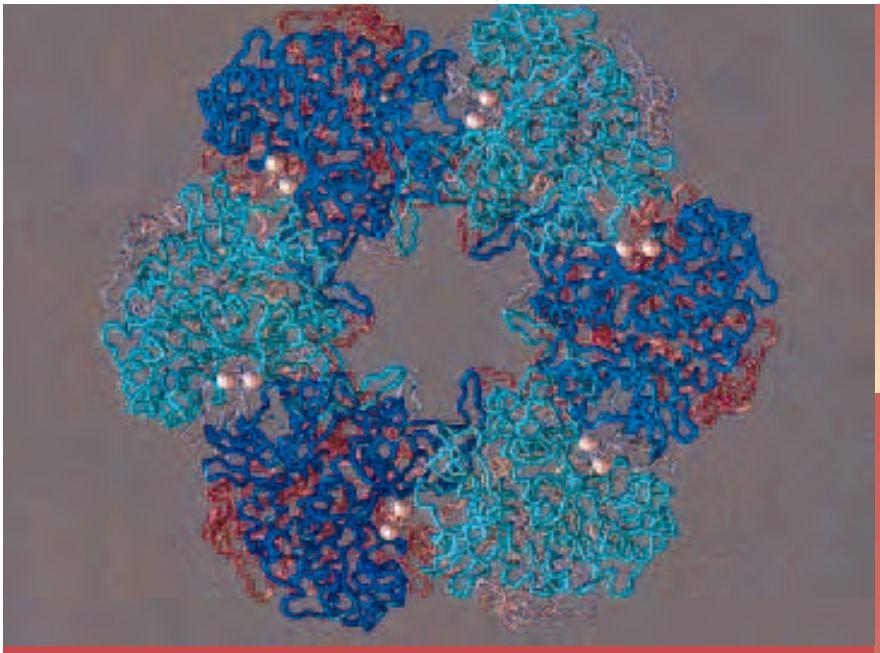

Molecular model of glutamine synthetase.

20

Enzymes

BIOCHEMISTRY

Outline

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Classification of Enzymes
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20.12 Uses of Enzymes in Medicine

Summary
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Critical Thinking Problems

Learning Goals

1. Classify enzymes according to the type of reaction catalyzed and the type of specificity.
2. Give examples of the correlation between an enzyme's common name and its function.
3. Describe the effect that enzymes have on the activation energy of a reaction.
4. Explain the effect of substrate concentration on enzyme-catalyzed reactions.
5. Discuss the role of the active site and the importance of enzyme specificity.
6. Describe the difference between the lock-and-key model and the induced fit model of enzyme-substrate complex formation.
7. Discuss the roles of cofactors and coenzymes in enzyme activity.
8. Explain how pH and temperature affect the rate of an enzyme-catalyzed reaction.
9. Describe the mechanisms used by cells to regulate enzyme activity.
10. Discuss the mechanisms by which certain chemicals inhibit enzyme activity.
11. Discuss the role of the enzyme chymotrypsin and other serine proteases.
12. Provide examples of medical uses of enzymes.

CHEMISTRY CONNECTION

Super Hot Enzymes and the Origin of Life

Imagine the earth about four billion years ago: It was young then, not even a billion years old. Beginning as a red-hot molten sphere, slowly the earth's surface had cooled and become solid rock. But the interior, still extremely hot, erupted through the crust spewing hot gases and lava. Eventually these eruptions produced craggy land masses and an atmosphere composed of gases like hydrogen, carbon dioxide, ammonia, and water vapor. As the water vapor cooled, it condensed into liquid water, forming ponds and shallow seas.

At the dawn of biological life, the surface of the earth was still very hot and covered with rocky peaks and hot shallow oceans. The atmosphere was not very inviting either—filled with noxious gases and containing no molecular oxygen. Yet this is the environment that fostered the beginnings of life on this planet.

Some scientists think that they have found bacteria—living fossils—that may be very closely related to the first inhabitants of earth. The bacteria thrive at temperatures higher than the boiling point of water. Some need only H_2, CO_2, and H_2O for their metabolic processes and they quickly die in the presence of molecular oxygen.

But this lifestyle raises some uncomfortable questions. For instance, how do these bacteria survive at these extreme temperatures that would cook the life-forms with which we are more familiar? Researcher Mike Adams of the University of Georgia has found some of the answers. Adams and his students have studied the structure of an enzyme, a protein that acts as a biological catalyst, from one of these extraordinary bacteria. He compared the structure of the super hot enzyme with that of the same enzyme purified from an organism that grows at "normal" temperatures. The overall three-dimensional structures of the two enzymes were very similar. This makes sense because they both catalyze the same reaction.

The question, then, is why is the super hot enzyme so stable at very high temperatures, while its low temperature counterpart is not. The answer lay in the tertiary structure of the enzyme. Adams observed that the three-dimensional structure of the super hot enzyme is held together by many more R group interactions than are found in the low-temperature version. These R group interactions, along with other differences, keep the protein stable and functional even at temperatures above 100°C.

In Chapter 19 we studied the structure and properties of proteins. We are now going to apply that knowledge to the study of a group of proteins that do the majority of the work for the cell. These special proteins, the enzymes, catalyze the biochemical reactions that break down food molecules to allow the cell to harvest energy. They also catalyze the biosynthetic reactions that produce the molecules required for cellular life. In this chapter we will study the properties of this extraordinary group of proteins and learn how they dramatically speed up biochemical reactions.

Introduction

The enzymes discussed in this chapter are proteins; however, several ribonucleic acid (RNA) molecules have been demonstrated to have the ability to catalyze biological reactions. These are *ribozymes*.

An **enzyme** is a biological molecule that serves as a catalyst for a biochemical reaction. The majority of enzymes are proteins. Without enzymes to speed up biochemical reactions, life could not exist. The life of the cell depends on the simultaneous occurrence of hundreds of chemical reactions that must take place rapidly under mild conditions. It is possible, for example, to add water to an alkene. However, this reaction is usually carried out at a temperature of 100°C in aqueous sulfuric acid. Such conditions would kill a cell. The fragile cell must carry out its chemical reactions at body temperature (37°C) and in the absence of any strong acids or bases. How can this be accomplished? In Section 8.3 we saw that catalysts lower the energy of activation of a chemical reaction and thereby increase the rate of the reaction. This allows reactions to occur under milder conditions. The cell uses enzymes to solve the problem of chemical reactions that must occur rapidly under the mild conditions found within the cell. The enzyme *facilitates* a biochemical reaction, lowering the energy of activation and increasing the rate of the reaction. The efficient functioning of enzymes is essential for the life of the cell and of the organism.

The twin phenomena of high specificity and rapid reaction rates are the cornerstones of enzyme activity and the topic of this chapter. A typical cell contains thousands of different molecules, each of which is important to the chemistry of life processes. Each enzyme "recognizes" only one, or occasionally a few, of these

molecules. One of the most remarkable features of enzymes is this *specificity.* Each can recognize and bind to a single type of *substrate* or reactant. The molecular size, shape, and charge distribution of both the enzyme and substrate must be compatible for this selective binding process to occur. The enzyme then transforms the substrate into the *product* with lightning speed. In fact, enzyme-catalyzed reactions often occur from one million to one hundred million times faster than the corresponding uncatalyzed reaction.

The enzyme *catalase* provides one of the most spectacular examples of the increase in reaction rates brought about by enzymes. This enzyme is required for life in an oxygen-containing environment. In this environment the process of the aerobic (oxygen-requiring) breakdown of food molecules produces hydrogen peroxide (H_2O_2). Because H_2O_2 is toxic to the cell, it must be destroyed. One molecule of catalase converts *forty million* molecules of hydrogen peroxide to harmless water and oxygen every second:

$$2H_2O_2 \xrightarrow[\text{(an enzyme)}]{\text{Catalase}} 2H_2O + O_2$$

Reaction occurs forty million times every second!

This is the same reaction that you witness when you pour hydrogen peroxide on a wound. The catalase released from injured cells rapidly breaks down the hydrogen peroxide. The bubbles that you see are oxygen gas released as a product of the reaction.

20.1 Nomenclature and Classification

Classification of Enzymes

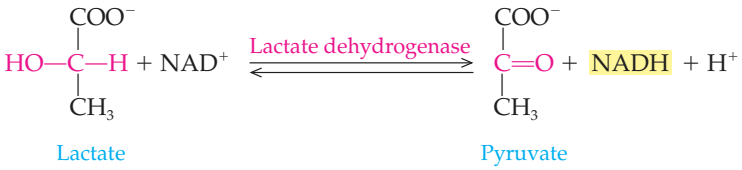
Enzymes may be classified according to the type of reaction that they catalyze. The six classes are as follows.

Learning Goal 1

Oxidoreductases

Oxidoreductases are enzymes that catalyze oxidation–reduction (redox) reactions. *Lactate dehydrogenase* is an oxidoreductase that removes hydrogen from a molecule of lactate. Other subclasses of the oxidoreductases include oxidases and reductases.

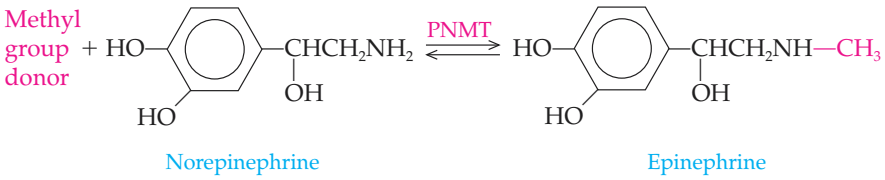
Recall that redox reactions involve electron transfer from one substance to another (Section 9.5).



Transferases

Transferases are enzymes that catalyze the transfer of functional groups from one molecule to another. For example, a *transaminase* catalyzes the transfer of an amino functional group, and a *kinase* catalyzes the transfer of a phosphate group. Kinases play a major role in energy-harvesting processes involving ATP. In the adrenal glands, norepinephrine is converted to epinephrine by the enzyme *phenylethanolamine-N-methyltransferase* (PNMT), a *transmethylase.*

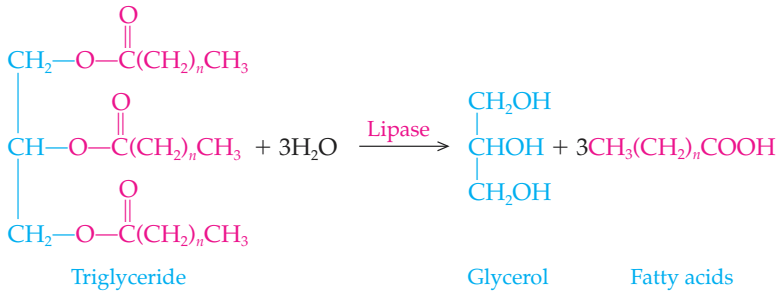
The significance of phosphate group transfers in energy metabolism is discussed in Sections 21.1 and 21.3.



Hydrolysis of esters is described in Section 15.2. The action of lipases in digestion is discussed in Section 23.1.

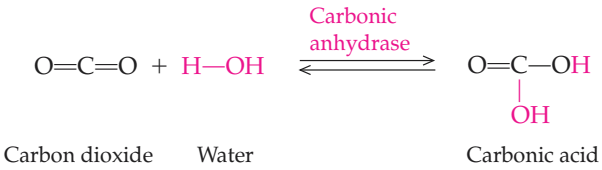
Hydrolases

Hydrolases catalyze hydrolysis reactions, that is, the addition of a water molecule to a bond resulting in bond breakage. These reactions are important in the digestive process. For example, *lipases* catalyze the hydrolysis of the ester bonds in triglycerides:

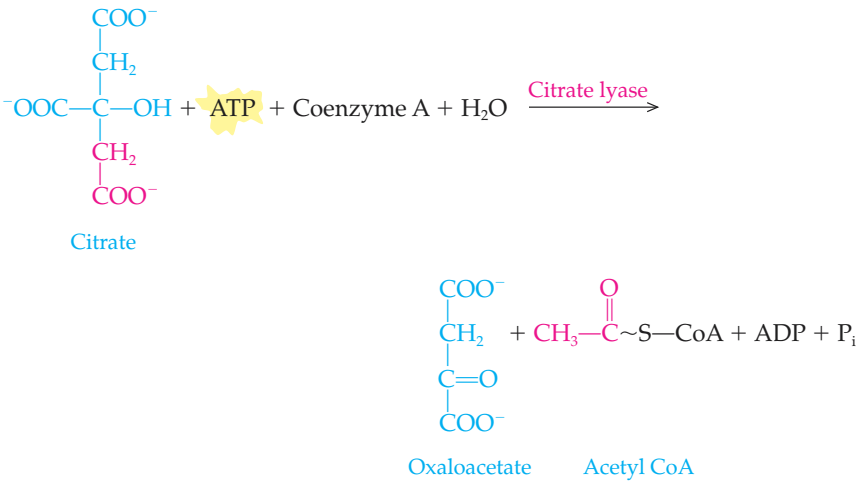


Lyases

Lyases catalyze the addition of a group to a double bond or the removal of a group to form a double bond. *Carbonic anhydrase* is an example of a lyase. This reaction, which we studied in Section 19.9, occurs in the blood and is one of the body's mechanisms for buffering body fluids. In this reaction we see the addition of a group to a double bond.



