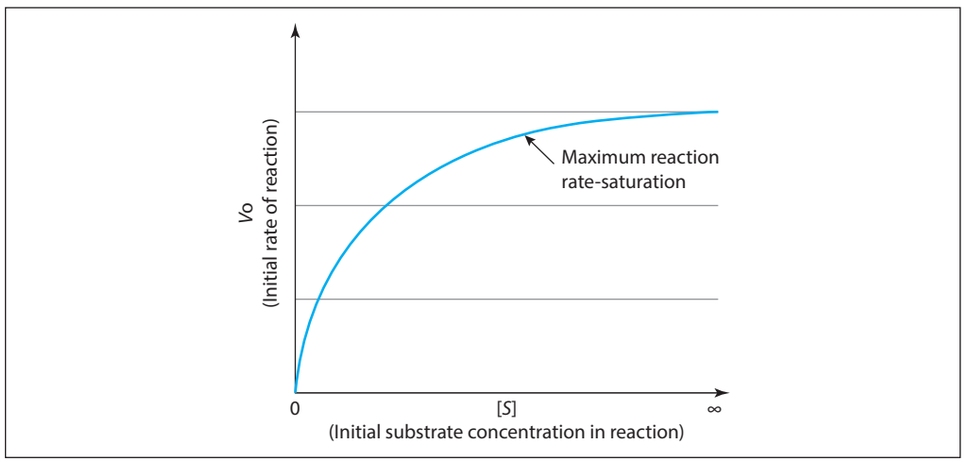

FIGURE 1-11 Enzyme catalysis as a function of substrate concentration.

Michaelis–Menten Kinetics

Enzymes can exhibit a wide variety of kinetic behavior, but one of the most common paradigms is known as the **Michaelis–Menten model**. In this type of system a substrate

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(S) and enzyme (E) engage in a pre-equilibrium to form an enzyme-substrate complex (ES)—also called the **Michaelis complex**—which then undergoes conversion to the product (P).

$$E + S \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P \quad \Rightarrow \quad E + S \overset{K_m}{\rightleftharpoons} ES \xrightarrow{k_{cat}} E + P$$

For systems that obey Michaelis–Menten kinetics, when the initial velocity of product formation (v) is plotted against the initial substrate concentration ($\{S\}$), a data set is obtained that can be fit to a rectangular parabolic function, as shown in Figure 1-12. This function asymptotically approaches a maximum velocity (V_{max}) as $\{S\}$ approaches infinity. The concentration corresponding to exactly half the V_{max} is defined as the Michaelis constant, or K_m. On one hand, this constant is a measure of the stability of the Michaelis complex (ES); another interpretation is that K_m represents the concentration of substrate necessary for effective catalysis to be observed. In other words, an enzyme with a very low K_m will catalyze reactions with very low substrate concentrations. Often, K_m is referred to as the **binding affinity**—that is, enzymes with a low K_m have a high binding affinity. However, the latter description holds true only if $k_{off} \gg k_{cat}$.

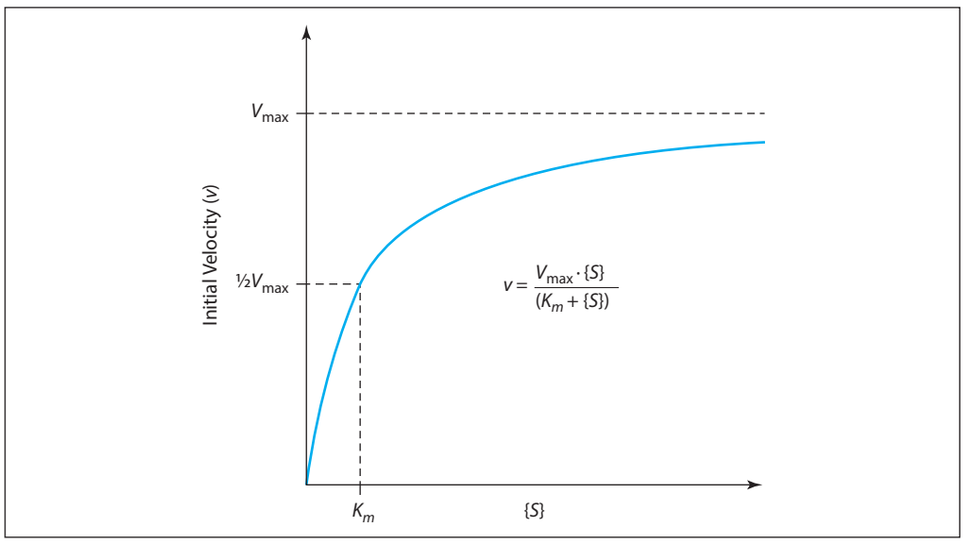

FIGURE 1-12 An enzyme system obeying Michaelis–Menten kinetics.