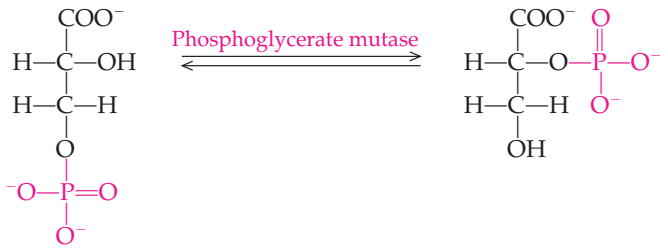
Citrate lyase catalyzes a far more complicated reaction in which we see the removal of a group and formation of a double bond. Specifically, citrate lyase catalyzes the removal of an acetyl group from a molecule of citrate. The products of this reaction include oxaloacetate, acetyl CoA, ADP, and an inorganic phosphate group (P_i):



Recall that the squiggle (~) represents a high-energy bond.

Isomerases

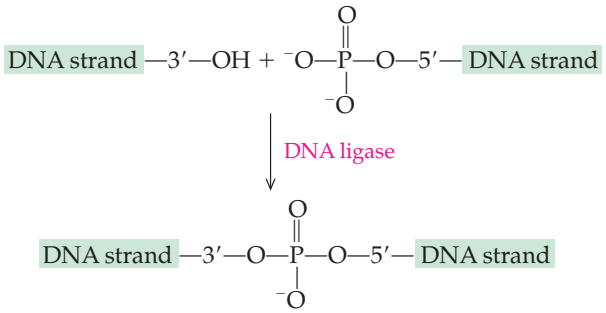
Isomerases rearrange the functional groups within a molecule and catalyze the conversion of one isomer into another. For example, *phosphoglycerate mutase* converts one structural isomer, 3-phosphoglycerate, into another, 2-phosphoglycerate:



3-Phosphoglycerate 2-Phosphoglycerate

Ligases

Ligases are enzymes that catalyze a reaction in which a C—C, C—S, C—O, or C—N bond is made or broken. This is accompanied by an ATP-ADP interconversion. For example, *DNA ligase* catalyzes the joining of the hydroxyl group of a nucleotide in a DNA strand with the phosphoryl group of the adjacent nucleotide to form a phosphoester bond:

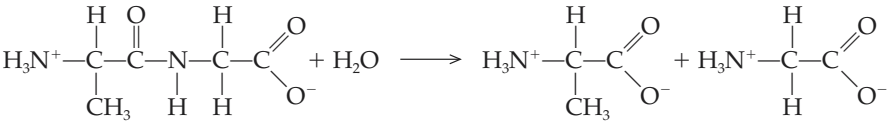


The use of DNA ligase in recombinant DNA studies is detailed in Section 24.8.

ATP, adenosine triphosphate, is the universal energy currency for all life-forms we have studied. It is formed by the addition of a phosphoryl group to a molecule of ADP, adenosine diphosphate. Formation of ATP requires energy. Thus, when a ligase breaks a bond, the energy released is used to form ATP. When a ligase forms a bond, energy is required. That energy is provided by the hydrolysis of the terminal phosphoric anhydride bond in ATP.

Classifying Enzymes According to the Type of Reaction That They Catalyze — EXAMPLE 20.1

Classify the enzyme that catalyzes each of the following reactions, and explain your reasoning.



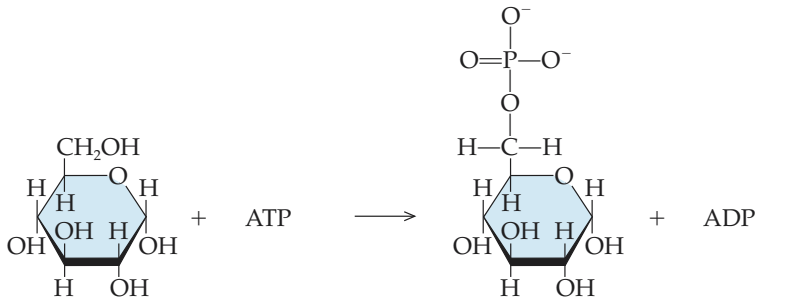
Alanylglycine Alanine Glycine

Solution

The reaction occurring here involves breaking a bond, in this case a peptide bond, by the addition of a water molecule. The enzyme is classified as a hydrolase, specifically a peptidase.

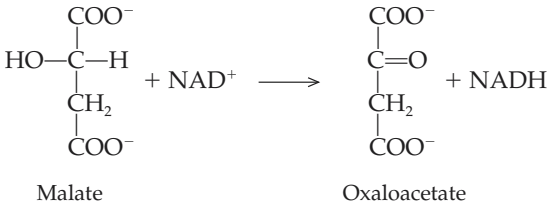
Continued—

EXAMPLE 20.1 *—Continued*



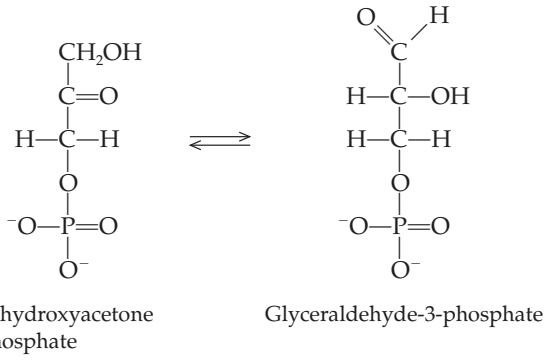
Solution

This is the first reaction in the biochemical pathway called *glycolysis*. A phosphoryl group is transferred from a donor molecule, adenosine triphosphate, to the recipient molecule, glucose. The products are glucose-6-phosphate and adenosine diphosphate. This enzyme, called *hexokinase*, is an example of a transferase.



Solution

In this reaction, the reactant malate is oxidized and the coenzyme NAD^+ is reduced. The enzyme that catalyzes this reaction, malate dehydrogenase, is an oxidoreductase.



Solution

Careful inspection of the structure of the reactant and the product reveals that they each have the same number of carbon, hydrogen, oxygen, and phosphorus atoms; thus, they must be structural isomers. The enzyme must be an isomerase. Its name is triose phosphate isomerase.

Question 20.1

To which class of enzymes does each of the following belong?

a. Pyruvate kinase
b. RNA ligase
c. Triose isomerase
d. Pyruvate dehydrogenase
e. Phosphoglucoisomerase

Question 20.2

To which class of enzymes does each of the following belong?

a. Phosphofructokinase
b. Lipase
c. Acetoacetate decarboxylase
d. Succinate dehydrogenase
e. Alanine transaminase

Nomenclature of Enzymes

Learning Goal 2

The common name for a hydrolase is derived from the name of the **substrate,** the reactant that binds to the enzyme and is converted to product. Names of other enzymes reflect the type of reactions that they catalyze. Because of this, the function of the enzyme is generally conveyed directly by its common name.

Let's look at a few examples of this simple concept. *Urea* is the substrate acted on by the enzyme *urease:*

$$\underset{\text{Substrate}}{\text{Urea} - \text{a}} + \text{ase} = \underset{\text{Enzyme}}{\text{Urease}}$$

Note that the name of this enzyme is simply the name of the substrate with the ending *-ase* added. With the exception of some historical common names, the general ending for the name of an enzyme is *-ase.* For instance, *lactose* is the substrate of *lactase:*

$$\underset{\text{Substrate}}{\text{Lactose} - \text{ose}} + \text{ase} = \underset{\text{Enzyme}}{\text{Lactase}}$$

Other enzymes may be named for the reactions they catalyze. For example,

Dehydrogenases remove hydrogen atoms, transferring them to a coenzyme.

Decarboxylases remove carboxyl groups.

Coenzymes are molecules required by some enzymes to serve as donors or acceptors of electrons, hydrogen atoms, or other functional groups during a chemical reaction. Coenzymes are discussed in Section 20.7.

The prefix *de-* indicates that a functional group is being removed. Hydrogenases and carboxylases, on the other hand, add hydrogen or carboxyl groups. Some enzyme names include *both* the names of the substrate and of the reaction type. For example, *lactate dehydrogenase* removes hydrogen atoms from lactate ions, and *pyruvate decarboxylase* removes the carboxyl group from pyruvate.

The complete name for lactate dehydrogenase is lactate: NAD oxidoreductase. This systematic name tells us the substrate, coenzyme, and type of reaction catalyzed.

As in other areas of chemistry, historical names, having no relationship to either substrate or reaction, continue to be used. In these cases the substrates and reactions must simply be memorized. Examples of some historical common names include catalase, pepsin, chymotrypsin, and trypsin.

Question 20.3

What is the substrate for each of the following enzymes?

a. Sucrase
b. Pyruvate decarboxylase
c. Succinate dehydrogenase

Question 20.4

What chemical reaction is mediated by each of the enzymes in Question 20.3?

20.2 The Effect of Enzymes on the Activation Energy of a Reaction

Learning Goal 3

How does an enzyme speed up a chemical reaction? It changes the path by which the reaction occurs, providing a lower energy route for the conversion of the substrate into the **product,** the chemical species that results from the enzyme-catalyzed reaction. Thus enzymes speed up reactions by lowering the activation energy of the reaction.

Recall that every chemical reaction is characterized by an equilibrium constant. Consider, for example, the simple equilibrium

The activation energy (Section 8.3) of a reaction is the threshold energy that must be overcome to produce a chemical reaction.

$$aA \rightleftharpoons bB$$

The equilibrium constant for this reaction, K_{eq}, is defined as

Equilibrium constants are described in Section 8.4.

$$K_{eq} = \frac{[B]^b}{[A]^a} = \frac{[\text{product}]^b}{[\text{reactant}]^a}$$

Energy, rate, and equilibrium are described in Chapter 8.

This equilibrium constant is actually a reflection of the difference in energy between reactants and products. It is a measure of the relative stabilities of the reactants and products. No matter how the chemical reaction occurs (which path it follows), the difference in energy between the reactants and the products is always the same. **An enzyme cannot therefore alter the equilibrium constant for the reaction that it catalyzes.** An enzyme does, however, change the path by which the process occurs, providing a lower energy route for the conversion of the substrate into the product. An enzyme increases the rate of a chemical reaction by lowering the activation energy for the reaction (Figure 20.1). An enzyme thus increases the rate at which the reaction it catalyzes reaches equilibrium.

20.3 The Effect of Substrate Concentration on Enzyme-Catalyzed Reactions

Learning Goal 4

The rates of uncatalyzed chemical reactions often double every time the substrate concentration is doubled (Figure 20.2a). Therefore as long as the substrate concentration increases, there is a direct increase in the rate of the reaction. For enzyme-

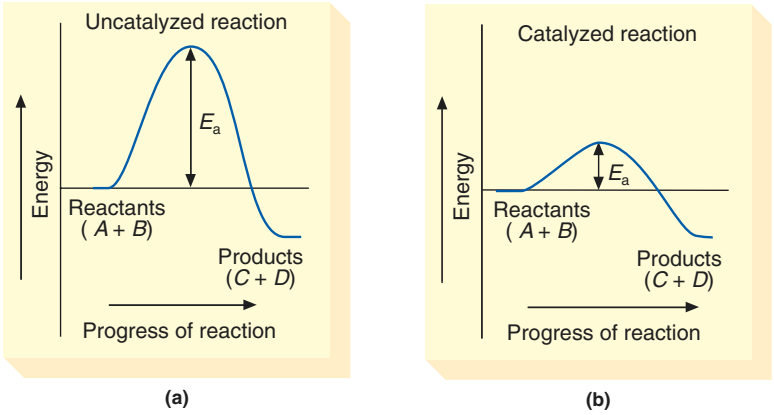


Figure 20.1
Diagram of the difference in energy between the reactants (*A* and *B*) and products (*C* and *D*) for a reaction. Enzymes cannot change this energy difference but act by lowering the activation energy (E_a) for the reaction, thereby speeding up the reaction.

catalyzed reactions, however, this is not the case. Although the rate of the reaction is initially responsive to the substrate concentration, at a certain concentration of substrate the rate of the reaction reaches a maximum value. A graph of the rate of reaction, V, versus the substrate concentration, [S], is shown in Figure 20.2b. We see that the rate of the reaction initially increases rapidly as the substrate concentration is increased but that the rate levels off at a maximum value. At its maximum rate the active sites of all the enzyme molecules in solution are occupied by a substrate molecule. The active site is the region of the enzyme that specifically binds the substrate and catalyzes the reaction. A new molecule of substrate cannot bind to a given enzyme molecule until the substrate molecule already bound to the enzyme is converted to product. Thus it appears that the enzyme-catalyzed reaction occurs in two stages. The first, rapid step is the formation of the *enzyme-substrate complex*. The second step is slower and thus controls the rate at which the reaction can occur. It is said to be *rate-limiting* and involves conversion of the substrate to product and the release of the product and enzyme from the resulting enzyme-product complex. In effect, the rate of the reaction is limited by the speed with which the substrate is converted into product and the product is released. Thus the reaction rate is dependent on the availability of the enzyme.

20.4 The Enzyme-Substrate Complex

Learning Goal 5

The following series of reversible reactions represents the steps in an enzyme-catalyzed reaction. The first step (highlighted in blue) involves the encounter of the enzyme with its substrate and the formation of an **enzyme-substrate complex.**

$$\underset{\text{Enzyme + substrate}}{E + S} \underset{}{\overset{\text{Step I}}{\rightleftharpoons}} \underset{\text{Enzyme–substrate complex}}{ES} \overset{\text{Step II}}{\rightleftharpoons} \underset{\text{Transition state}}{ES^*} \overset{\text{Step III}}{\rightleftharpoons} \underset{\text{Enzyme–product complex}}{EP} \overset{\text{Step IV}}{\rightleftharpoons} \underset{\text{Enzyme + product}}{E + P}$$

The part of the enzyme that binds with the substrate is called the **active site.** We find that the properties of the active site are crucial to the function of the enzyme and have the following general characteristics:

- Enzyme active sites are pockets or clefts in the surface of the enzyme. The R groups in the active site that are involved in catalysis are called *catalytic groups*.
- The shape of the active site is complementary to the shape of the substrate. That is, the substrate fits neatly into the active site of the enzyme.

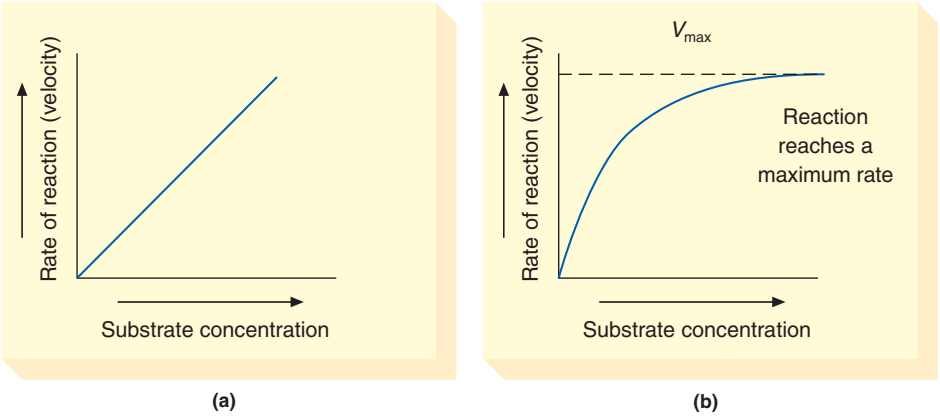


Figure 20.2
Plot of the rate or velocity, V, of a reaction versus the concentration of substrate, [S], for (a) an uncatalyzed reaction and (b) an enzyme-catalyzed reaction. For an enzyme-catalyzed reaction the rate is at a maximum when all of the enzyme molecules are bound to the substrate. Beyond this concentration of substrate, further increases in substrate concentration have no effect on the rate of the reaction.

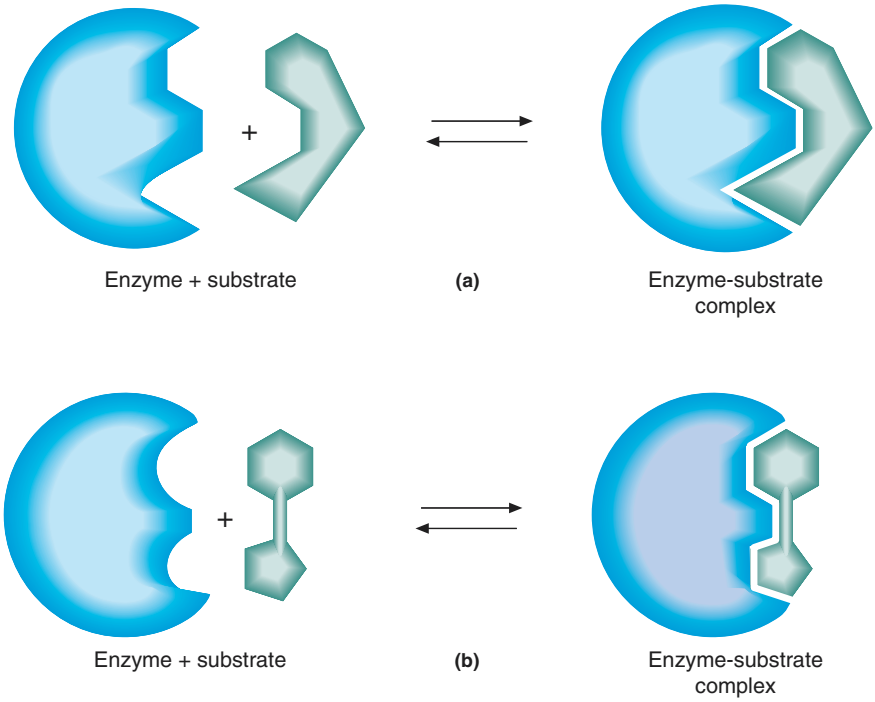


Figure 20.3
(a) The lock-and-key model of enzyme-substrate binding assumes that the enzyme active site has a rigid structure that is precisely complementary in shape and charge distribution to the substrate. (b) The induced fit model of enzyme-substrate binding. As the enzyme binds to the substrate, the shape of the active site conforms precisely to the shape of the substrate. The shape of the substrate may also change.

The overall shape of a protein is maintained by many weak interactions. At any time a few of these weak interactions may be broken by heat energy or a local change in pH. If only a few bonds are broken, they will re-form very quickly. The overall result is that there is a brief change in the shape of the enzyme. Thus the protein or enzyme can be viewed as a flexible molecule, changing shape slightly in response to minor local changes.

- An enzyme attracts and holds its substrate by weak, noncovalent interactions. The R groups involved in substrate binding, and not necessarily catalysis, make up the *binding site.*
- The conformation of the active site determines the specificity of the enzyme because only the substrate that fits into the active site will be used in a reaction.

The **lock-and-key model** of enzyme activity, shown in Figure 20.3a, was devised by Emil Fischer in 1894. At that time it was thought that the substrate simply snapped into place like a piece of a jigsaw puzzle or a key into a lock.

Today we know that proteins are flexible molecules. This led Daniel E. Koshland, Jr., to propose a more sophisticated model of the way enzymes and substrates interact. This model, proposed in 1958, is called the **induced fit model** (Figure 20.3b). In this model the active site of the enzyme is not a rigid pocket into which the substrate fits precisely; rather, it is a flexible pocket that *approximates* the shape of the substrate. When the substrate enters the pocket, the active site "molds" itself around the substrate. This produces the perfect enzyme-substrate "fit."

Learning Goal 6

Question 20.5 Compare the lock-and-key and induced fit models of enzyme-substrate binding.

Question 20.6 What is the relationship between an enzyme active site and its substrate?

20.5 Specificity of the Enzyme-Substrate Complex

Learning Goal 1 Learning Goal 5

For an enzyme-substrate interaction to occur, the surfaces of the enzyme and substrate must be complementary. It is this requirement for a specific fit that determines whether an enzyme will bind to a particular substrate and carry out a chemical reaction.

Enzyme specificity is the ability of an enzyme to bind only one, or a very few, substrates and thus catalyze only a single reaction. To illustrate the specificity of enzymes, consider the following reactions.

The enzyme urease catalyzes the decomposition of urea to carbon dioxide and ammonia as follows:

$$\underset{\text{Urea}}{H_2N{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}NH_2} + H_2O \xrightarrow{\text{Urease}} CO_2 + 2NH_3$$

This reaction is one that you may have observed if you have a cat. Urea is a waste product of the breakdown of proteins and is removed from the body in urine. Bacteria in kitty litter produce urease. As the urease breaks down the urea in the cat urine, the ammonia released produces the distinctive odor of an untended litter box.

Methylurea, in contrast, though structurally similar to urea, is not affected by urease:

$$\underset{\text{Methylurea}}{H_2N{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}NHCH_3} + H_2O \xrightarrow{\text{Urease}} \text{no reaction}$$

Not all enzymes exhibit the same degree of specificity. Four classes of enzyme specificity have been observed.

- **Absolute specificity:** An enzyme that catalyzes the reaction of only one substrate has **absolute specificity.** Aminoacyl tRNA synthetases exhibit absolute specificity. Each must attach the correct amino acid to the correct transfer RNA molecule. If the wrong amino acid is attached to the transfer RNA, it will mistakenly be added to a peptide chain, producing a nonfunctional protein.
- **Group specificity:** An enzyme that catalyzes processes involving similar molecules containing the same functional group has **group specificity.** Hexokinase is a group-specific enzyme that catalyzes the addition of a phosphoryl group to the hexose sugar glucose in the first step of glycolysis. Hexokinase can also add a phosphoryl group to several other six-carbon sugars.
- **Linkage specificity:** An enzyme that catalyzes the formation or breakage of only certain bonds in a molecule has **linkage specificity.** Proteases, such as trypsin, chymotrypsin, and elastase, are enzymes that selectively hydrolyze peptide bonds. Thus these enzymes are linkage specific.
- **Stereochemical specificity:** An enzyme that can distinguish one enantiomer from the other has **stereochemical specificity.** Most of the enzymes of the human body show stereochemical specificity. Because we use only D-sugars and L-amino acids, the enzymes involved in digestion and metabolism recognize only those particular stereoisomers.

Aminoacyl tRNA synthetases are discussed in Section 24.6. Aminoacyl group transfer reactions were described in Section 16.4.

Hexokinase activity is described in Section 21.3.

Proteolytic enzymes are discussed in Section 20.11.

20.6 The Transition State and Product Formation

How does enzyme-substrate binding result in a faster chemical reaction? The precise answer to this question is probably different for each enzyme-substrate pair, and, indeed, we understand the exact mechanism of catalysis for very few enzymes. Nonetheless, we can look at the general features of enzyme-substrate interactions that result in enhanced reaction rate and product formation. To do this, we must once again look at the steps of an enzyme-catalyzed reaction, focusing on the remaining steps highlighted in blue:

$$\underset{\substack{\text{Enzyme}\\+\\\text{substrate}}}{E + S} \underset{}{\overset{\text{Step I}}{\rightleftharpoons}} \underset{\substack{\text{Enzyme–}\\\text{substrate}\\\text{complex}}}{ES} \overset{\text{Step II}}{\rightleftharpoons} \underset{\substack{\text{Transition}\\\text{state}}}{ES^*} \overset{\text{Step III}}{\rightleftharpoons} \underset{\substack{\text{Enzyme–}\\\text{product}\\\text{complex}}}{EP} \overset{\text{Step IV}}{\rightleftharpoons} \underset{\substack{\text{Enzyme}\\+\\\text{product}}}{E + P}$$

A MEDICAL PERSPECTIVE

HIV Protease Inhibitors and Pharmaceutical Drug Design

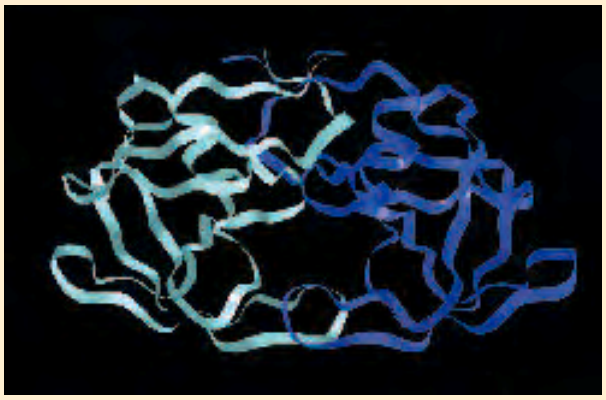
In 1981 the Centers for Disease Control in Atlanta, Georgia, recognized a new disease syndrome, acquired immune deficiency syndrome (AIDS). The syndrome is characterized by an impaired immune system, a variety of opportunistic infections and cancer, and brain damage that results in dementia. It soon became apparent that the disease was being transmitted by blood and blood products, as well as by sexual conduct.

The earliest drugs that proved effective in the treatment of HIV infections were all inhibitors of replication of the genetic material of the virus. While these treatments were initially effective, prolonging the lives of many, it was not long before viral mutants resistant to these drugs began to appear.

In 1989 a group of scientists revealed the three-dimensional structure of the HIV protease. This structure is shown in the accompanying figure. This enzyme is necessary for viral replication because the virus has an unusual strategy for making all of its proteins. Rather than make each protein individually, it makes large "polyproteins" that must then be cut by the HIV protease to form the final proteins required for viral replication.

Since scientists realized that this enzyme was essential for HIV replication, they decided to engineer a substance that would inhibit the enzyme by binding irreversibly to the active site, in essence plugging it up. The challenge, then, was to design a molecule that would be the plug. Researchers knew the primary structure (amino acid sequence) of the HIV protease from earlier nucleic acid sequencing studies. By 1989 they also had a very complete picture of the three-dimensional nature of the molecule, which they had obtained by X-ray crystallography.

Putting all of this information into a sophisticated computer modeling program, they could look at the protease from any angle. They could see the location of each of the R groups of each of the amino acids in the active site. This kind of information allowed the scientists to design molecules that would be complementary to the shape and charge distribution of the enzyme active site—in other words, structural analogs of the normal protease substrate. It was not long before the scientists had produced several candidates for the HIV protease inhibitor.



The human immunodeficiency virus protease.

But, there are many tests that a drug candidate must pass before it can be introduced into the market as safe and effective. Scientists had to show that the candidate drugs would bind effectively to the HIV protease and block its function, thereby inhibiting virus replication. Properties such as the solubility, the efficiency of absorption by the body, the period of activity in the body, and the toxicity of the drug candidates all had to be determined.

By 1996 there were three protease inhibitors available to those with HIV infection. There are currently seven of these drugs on the market. In many cases development and testing of a drug candidate can take up to fifteen years. In the case of the first HIV protease inhibitors, the first three drugs were on the market in less than eight years. This is a testament both to the urgent need for HIV treatments and to the technology available to attack the problem.

In Section 20.4 we examined the events of step I by which the enzyme and substrate interact to form the enzyme-substrate complex. In this section we will look at the events that lead to product formation. We have already described the enzyme as a flexible molecule; the substrate also has a degree of flexibility. The continued interaction between the enzyme and substrate changes the shape or position of the substrate in such a way that the molecular configuration is no longer energetically stable (step II). In this state, the **transition state,** the shape of the substrate is altered, because of its interaction with the enzyme, into an intermediate form having features of both the substrate and the final product. This transition state, in turn, favors the conversion of the substrate into product (step III). The product remains bound to the enzyme for a very brief time, then in step IV the product and enzyme dissociate from one another, leaving the enzyme completely unchanged.