One classical way to estimate these constants with a linear fit is through the **Lineweaver–Burk plot** (see Figure 1-13), in which the reciprocal of velocity is plotted against the reciprocal of the substrate concentration (for this reason, it is sometimes called a **double reciprocal plot**). On an L–B plot, the y-intercept is the reciprocal of V_{max}, the x-intercept is the reciprocal of K_m, and the slope is the ratio of K_m to V_{max}.

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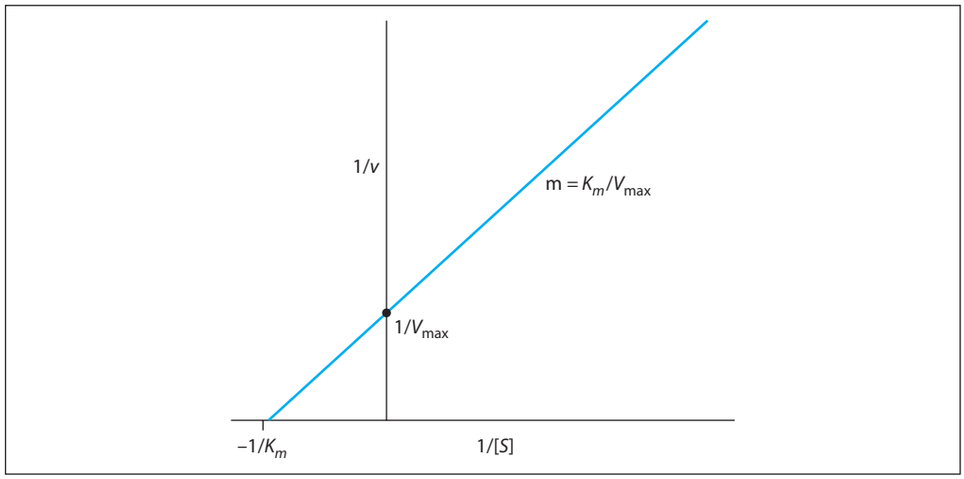

FIGURE 1-13 A Lineweaver-Burk plot.

The **catalytic rate constant** (k_{cat}) can be estimated from V_{max} through the following relationship:

$$V_{max} = k_{cat} \cdot \{E\}_T$$

where $\{E\}_T$ represents the total number of binding sites, or the sum of bound and unbound enzyme. The catalytic rate constant is also called the **turnover number**, which is a measure of how many substrate molecules can be converted into product in a given amount of time when the enzyme is saturated with substrate. The units of k_{cat} are sec^{-1}, and the reciprocal of this value is a measure of the time it takes for one enzyme molecule to turn over (i.e., become available for the next substrate molecule). Therefore, enzymes with high k_{cat} values turn over very quickly (i.e., in a very short amount of time).

The ratio of k_{cat}/K_m is often used as a measure of the enzyme's efficiency: the higher the ratio, the more efficient the enzyme. If an enzyme operates on a variety of substrates, this ratio can also reflect the selectivity of an enzyme for one substrate over another. For example, the k_{cat}/K_m ratio exhibited by chymotrypsin for phenylalanine is on the order of 10^5, whereas the k_{cat}/K_m ratio for glycine is on the order of 10^{-1}, meaning chymotrypsin shows a millionfold selectivity for phenylalanine vs. glycine.

The Michaelis–Menten model is based on a few simplifying assumptions, including:

1. **The steady-state approximation**, which assumes that the concentration of ES remains constant even though the concentration of substrate and product are changing.
2. **The free ligand approximation**, which assumes that the concentration of the free substrate approximates the total substrate concentration, a premise that holds as long as the enzyme concentration is well below K_m.

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3. **The rapid equilibrium approximation**, which assumes the turnover rate (k_{cat}) is much smaller than the reverse equilibrium rate constant (k_{off}).

Cooperativity

The reaction rate of an enzyme can be influenced by multiple substrate binding sites. When enzymes have multiple substrate binding sites, the affinity of those binding sites can be altered upon binding to a single site. For example, hemoglobin has four binding sites. The binding of oxygen to the first binding site increases the affinity of the other binding sites on hemoglobin. This is termed **cooperative binding**. In some cases, binding of one substrate decreases the affinity of other bonding sites. This is called **negative cooperativity**.

Control of Enzyme Activity

It is critical to be able to regulate the activity of enzymes in cells to maintain efficiency. This regulation can be carried out in a variety of ways.

FEEDBACK REGULATION

In addition to an active site, allosteric enzymes have another site for the attachment of regulatory molecules. Many enzymes contain allosteric binding sites and require signal molecules such as repressors and activators to function. Feedback regulation, illustrated in Figure 1-14, acts somewhat like a thermostat to regulate enzyme activity. As the product of a reaction builds up, repressor molecules can bind to the allosteric site of the enzyme, causing a change in the shape of the active site. The consequence of this binding is that the substrate can no longer interact with the active site of the enzyme, and the activity of the enzyme is temporarily slowed or halted. When the product of the reaction declines, the repressor molecule dissociates from the allosteric site. This allows the active site of the enzyme to resume its normal shape and normal activity.

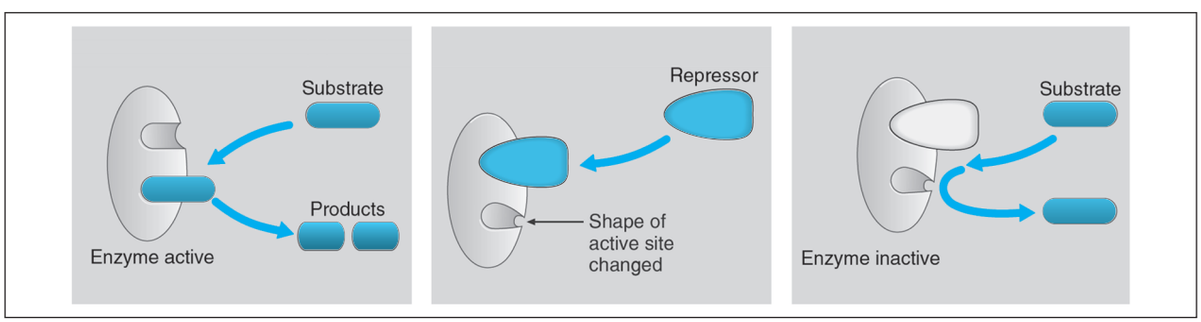

FIGURE 1-14 Allosteric inhibition of an enzyme. Repressors can be used to regulate the activity of an enzyme. *Source:* From George B. Johnson. *The Living World*, 3rd ed., McGraw-Hill, 2003; reproduced with permission of The McGraw-Hill Companies.

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Some allosteric enzymes stay inactive unless activator molecules are present to allow the active site to function.

ENZYME INHIBITION

Inhibitor molecules also regulate enzyme action. A **competitive inhibitor** is a molecule that resembles the substrate in shape so much that it binds to the active site of the enzyme, thus preventing the substrate from binding. This halts the activity of the enzyme until the competitive inhibitor is removed or is outcompeted by an increasing amount of substrate. **Noncompetitive inhibitors** bind to allosteric sites and change the shape of the active site, thereby decreasing the functioning of the enzyme. Increasing levels of substrate have no effect on noncompetitive inhibitors, but the activity of the enzyme can be restored when the noncompetitive inhibitor is removed.

In contrast to competitive inhibition, which allows an inhibitor to bind to the active site in order to block substrate binding, during **uncompetitive inhibition** an inhibitor binds to the enzyme if the substrate is already bound. During **mixed inhibition**, the inhibitor may bind whether the enzyme is bound to the substrate or not.

COVALENT MODIFICATIONS

One means of covalent modification of enzymes involves the transfer of an atom or molecule to the enzyme from a donor or proteolytic cleavage of the amino acid sequence of the enzyme. The **phosphorylation** (transfer of inorganic phosphate) of enzymes by kinases and the **dephosphorylation** of enzymes by phosphatases are examples of covalent modification.

Zymogens are enzyme precursors found in an inactive form. In order for the zymogen to be activated, a biochemical change must occur to expose the active site of the enzyme. This activation often involves proteolytic cleavage of the enzyme and occurs in the lysosomes of eukaryotic cells. The digestive enzyme pepsin is secreted in zymogen form (called pepsinogen) to prevent the enzyme from digesting proteins in the cells of the pancreas where the enzyme is produced.