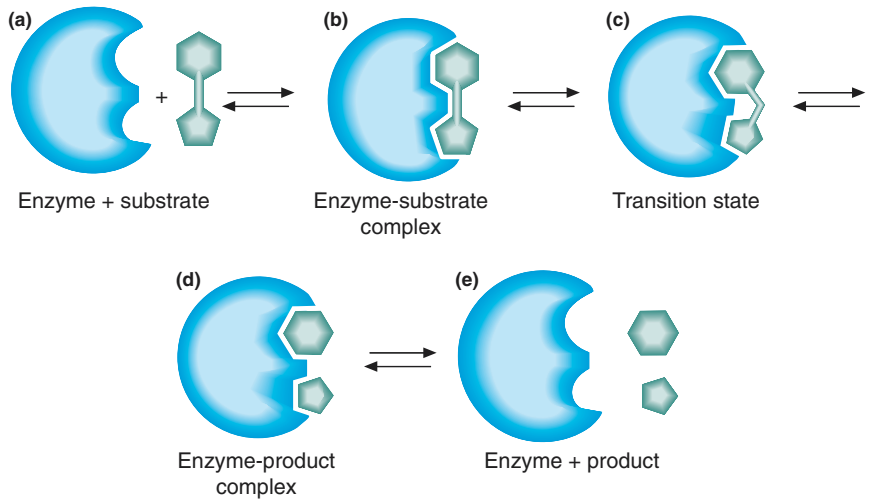
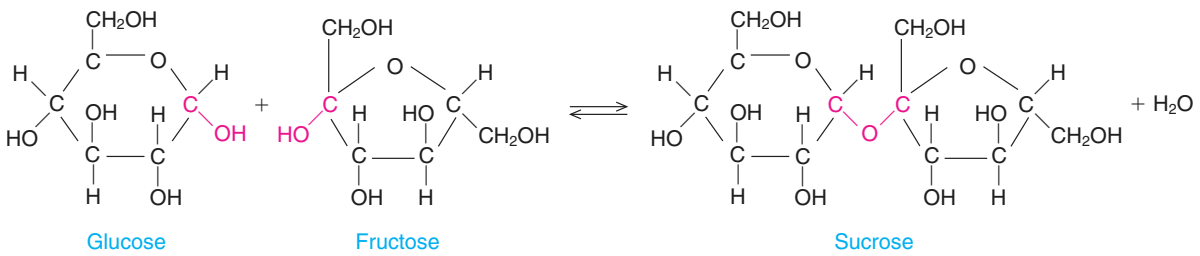


Figure 20.4
Bond breakage is facilitated by the enzyme as a result of stress on a bond. (a, b) The enzyme-substrate complex is formed. (c) In the transition state, the enzyme changes shape and thereby puts stress on the O-glycosidic linkage holding the two monosaccharides together. This lowers the energy of activation of this reaction. (d, e) The bond is broken, and the products are released.

What kinds of transition state changes might occur in the substrate that would make a reaction proceed more rapidly?

1. The enzyme might put "stress" on a bond and thereby facilitate bond breakage. Consider the hydrolysis of the sugar sucrose by the enzyme sucrase. The enzyme catalyzes the hydrolysis of the disaccharide sucrose into the monosaccharides glucose and fructose. The formation of the enzyme-substrate complex (Figures 20.4a and 20.4b) results in a change in the shape of the enzyme. This, in turn, may stretch or put pressure on one of the bonds of the substrate. Such a stress weakens the bond, allowing it to be broken much more easily than in the absence of the enzyme. This is represented in Figure 20.4c as the bending of the O-glycosidic bond between the fructose and the glucose. In the transition state the substrate has a molecular form resembling both the disaccharide, the original substrate, and the two monosaccharides, the eventual products. Clearly, the stress placed on the bond weakens it, and much less energy is required to break the bond to form products (Figures 20.4d and 20.4e). This also has the effect of speeding up the reaction.
2. An enzyme may facilitate a reaction by bringing two reactants into close proximity and in the proper orientation for reaction to occur. Consider now the condensation reaction between glucose and fructose to produce sucrose (Figure 20.5a). Each of the sugars has five hydroxyl groups that could undergo condensation to produce a disaccharide. But the purpose is to produce sucrose, not some other disaccharide. By random molecular collision there is a one in twenty-five chance that the two molecules will collide in the proper orientation to produce sucrose. The probability that the two will react is actually much less than that because of a variety of conditions in addition to orientation that must be satisfied for the reaction to occur. For example, at body temperature, most molecular collisions will not have a sufficient amount of energy to overcome the energy of activation, even if the molecules are in the proper orientation. The enzyme can facilitate the reaction by bringing the two molecules close together in the correct alignment (Figure 20.5b), thereby forcing the desired reactive groups of the two molecules together in the transition state and greatly speeding up the reaction.
3. The active site of an enzyme may modify the pH of the microenvironment surrounding the substrate. To accomplish this, the enzyme may, for example, serve as a donor or an acceptor of H^+. As a result, there would be a change in the pH in the vicinity of the substrate without disturbing the normal pH elsewhere in the cell.



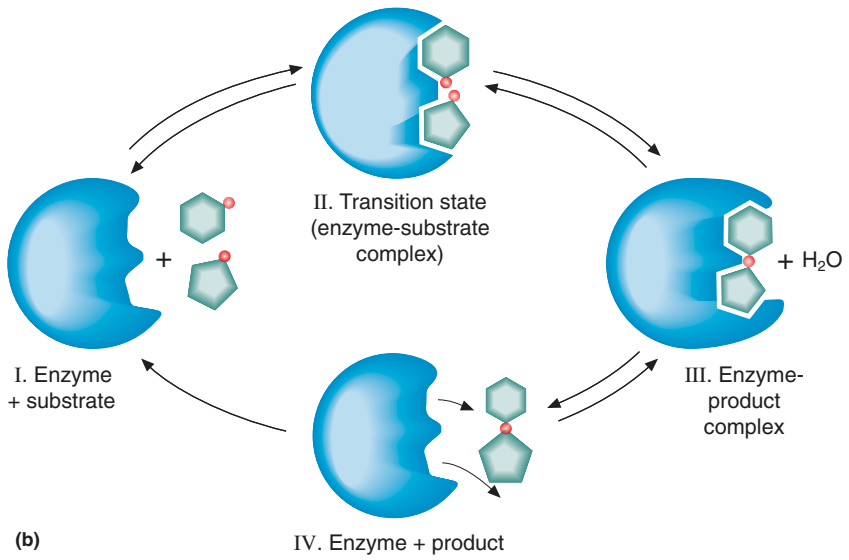


Figure 20.5
An enzyme may lower the energy of activation required for a reaction by holding the substrates in close proximity and in the correct orientation. (a) A condensation reaction in which glucose and fructose are joined in O-glycosidic linkage to produce sucrose. (b) The enzyme-substrate complex forms, bringing the two monosaccharides together with the hydroxyl groups involved in the linkage extended toward one another.

Question 20.7 Summarize three ways in which an enzyme might lower the energy of activation of a reaction.

Question 20.8 What is the transition state in an enzyme-catalyzed reaction?

20.7 Cofactors and Coenzymes

Learning Goal 7

In Section 19.7 we saw that some proteins require an additional nonprotein prosthetic group to function. The same is true of some enzymes. The polypeptide portion of such an enzyme is called the **apoenzyme,** and the nonprotein prosthetic group is called the **cofactor.** Together they form the active enzyme called the **holoenzyme.** Cofactors may be metal ions, organic compounds, or organometallic compounds. They must be bound to the enzyme to maintain the correct configuration of the enzyme active site (Figure 20.6). When the cofactor is bound and the active site is in the proper conformation, the enzyme can bind the substrate and catalyze the reaction.

Other enzymes require the temporary binding of a **coenzyme.** Such binding is generally mediated by weak interactions like hydrogen bonds. The coenzymes are organic molecules that generally serve as carriers of electrons or chemical groups.

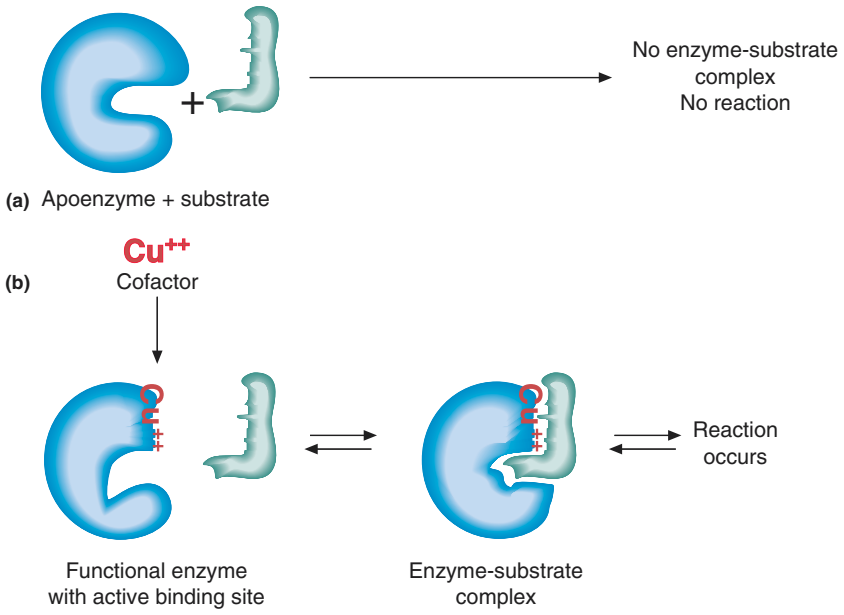


Figure 20.6
(a) The apoenzyme is unable to bind to its substrate. (b) When the required cofactor, in this case a copper ion, Cu^{2+}, is available, it binds to the apoenzyme. Now the active site takes on the correct configuration, the enzyme-substrate complex forms, and the reaction occurs.

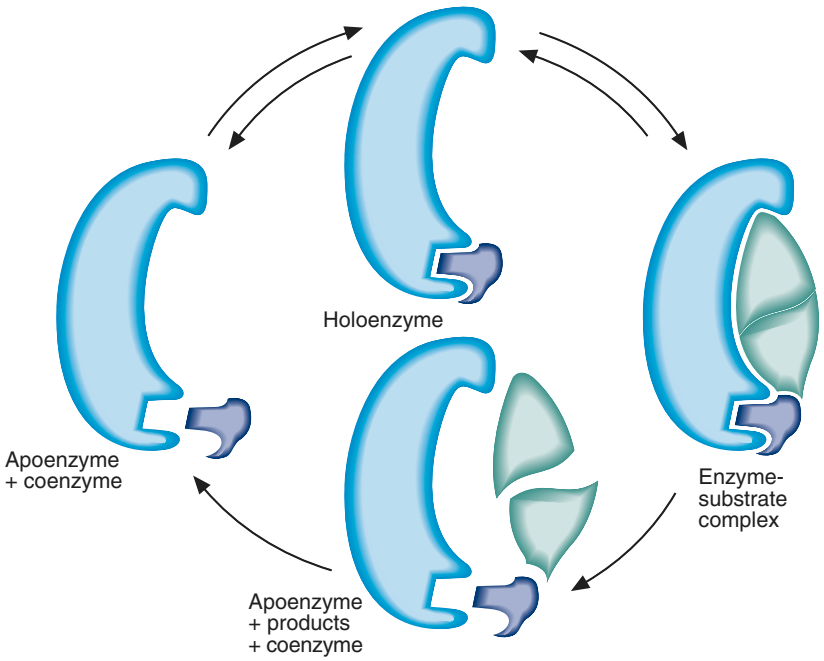


Figure 20.7
Some enzymes require a coenzyme to facilitate the reaction. The apoenzyme binds the coenzyme and then the substrate. The coenzyme is a part of the catalytic domain and will either donate or accept functional groups, allowing the reaction to occur. Once the product is formed, both the product and the coenzyme are released.

In chemical reactions they may either donate groups to the substrate or serve as recipients of groups that are removed from the substrate (Figure 20.7).

Often coenzymes contain modified vitamins as part of their structure. A **vitamin** is an organic substance that is required in the diet in only small amounts. Of the water-soluble vitamins, only vitamin C has not been associated with a coenzyme. Table 20.1 is a summary of some coenzymes and the water-soluble vitamins from which they are made.

Water soluble vitamins are discussed in greater detail in Appendix F.

Table 20.1 The Water-Soluble Vitamins and the Coenzymes of Which They Are Structural Components

Vitamin	Coenzyme	Function
Thiamine (B_1)	Thiamine pyrophosphate	Decarboxylation reactions
Riboflavin (B_2)	Flavin mononucleotide (FMN)	Carrier of H atoms
	Flavin adenine dinucleotide (FAD)	
Niacin (B_3)	Nicotinamide adenine dinucleotide (NAD^+)	Carrier of hydride ions
	Nicotinamide adenine dinucleotide phosphate ($NADP^+$)	
Pyridoxine (B_6)	Pyridoxal phosphate	Carriers of amino and carboxyl groups
	Pyridoxamine phosphate	
Cyanocobalamin (B_{12})	Deoxyadenosyl cobalamin	Coenzyme in amino acid metabolism
Folic acid	Tetrahydrofolic acid	Coenzyme for 1-C transfer
Pantothenic acid	Coenzyme A	Acyl group carrier
Biotin	Biocytin	Coenzyme in CO_2 fixation
Ascorbic acid	Unknown	Hydroxylation of proline and lysine in collagen

Nicotinamide adenine dinucleotide (NAD^+), shown in Figure 20.8, is an example of a coenzyme that is of critical importance in the oxidation reactions of the cellular energy-harvesting processes. The NAD^+ molecule has the ability to accept a hydride ion, a hydrogen atom with two electrons, from the substrate of the energy-harvesting reactions. The substrate is oxidized, and the portion of NAD^+ that is derived from the vitamin *niacin* is reduced to produce NADH. The NADH subsequently yields the hydride ion to the first acceptor in an electron transport chain. This regenerates the NAD^+ and provides electrons for the generation of ATP, the chemical energy required by the cell. Also shown in Figure 20.8 is the hydride carrier $NADP^+$ and the hydrogen atom carrier FAD. Both are used in the oxidation-reduction reactions that harvest energy for the cell. Unlike NADH and $FADH_2$, NADPH serves as "reducing power" for the cell by donating hydride ions in biochemical reactions. Like NAD^+, $NADP^+$ is derived from niacin. FAD is made from the vitamin *riboflavin*.

Question 20.9

Why does the body require the water-soluble vitamins?

Question 20.10

What are the coenzymes formed from each of the following vitamins? What are the functions of each of these coenzymes?

a. Pantothenic acid
b. Niacin
c. Riboflavin

20.8 Environmental Effects

Effect of pH

Learning Goal 8

Most enzymes are active only within a very narrow pH range. The cellular cytoplasm has a pH of 7, and most cytoplasmic enzymes function at a maximum efficiency at this pH. A plot of the relative rate at which a typical cytoplasmic enzyme catalyzes its specific reaction versus pH is provided in Figure 20.9.

The pH at which an enzyme functions optimally is called the **pH optimum.** Making the solution more basic or more acidic sharply decreases the rate of the

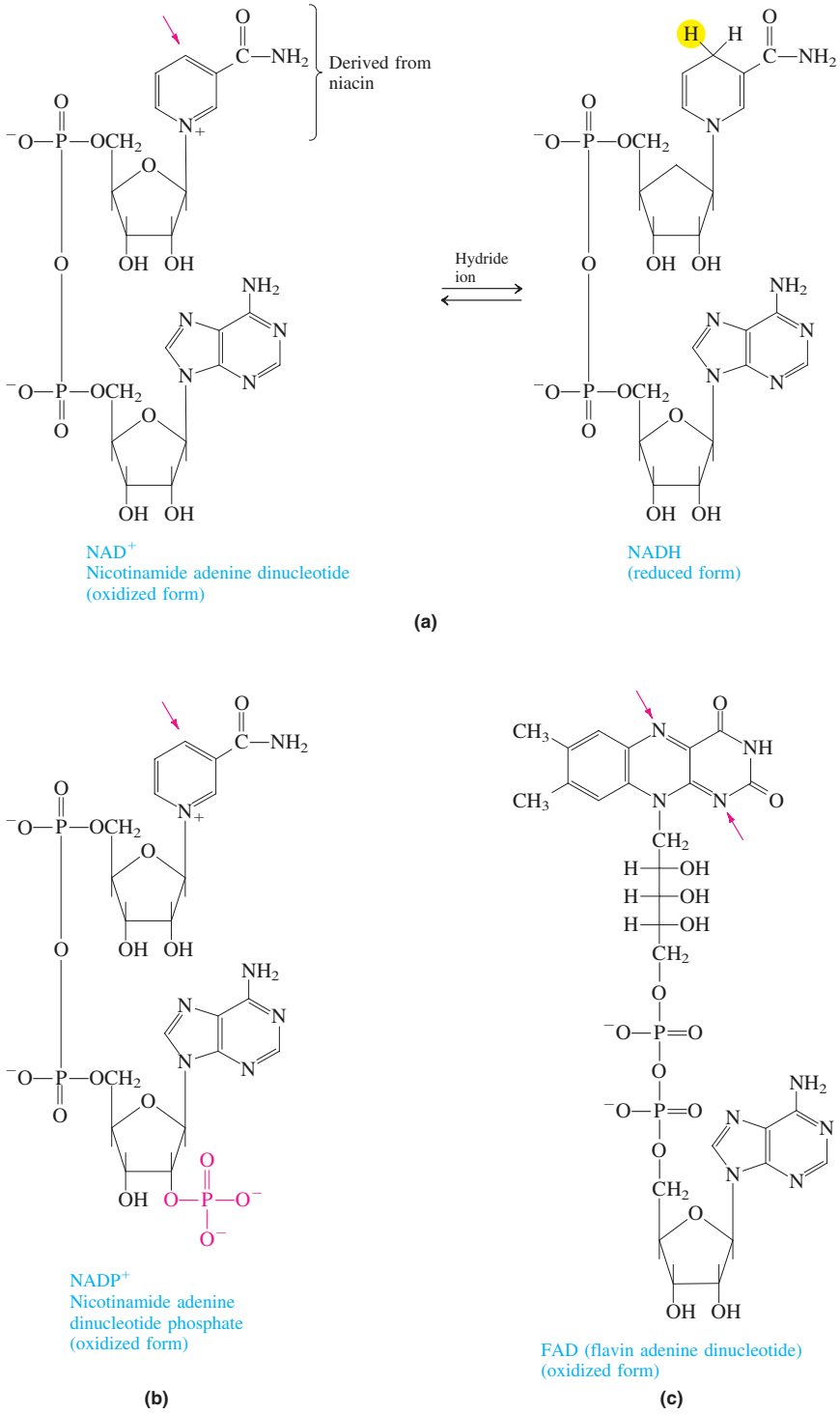


Figure 20.8
The structure of three coenzymes. (a) The oxidized and reduced forms of nicotinamide adenine dinucleotide. (b) The oxidized form of the closely related hydride ion carrier, nicotinamide adenine dinucleotide phosphate ($NADP^+$), which accepts hydride ions at the same position as NAD^+ (colored arrow). (c) The oxidized form of flavin adenine dinucleotide (FAD) accepts hydrogen atoms at the positions indicated by the colored arrows.

reaction. As was discussed in Section 19.10, at extremes of pH the enzyme actually loses its biologically active conformation and is *denatured*. This is because pH changes alter the degree of ionization of the R groups of the amino acids within the protein chain, as well as the extent to which they can hydrogen bond. Just as these

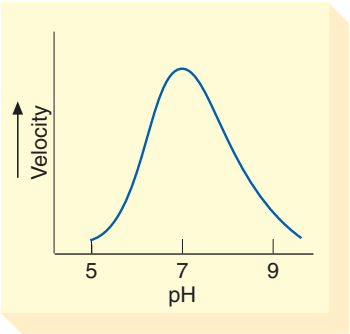


Figure 20.9
Effect of pH on the rate of an enzyme-catalyzed reaction. The enzyme functions most efficiently at pH 7. The rate of the reaction falls rapidly as the solution is made either more acidic or more basic.

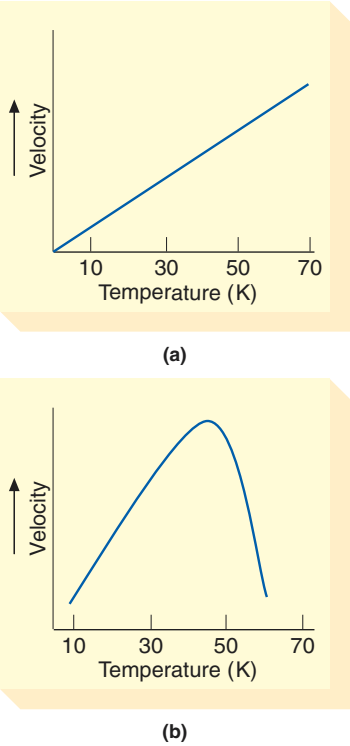


Figure 20.10
Effect of temperature on (a) uncatalyzed reactions and (b) enzyme-catalyzed reactions.

interactions can drastically alter the overall configuration of a protein, so too can less drastic changes in the R groups of an enzyme active site destroy the ability to form the enzyme-substrate complex.

Although the cytoplasm of the cell and the fluids that bathe the cells have a pH that is carefully controlled so that it remains at about pH 7, there are environments within the body in which enzymes must function at a pH far from 7. Protein sequences have evolved that can maintain the proper three-dimensional structure under extreme conditions of pH. For instance, the pH of the stomach is approximately 2 as a result of the secretion of hydrochloric acid by specialized cells of the stomach lining. The proteolytic digestive enzyme *pepsin* must effectively degrade proteins at this extreme pH. In the case of pepsin the enzyme has evolved in such a way that it can maintain a stable tertiary structure at a pH of 2 and is catalytically most active in the hydrolysis of peptides that have been denatured by very low pH. Thus pepsin has a pH optimum of 2.

In a similar fashion, another proteolytic enzyme, *trypsin,* functions under the conditions of higher pH found in the intestine. Both pepsin and trypsin cleave peptide bonds by virtually identical mechanisms, yet their amino acid sequences have evolved so that they are stable and active in very different environments.

The body has used adaptation of enzymes to different environments to protect itself against one of its own destructive defense mechanisms. Within the cytoplasm of a cell are organelles called *lysosomes.* Christian de Duve, who discovered lyosomes in 1956, called them "suicide bags" because they are membrane-bound vesicles containing about fifty different kinds of hydrolases or hydrolytic enzymes. The purpose of these enzymes is to degrade large molecules into small molecules that are useful for energy-harvesting reactions. For instance, some of the enzymes in the lysosomes can degrade proteins to amino acids, and others hydrolyze polysaccharides into monosaccharides. Certain cells of the immune defense system engulf foreign invaders, such as bacteria and viruses. They then use the hydrolytic enzymes in the lysosomes to degrade and destroy the invaders and use the simple sugars, amino acids, and lipids that are produced as energy sources.

What would happen if the hydrolytic enzymes of the lysosome were accidentally released into the cytoplasm of the cell? Certainly, the result would be the destruction of cellular macromolecules and death of the cell. Because of this danger, the cell invests a great deal of energy in maintaining the integrity of the lysosomal membranes. An additional protective mechanism relies on the fact that lysosomal enzymes function optimally at an acid pH (pH 4.8). Should some of these enzymes leak out of the lysosome or should a lysosome accidentally rupture, the cytoplasmic pH of 7.0–7.3 renders them inactive.

Effect of Temperature

Enzymes are rapidly destroyed if the temperature of the solution rises much above 37°C, but they remain stable at much lower temperatures. It is for this reason that solutions of enzymes used for clinical assays are stored in refrigerators or freezers before use. Figure 20.10 shows the effects of temperature on enzyme-catalyzed and uncatalyzed reactions. The rate of the uncatalyzed reaction steadily increases with increasing temperature because more collisions occur with sufficient energy to overcome the energy barrier for the reaction. The rate of an enzyme-catalyzed reaction also increases with modest increases in temperature because there are increasing numbers of collisions between the enzyme and the substrate. At the **temperature optimum** the enzyme is functioning optimally and the rate of the reaction is maximal. Above the temperature optimum, increasing temperature begins to increase the vibrational energy of the bonds within the enzyme. Eventually, so many bonds and weak interactions are disrupted that the enzyme becomes denatured, and the reaction stops.

A MEDICAL PERSPECTIVE

α_1-Antitrypsin and Familial Emphysema

Nearly two million people in the United States suffer from emphysema. Emphysema is a respiratory disease caused by destruction of the alveoli, the tiny, elastic air sacs of the lung. This damage results from the irreversible destruction of a protein called elastin, which is needed for the strength and flexibility of the walls of the alveoli. When elastin is destroyed, the small air passages in the lungs, called bronchioles, become narrower or may even collapse. This severely limits the flow of air into and out of the lung, causing respiratory distress, and in extreme conditions, death.

Some people have a genetic predisposition to emphysema. This is called familial emphysema. These individuals have been found to have a genetic defect in the gene that encodes the human plasma protein α_1-antitrypsin. As the name suggests, α_1-antitrypsin is an inhibitor of the proteolytic enzyme trypsin. But, as we have seen in this chapter, trypsin is just one member of a large family of proteolytic enzymes called the serine proteases. In the case of the α_1-antitrypsin activity in the lung, it is the inhibition of the enzyme elastase that is the critical event.

Elastase damages or destroys elastin, which in turn promotes the development of emphysema. People with normal levels of α_1-antitrypsin are protected from familial emphysema because their α_1-antitrypsin inhibits elastase and, thus, protects the elastin. The result is healthy alveoli in the lungs. However, individuals with a genetic predisposition to emphysema have very low levels of α_1-antitrypsin. This is due to a mutation that causes a single amino acid substitution in the protein chain. Because elastase in the lungs is not effectively controlled, severe lung damage characteristic of emphysema occurs.

Emphysema is also caused by cigarette smoking. Is there a link between these two forms of emphysema? The answer is yes; research has revealed that components of cigarette smoke cause the oxidation of a methionine near the amino terminus of the α_1-antitrypsin. This chemical damage destroys α_1-antitrypsin activity. There are enzymes in the lung that reduce the methionine, converting it back to its original chemical form and restoring α_1-antitrypsin activity. However, it is obvious that over a long period, smoking seriously reduces the level of α_1-antitrypsin activity. The accumulated lung damage results in emphysema in many chronic smokers.

At the current time the standard treatment of emphysema is the use of inhaled oxygen. It has been found that α_1-antitrypsin can be isolated from blood. Studies have shown that introduction of this material by intravenous infusion is both safe and effective. However, the level of α_1-antitrypsin in the blood must be maintained by repeated administration.

The α_1-antitrypsin gene has been cloned. In experiments with sheep it was shown that the protein remains stable when administered as an aerosol. It is still functional after it has passed through the pulmonary epithelium. This research offers hope of an effective treatment for this frightful disease.

Because heating enzymes and other proteins destroys their characteristic three-dimensional structure, and hence their activity, a cell cannot survive very high temperatures. Because cells cannot function without proper enzyme activity, heat is an effective means of sterilizing medical instruments and solutions for transfusion or clinical tests. Although instruments can be sterilized by dry heat (160°C) applied for at least two hours in a dry air oven, autoclaving is a quicker, more reliable procedure. The autoclave works on the principle of the pressure cooker. Air is pumped out of the chamber, and steam under pressure is pumped into the chamber until a pressure of two atmospheres is achieved. The pressure causes the temperature of the steam, which would be 100°C at atmospheric pressure, to rise to 121°C. Within twenty minutes, all the bacteria and viruses are killed. This is the most effective means of destroying the very heat-resistant endospores that are formed by many bacteria of clinical interest. These bacteria include the genera *Bacillus* and *Clostridium*, which are responsible for such unpleasant and deadly diseases as anthrax, gas gangrene, tetanus, and botulism food poisoning.

However, not all enzymes are inactivated by heating, even to rather high temperatures. Certain bacteria live in such out-of-the-way places as coal slag heaps, which are actually burning. Others live in deep vents on the ocean floor where temperatures and pressures are extremely high. Still others grow in the hot springs of Yellowstone National Park, where some bacteria thrive at temperatures near the

boiling point of water. These organisms, along with their enzymes, survive under such incredible conditions because the amino acid sequences of their proteins dictate structures that are stable at such seemingly impossible temperature extremes.

Question 20.11

How does a decrease in pH alter the activity of an enzyme?

Question 20.12

Heating is an effective mechanism for killing bacteria on surgical instruments. How does elevated temperature result in cellular death?

20.9 Regulation of Enzyme Activity

Learning Goal 9

One of the major ways in which enzymes differ from nonbiological catalysts is that the activity of the enzyme is often regulated by the cell. There are many reasons for this control of enzyme function. Some involve energy considerations. If the cell runs out of chemical energy, it will die; therefore many mechanisms exist to conserve cellular energy. For instance, it is a great waste of energy to produce an enzyme if the substrate is not available. Similarly, if the product of an enzyme-catalyzed reaction is present in excess, it is a waste of energy for the enzyme to continue to catalyze the reaction, thereby producing more of the unwanted product.

Just as there are many reasons for regulation of enzyme activity, there are many mechanisms for such regulation. The simplest mechanism is to produce the enzyme only when the substrate is present. This mechanism is used by bacteria to regulate the enzymes needed to break down various sugars to yield ATP for cellular work. The bacteria have no control over their environment or over what food sources, if any, might be available. It would be an enormous waste of energy to produce all of the enzymes that are needed to break down all the possible sugars. Thus the bacteria save energy by producing the enzymes only when a specific sugar substrate is available. Other mechanisms for regulating enzyme activity include use of allosteric enzymes, feedback inhibition, production of zymogens, and protein modification. Let's take a look at these regulatory mechanisms in some detail.

Allosteric Enzymes

One type of enzyme regulation involves enzymes that have more than a single binding site. These enzymes, called **allosteric enzymes,** meaning "other forms," are enzymes whose active sites can be altered by binding of small molecules called *effector molecules.* As shown in Figure 20.11, the effector binding to its binding site alters the shape of the active site of the enzyme. The result can be to convert the active site to an inactive configuration, **negative allosterism,** or to convert the active site to the active configuration, **positive allosterism.** In either case, binding of the effector molecule regulates enzyme activity by determining whether it will be active or inactive.

In upcoming chapters we will begin our study of metabolic pathways. A metabolic pathway is a series of biochemical reactions that accomplishes the breakdown or synthesis of one or more biological molecules. Consider the pathway glycolysis, which is the first stage of the breakdown of carbohydrates to release energy that can be used by the cell (adenosine triphosphate or ATP). Such a pathway must be attuned to the demands of the body. When more energy is required, the reactions of the pathway should occur more quickly, releasing more energy. However, if the energy demand is low, the reactions should slow down, producing less ATP.

The third reaction in glycolysis is the transfer of a phosphoryl group from an ATP molecule to a molecule of fructose-6-phosphate. This reaction, shown here, is catalyzed by an enzyme called *phosphofructokinase:*

Fructose-6-phosphate Fructose-1,6-bisphosphate

Phosphofructokinase activity is sensitive to both positive and negative allosterism. For instance, when ATP is present in abundance, a signal that the body has sufficient energy, it binds to an effector binding site on phosphofructokinase. This inhibits the activity of the enzyme and, thus, slows the entire pathway. An abundance of AMP (adenosine monophosphate), which is a precursor of ATP, is evidence that the body needs to make ATP to have a sufficient energy supply. When AMP binds to an effector binding site on phosphofructokinase, enzyme activity is increased, speeding up the reaction and the entire pathway.

Feedback Inhibition

Allosteric enzymes are the basis for **feedback inhibition** of biochemical pathways. This system functions on the same principle as the thermostat on your furnace. You set the thermostat at 70°F; the furnace turns on and produces heat until the sensor in the thermostat registers a room temperature of 70°F. It then signals the furnace to shut off.

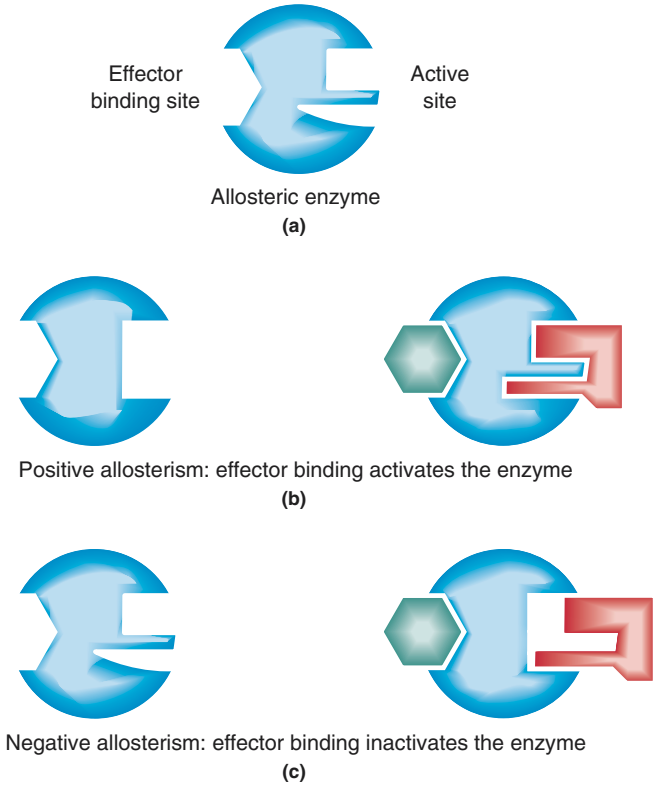


Figure 20.11
(a) Allosteric enzymes regulate a great many biochemical pathways. The allosteric enzyme has an active site and an effector binding site. (b) Positive allosterism. (c) Negative allosterism.

Feedback inhibition usually regulates pathways of enzymes involved in the synthesis of a biological molecule. Such a pathway can be shown schematically as follows:

$$A \xrightarrow{E_1} B \xrightarrow{E_2} C \xrightarrow{E_3} D \xrightarrow{E_4} E \xrightarrow{E_5} F$$

In this pathway the starting material, *A*, is converted to *B* by the enzyme E_1. Enzyme E_2 immediately converts *B* to *C*, and so on until the final product, *F*, has been synthesized. If *F* is no longer needed, it is a waste of cellular energy to continue to produce it.

To avoid this waste of energy, the cell uses feedback inhibition, in which the product can shut off the entire pathway for its own synthesis. This is the result of the fact that the product, *F*, acts as a negative allosteric effector on one of the early enzymes of the pathway. For instance, enzyme E_1 may have an effector-binding site for *F* in addition to the active site that binds to *A*. When *F* is present in excess, it binds to the effector-binding site. This binding causes the active site to close so that it cannot bind to substrate *A*. Thus *A* is not converted to *B*. If no *B* is produced, there is no substrate for enzyme E_2, and the entire pathway ceases to operate. The product, *F*, has turned off all the steps involved in its own synthesis, just as the heat produced by the furnace is ultimately responsible for turning off the furnace itself.

Zymogens

Another means of regulating enzyme activity involves the production of the enzyme in an inactive form called a **zymogen,** or a *proenzyme.* It is then converted, usually by proteolysis (hydrolysis of the protein), to the active form when it has reached the site of its activity. What is the purpose of this type of mechanism? On first examination it seems wasteful to add a step to the synthesis of an enzyme. But consider for a moment the very destructive nature of some of the enzymes that are necessary for life. The enzymes pepsin, trypsin, and chymotrypsin are all proteolytic enzymes of the digestive tract. They are necessary to life because they degrade dietary proteins into amino acids that are used by the cell. But what would happen to the cells that produce these enzymes if they were synthesized in active form? Those cells would be destroyed. Thus the cells of the stomach that produce pepsin actually produce an inactive zymogen, called *pepsinogen.* Pepsinogen has an additional forty-two amino acids. In the presence of stomach acid and previously activated pepsin, the extra forty-two amino acids are cleaved off, and the zymogen is transformed into the active enzyme. Table 20.2 lists several other zymogens and the enzymes that convert them to active form.

Protein Modification

Protein modification is another mechanism that the cell can use to turn an enzyme on or off. This is a process in which a chemical group is covalently added to or

Table 20.2 Zymogens of the Digestive Tract

Zymogen	Activator	Enzyme
Proelastase	Trypsin	Elastase
Trypsinogen	Trypsin	Trypsin
Chymotrypsinogen A	Trypsin + chymotrypsin	Chymotrypsin
Pepsinogen	Acid pH + pepsin	Pepsin
Procarboxypeptidases	Trypsin	Carboxypeptidase A, carboxypeptidase B

removed from the protein. This covalent modification either activates the enzyme or turns it off.

The most common type of protein modification is phosphorylation or dephosphorylation of an enzyme. Typically, the phosphoryl group is added to (or removed from) the R group of a serine, tyrosine, or threonine in the protein chain of the enzyme. Notice that these three amino acids have a free —OH in their R group, which serves as the site for the addition of the phosphoryl group.

The covalent modification of an enzyme's structure is catalyzed by other enzymes. *Protein kinases* add phosphoryl groups to a target enzyme, while *phosphatases* remove them. For some enzymes it is the phosphorylated form that is active. For instance, in adipose tissue, phosphorylation activates the enzyme triacylglycerol lipase, an enzyme that breaks triglycerides down to fatty acids and glycerol. Glycogen phosphorylase, an enzyme involved in the breakdown of glycogen, is also activated by the addition of a phosphoryl group. However, for some enzymes phosphorylation inactivates the enzyme. This is true for glycogen synthase, an enzyme involved in the synthesis of glycogen. When this enzyme is phosphorylated, it becomes inactive.

The convenient aspect of this type of regulation is the reversibility. An enzyme can quickly be turned on or off in response to environmental or physiological conditions.

20.10 Inhibition of Enzyme Activity

Learning Goal 10

Many chemicals can bind to enzymes and either eliminate or drastically reduce their catalytic ability. These chemicals, called *enzyme inhibitors,* have been used for hundreds of years. When she poisoned her victims with arsenic, Lucretia Borgia was unaware that it was binding to the thiol groups of cysteine amino acids in the proteins of her victims and thus interfering with the formation of disulfide bonds needed to stabilize the tertiary structure of enzymes. However, she was well aware of the deadly toxicity of heavy metal salts like arsenic and mercury. When you take penicillin for a bacterial infection, you are taking another enzyme inhibitor. Penicillin inhibits several enzymes that are involved in the synthesis of bacterial cell walls.

The effect of penicillin on bacterial cell wall biosynthesis is discussed in A Clinical Perspective: The Bacterial Cell Wall in Chapter 17.

Enzyme inhibitors are classified on the basis of whether the inhibition is reversible or irreversible, competitive or noncompetitive. Reversibility deals with whether the inhibitor will eventually dissociate from the enzyme, releasing it in the active form. Competition refers to whether the inhibitor is a structural analog, or look-alike, of the natural substrate. If so, the inhibitor and substrate will compete for the enzyme active site.

Irreversible Inhibitors

Irreversible enzyme inhibitors, such as arsenic, usually bind very tightly, sometimes even covalently, to the enzyme. This generally involves binding of the inhibitor to one of the R groups of an amino acid in the active site. Inhibitor binding may block the active site binding groups so that the enzyme-substrate complex cannot form. Alternatively, an inhibitor may interfere with the catalytic groups of the active site, thereby effectively eliminating catalysis. Irreversible inhibitors generally inhibit many different enzymes. Examples include snake venoms and nerve gases.

Reversible, Competitive Inhibitors

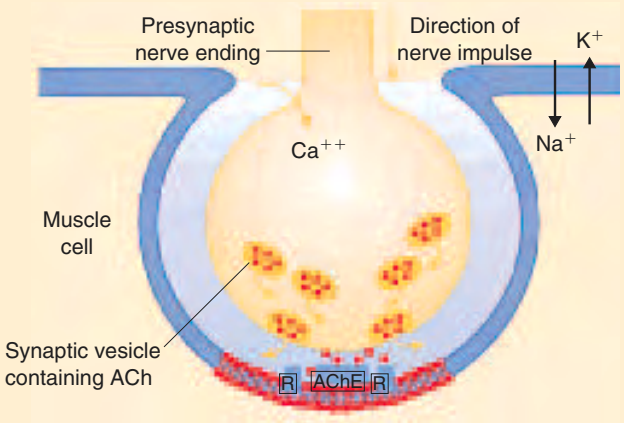
Reversible, competitive enzyme inhibitors are often referred to as **structural analogs,** that is, they are molecules that resemble the structure and charge distribution of the natural substrate for a particular enzyme. Because of this resemblance, the inhibitor can occupy the enzyme active site. However, no reaction can occur, and enzyme activity is inhibited. This inhibition is said to be competitive because

See A Human Perspective: Fooling the AIDS Virus with "Look-Alike" Nucleotides in Chapter 24.

A CLINICAL PERSPECTIVE

Enzymes, Nerve Transmission, and Nerve Agents

The transmission of nerve impulses at the *neuromuscular junction* involves many steps, one of which is the activity of a critical enzyme, called *acetylcholinesterase,* which catalyzes the hydrolysis of the chemical messenger, *acetylcholine,* that initiated the nerve impulse. The need for this enzyme activity becomes clear when we consider the events that begin with a message from the nerve cell and end in the appropriate response by the muscle cell. Acetylcholine is a chemical messenger that transmits a message from the nerve cell to the muscle cell. Such a molecule is known as a *neurotransmitter.* Acetylcholine is stored in membrane-bound bags, called *synaptic vesicles,* in the nerve cell ending.



Schematic diagram of the synapse at the neuromuscular junction. The nerve impulse causes acetylcholine (ACh) to be released from synaptic vesicles. Acetylcholine diffuses across the synaptic cleft and binds to a specific receptor protein (R) on the postsynaptic membrane. A channel opens that allows Na^+ ions to flow into the cell and K^+ ions to flow out of the cell. This results in muscle contraction. Any ACh remaining in the synaptic cleft is destroyed by acetylcholinesterase (AChE) to terminate the stimulation of the muscle cell.

Acetylcholinesterase comes into play in the following way. The arrival of a nerve impulse at the end plate of the nerve axon causes an influx of Ca^{2+}. This causes the acetylcholine-containing vesicles to migrate to the nerve cell membrane that is in contact with the muscle cell. This is called the *presynaptic membrane.* The vesicles fuse with the *presynaptic membrane* and release the neurotransmitter. The acetylcholine then diffuses across the *nerve synapse* (the space between the nerve and muscle cells) and binds to the acetylcholine receptor protein in the *postsynaptic membrane* of the muscle cell. This receptor then opens pores in the membrane through which Na^+ and K^+ ions flow into and out of the cell, respectively. This generates the nerve impulse and causes the muscle to contract. If acetylcholine remains at the neuromuscular junction, it will continue to stimulate the muscle contraction. To stop this continued stimulation, acetylcholine is hydrolyzed, and hence, destroyed by acetylcholinesterase. When this happens, choline is no longer able to bind to the acetylcholine receptor and nerve stimulation ceases.

$$H_3C{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}O{-}CH_2CH_2{-}N^+{-}(CH_3)_3 + H_2O$$

Acetylcholine

$\rightleftarrows$ Acetylcholinesterase

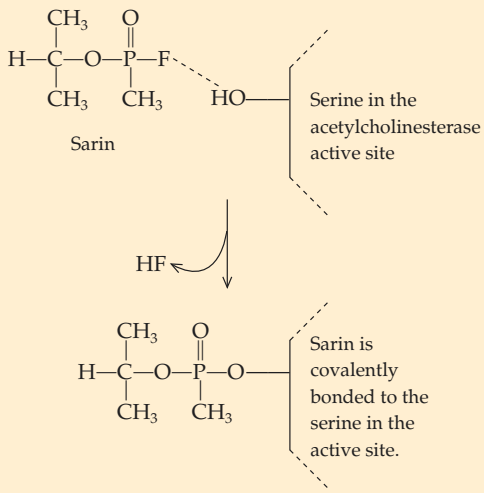
$$H_3C{-}C\begin{matrix}{=}O\\{-}O^-\end{matrix} + HO{-}CH_2CH_2{-}N^+{-}(CH_3)_3 + H^+$$

Acetate Choline

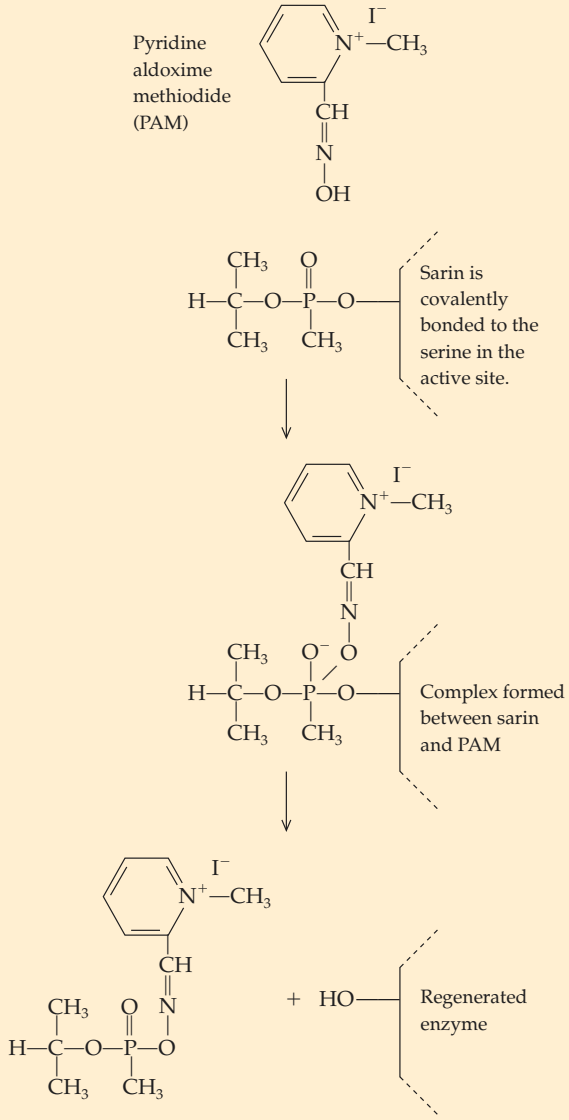
the inhibitor and the substrate compete for binding to the enzyme active site. Thus, the degree of inhibition depends on their relative concentrations. If the inhibitor is in excess or binds more strongly to the active site, it will occupy the active site more frequently, and enzyme activity will be greatly decreased. On the other hand, if the natural substrate is present in excess, it will more frequently occupy the active site, and there will be little inhibition.

The sulfa drugs, the first antimicrobics to be discovered, are **competitive inhibitors** of a bacterial enzyme needed for the synthesis of the vitamin folic acid.

Inhibitors of acetylcholinesterase are used both as poisons and as drugs. Among the most important inhibitors of acetylcholinesterase are a class of compounds known as *organophosphates*. One of these is the nerve agent Sarin (isopropylmethylfluorophosphate). Sarin forms a covalently bonded intermediate with the active site of acetylcholinesterase. Thus, it acts as an irreversible, noncompetitive inhibitor.



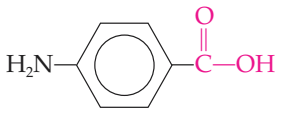
The covalent intermediate is stable, and acetylcholinesterase is therefore inactive. It is unable to react with other substrates. Nerve transmission continues, resulting in muscle spasm. Death may occur as a result of laryngeal spasm. Antidotes for poisoning by organophosphates, which include many insecticides and nerve gases, have been developed. The antidotes work by reversing the effects of the inhibitor. One of these antidotes is known as *PAM*, an acronym for *pyridine aldoxime methiodide*. This molecule displaces the organophosphate group from the active site of the enzyme, alleviating the effects of the poison.



Folic acid is a vitamin required for the transfer of methyl groups in the biosynthesis of methionine and nitrogenous bases. Humans cannot synthesize folic acid and must obtain it from the diet. Bacteria, on the other hand, must make folic acid because they cannot take it in from the environment.

para-Aminobenzoic acid (PABA) is the substrate for an early step in folic acid synthesis. The sulfa drugs, the prototype for which was discovered by Gerhard Domagk in the 1930s, are structural analogs of PABA and thus competitive inhibitors of the enzyme that uses PABA as its normal substrate.

In addition to the folic acid supplied in the diet, we obtain folic acid from our intestinal bacteria.



p-Aminobenzoic acid

Sulfanilamide

If the correct substrate (PABA) is bound by the enzyme, the reaction occurs, and the bacterium lives. However, if the sulfa drug is present in excess over PABA, it binds more frequently to the active site of the enzyme. No folic acid will be produced, and the bacterial cell will die.

Because we obtain our folic acid from our diets, sulfa drugs do not harm us. However, bacteria are selectively killed. Luckily, we can capitalize on this property for the treatment of bacterial infections, and as a result, sulfa drugs have saved countless lives. Although bacterial infection was the major cause of death before the discovery of sulfa drugs and other antibiotics, death caused by bacterial infection is relatively rare at present.

Reversible, Noncompetitive Inhibitors

Reversible, noncompetitive enzyme inhibitors bind to R groups of amino acids or perhaps to the metal ion cofactors. Unlike the situation of irreversible inhibition, however, the binding is weak, and the enzyme activity is restored when the inhibitor dissociates from the enzyme-inhibitor complex. Although these inhibitors generally do not bind to the active site, they do modify the shape of the active site by binding elsewhere in the protein structure. Keep in mind that the entire three-dimensional structure of an enzyme is needed to maintain the correct shape of the active site. Ionic bonding or weak bonding of the inhibitor to one or more sites on the enzyme surface can alter the shape of the active site in a fashion analogous to that of an allosteric effector. These inhibitors also inactivate a broad range of enzymes.

Question 20.13 Why are irreversible inhibitors considered to be poisons?

Question 20.14 Explain the difference between an irreversible inhibitor and a reversible, noncompetitive inhibitor.

Question 20.15 What is a structural analog?

Question 20.16 How can structural analogs serve as enzyme inhibitors?

20.11 Proteolytic Enzymes

Learning Goal 11

Proteolytic enzymes are protein-cleaving enzymes, that is, they break the peptide bonds that maintain the primary protein structure. *Chymotrypsin*, for example, is an enzyme that hydrolyzes dietary proteins in the small intestine. It acts specifically at peptide bonds on the carbonyl side of the peptide bond. The C-terminal amino acids of the peptides released by bond cleavage are methionine, tyrosine, tryptophan, and phenylalanine. The specificity of chymotrypsin depends upon the presence of a *hydrophobic pocket*, a cluster of hydrophobic amino acids brought together by the three-dimensional folding of the protein chain. The flat aromatic side chains of certain amino acids (tyrosine, tryptophan, phenylalanine) slide into this pocket, providing the binding specificity required for catalysis (Figure 20.12).

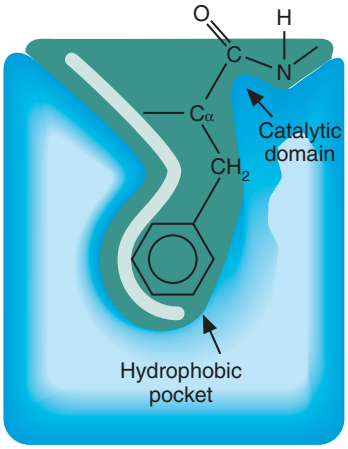
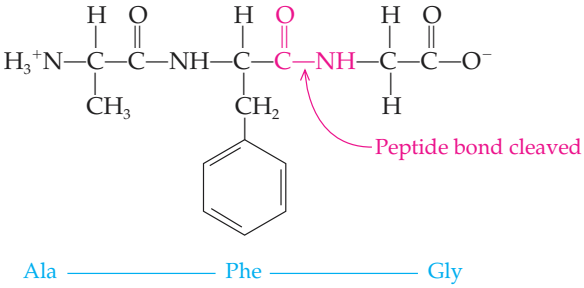


Figure 20.12
The specificity of chymotrypsin is determined by a hydrophobic pocket that holds the aromatic side chain of the substrate. This brings the peptide bond to be cleaved into the catalytic domain of the active site.

How can we determine which bond is cleaved by a protease such as chymotrypsin? To know which bond is cleaved, we must write out the sequence of amino acids in the region of the peptide that is being cleaved. This can be determined experimentally by amino acid sequencing techniques. Remember that the N-terminal amino acid is written to the left and the C-terminal amino acid to the right. Consider a protein having within it the sequence —Ala-Phe-Gly—. A reaction is set up in which the enzyme, chymotrypsin, is mixed with the protein substrate. After the reaction has occurred, the products are purified, and their amino acid sequences are determined. Experiments of this sort show that chymotrypsin cleaves the bond between phenylalanine and glycine, which is the peptide bond on the carbonyl side of amino acids having an aromatic side chain.



The **pancreatic serine proteases** trypsin, chymotrypsin, and elastase all hydrolyze peptide bonds. These enzymes are the result of *divergent evolution* in which a single ancestral gene first duplicated and then each copy evolved individually. They have similar primary structures, similar tertiary structures (Figure 20.13), and virtually identical mechanisms of action. However, as a result of evolution, these enzymes all have different specificities:

- Chymotrypsin cleaves peptide bonds on the carbonyl side of aromatic amino acids and large, hydrophobic amino acids such as methionine.
- Trypsin cleaves peptide bonds on the carbonyl side of basic amino acids.
- Elastase cleaves peptide bonds on the carbonyl side of glycine and alanine.

These enzymes are called *serine proteases* because they have the amino acid serine in the catalytic region of the active site that is essential for hydrolysis of the peptide bond.

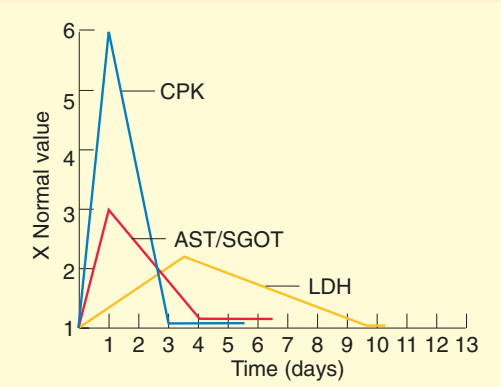
These enzymes have different pockets for the side chains of their substrates; *different keys fit different locks.* This difference manifests itself in the substrate specificity alluded to above. For example, the binding pocket of trypsin is long, narrow, and negatively charged to accommodate lysine or arginine R groups. Yet although the binding pockets have undergone divergent evolution, the catalytic sites have

A CLINICAL PERSPECTIVE

Enzymes, Isoenzymes, and Myocardial Infarction

A patient is brought into the emergency room with acute, squeezing chest pains; shallow, irregular breathing; and pale, clammy skin. The immediate diagnosis is myocardial infarction, a heart attack. The first thoughts of the attending nurses and physicians concern the series of treatments and procedures that will save the patient's life. It is a short time later, when the patient's condition has stabilized, that the doctor begins to consider the battery of enzyme assays that will confirm the diagnosis.

Myocardial infarction occurs when the blood supply to the heart muscle is blocked for an extended time. If this lack of blood supply, called *ischemia,* is prolonged, the myocardium suffers irreversible cell damage and muscle death, or infarction. When this happens, the concentration of cardiac enzymes in the blood rises dramatically as the dead cells release their contents into the bloodstream. Although many enzymes are liberated, three are of prime importance. These three enzymes, creatine phosphokinase (CPK), lactate dehydrogenase (LDH), and aspartate aminotransferase/serum glutamate–oxaloacetate transaminase (AST/SGOT), show a characteristic sequential rise in blood serum level following myocardial infarction and then return to normal. This enzyme profile, shown in the accompanying figure, is characteristic of and the basis for the diagnosis of a heart attack.



Characteristic pattern of serum cardiac enzyme concentrations following a myocardial infarction.

To ensure against misdiagnosis caused by tissue damage in other organs, the levels of other serum enzymes, including alanine aminotransferase/serum glutamate–pyruvate transaminase (ALT/SGPT) and isocitrate dehydrogenase (ICD), are also measured. ALT and AST are usually determined simultaneously to differentiate between cardiac and hepatic disease. The concentration of ALT is higher in liver disease, whereas the serum concentration of AST is higher following acute myocardial infarction. ICD is found primarily in the liver, and serum levels would not be elevated after a heart attack.

The use of LDH and CPK levels alone can also lead to a misdiagnosis because these enzymes are produced by many tissues. How can a clinician diagnose heart disease with confidence when the elevated serum enzyme levels could indicate coexisting disease in another tissue? The physician is able to make such a decision because of the presence of *isoenzymes,* which provide diagnostic accuracy because they reveal the tissue of origin.

Isoenzymes are forms of the same enzyme with slightly different amino acid sequences. The binding and catalytic sites are the same, but there are differences in the scaffolding sequences of the enzyme that maintain the three-dimensional structure of the protein. Each of the cells of the body contains the genes that could direct the production of all the different forms of these enzymes, yet the expression is *tissue-specific.* This means that the genes for certain isoenzymes are expressed preferentially in different types of tissue.

It is not clear why a certain isoenzyme is "turned on" in the liver whereas another predominates in the heart, but it is known that we can distinguish among the different forms in the laboratory on the basis of their migration through a gel placed in an electric field. This process is called *gel electrophoresis.* This test is based on the fact that each protein has a characteristic surface charge resulting from the R groups of the amino acids. If these proteins are placed in a gel matrix and an electrical current is applied, the proteins migrate as a function of that charge. The figure on the next page shows the position of the five isoenzymes of LDH following electrophoresis.

Imagine a mixture of serum proteins, each with a different overall charge, subjected to an electric field. The proteins with the greatest negative charge will be most strongly attracted to

the positive pole and will migrate rapidly toward it, whereas those with a lesser negative charge will migrate much more slowly. Thus, the proteins will be distributed throughout the gel based on their characteristic overall charge.

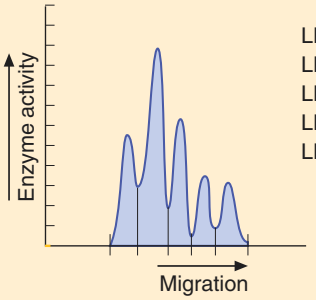
Once electrophoresis is terminated, the location of the bands of each isoenzyme must be determined and the amount of each must be measured. To do this an enzyme assay is carried out. The substrate is added to the gel in a solution that provides proper conditions for the enzyme. The product is a colored substance that can be seen visually. By inspecting the positions of the stained bands on the gel, one can determine which tissue isoenzymes are present.

To measure the amount of each, the intensity of the color can be measured with a spectrophotometer. The intensity of the color is directly proportional to the amount of the product, and thus to the amount of enzyme in the original sample. This allows the laboratory to calculate the concentration of each isoenzyme in the sample.

These data give the clinician an accurate picture of the nature of the diseased tissue. For a heart attack victim, only creatine phosphokinase isoenzyme 2 (CPK II, the predominant heart isoenzyme) will be elevated in the three days following the heart attack. CPK I (brain) and CPK III (skeletal muscle) levels will remain unchanged. Similarly, only LDH 1, the lactate dehydrogenase isoenzyme made in heart muscle, will be elevated. The levels of LDH 2–5 will remain within normal values.

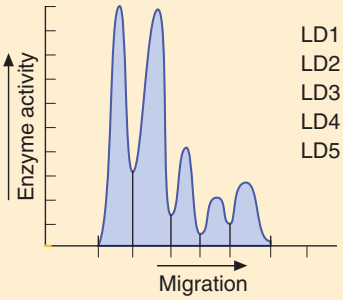
The physician also has enzymes available to treat a heart attack patient. Most myocardial infarctions are the result of a *thrombus*, or clot, within a coronary blood vessel. The clot restricts blood flow to the heart muscle. One technique that shows promise for treatment following a coronary thrombosis, a heart attack caused by the formation of a clot, is destruction of the clot by intravenous or intracoronary injection of an enzyme called *streptokinase*. This enzyme, formerly purified from the pathogenic bacterium *Streptococcus pyogenes* but now available through recombinant DNA techniques, catalyzes the production of the proteolytic enzyme plasmin from its zymogen, plasminogen. Plasmin has the ability to degrade a fibrin clot into subunits. Of course, this has the effect of dissolving the clot that is responsible for restricted blood flow to the heart, but there is an additional protective function as well. The subunits produced by plasmin degradation of fibrin clots are able to inhibit further clot formation by inhibiting thrombin.

Recombinant DNA technology has provided medical science with yet another, perhaps more promising, clot-dissolving enzyme. *Tissue-type plasminogen activator (TPA)* is a proteolytic enzyme that occurs naturally in the body as a part of the anticlotting mechanisms. TPA converts the zymogen, plasminogen, into the active enzyme, plasmin. Injection of TPA within two hours of the initial chest pain can significantly improve the circulation to the heart and greatly improve the patient's chances of survival.



(a)

Fraction	%	Reference range
LD1	19.8	13.5 – 28.8
LD2	37.3	26.9 – 43.5
LD3	19.4	15.9 – 25.2
LD4	11.1	6.0 – 14.0
LD5	12.5	4.9 – 20.8



(b)

Fraction	%	Reference range
LD1	37.1	13.5 – 28.8
LD2	34.0	26.9 – 43.5
LD3	12.0	15.9 – 25.2
LD4	6.3	6.0 – 14.0
LD5	10.6	4.9 – 20.8

A profile of the serum isoenzymes of lactate dehydrogenase. (a) The pattern of LDH isoenzymes from a normal individual. (b) The pattern of LDH isoenzymes of an individual suffering from a myocardial infarction.

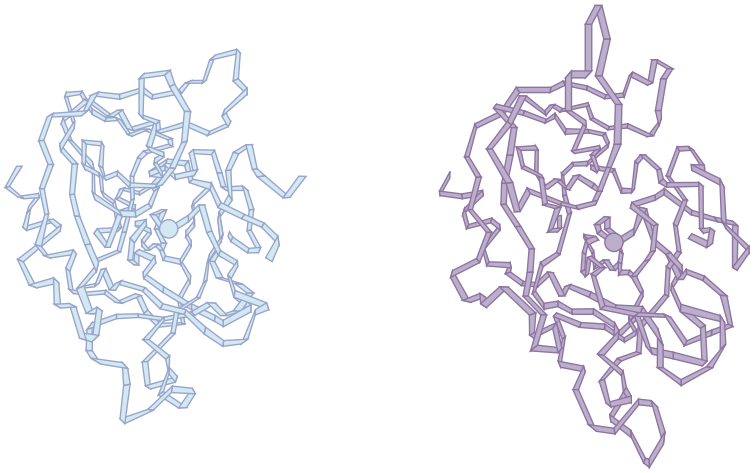


Figure 20.13
Structures of chymotrypsin and elastase are virtually identical, suggesting that these enzymes have evolved from a common ancestral protease.

remained unchanged, and the mechanism of proteolytic action is the same for all the serine proteases. In each case, the mechanism involves a serine R group.

Question 20.17

Draw the structural formulas of the following peptides and show which bond would be cleaved by chymotrypsin.

a. ala-phe-ala
b. tyr-ala-tyr
c. trp-val-gly
d. phe-ala-pro

Question 20.18

Draw the structural formula of the peptide val-phe-ala-gly-leu. Which bond would be cleaved if this peptide were reacted with chymotrypsin? With elastase?

20.12 Uses of Enzymes in Medicine

Learning Goal 12

Analysis of blood serum for levels (concentrations) of certain enzymes can provide a wealth of information about a patient's medical condition. Often, such tests are used to confirm a preliminary diagnosis based on the disease symptoms or clinical picture.

For example, when a heart attack occurs, a lack of blood supplied to the heart muscle causes some of the heart muscle cells to die. These cells release their contents, including their enzymes, into the bloodstream. Simple tests can be done to measure the amounts of certain enzymes in the blood. Such tests, called *enzyme assays*, are very precise and specific because they are based on the specificity of the enzyme-substrate complex. If you wish to test for the enzyme lactate dehydrogenase (LDH), you need only to add the appropriate substrate, in this case pyruvate and NADH. The reaction that occurs is the oxidation of NADH to NAD^+ and the reduction of pyruvate to lactate. To measure the rate of the chemical reaction, one can measure the disappearance of the substrate or the accumulation of one of the products. In the case of LDH, spectrophotometric methods (based on the light-absorbing properties of a substrate or product) are available to measure the rate of production of NAD^+. The choice of substrate determines what enzyme activity is to be measured.

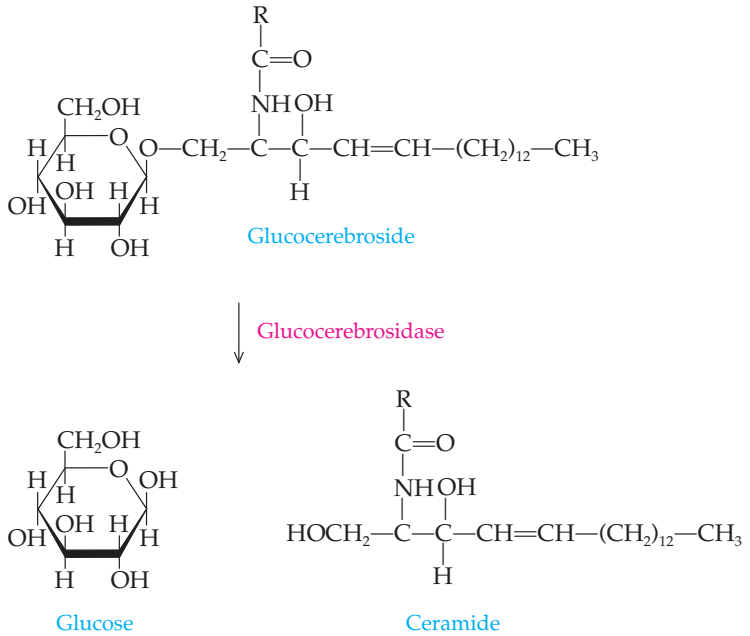
The role of LDH and other enzymes in disease diagnosis is discussed in A Clinical Perspective: Enzymes, Isoenzymes, and Myocardial Infarction.

Elevated blood serum concentrations of the enzymes amylase and lipase are indications of pancreatitis, an inflammation of the pancreas. Liver diseases such as cirrhosis and hepatitis result in elevated levels of one of the isoenzymes of lactate dehydrogenase (LDH_5), and elevated levels of alanine aminotransferase/serum glutamate–pyruvate transaminase (ALT/SGPT) and aspartate aminotransferase/serum glutamate–oxaloacetate transaminase (AST/SGOT) in blood serum. In fact, these latter two enzymes also increase in concentration following heart attack, but the physician can differentiate between these two conditions by considering the relative increase in the two enzymes. If ALT/SGPT is elevated to a greater extent than AST/SGOT, it can be concluded that the problem is liver dysfunction.

Enzymes are also used as analytical reagents in the clinical laboratory owing to their specificity. They often selectively react with one substance of interest, producing a product that is easily measured. An example of this is the clinical analysis of urea in blood. The measurement of urea levels in blood is difficult because of the complexity of blood. However, if urea is converted to ammonia using the enzyme urease, the ammonia becomes an *indicator* of urea, because it is produced from urea, and it is easily measured. This test, called the *blood urea nitrogen (BUN) test*, is useful in the diagnosis of kidney malfunction and serves as one example of the utility of enzymes in clinical chemistry.

Enzyme replacement therapy can also be used in the treatment of certain diseases. One such disease, Gaucher's disease, is a genetic disorder resulting in a deficiency of the enzyme *glucocerebrosidase*. In the normal situation, this enzyme breaks down a glycolipid called *glucocerebroside*, which is an intermediate in the synthesis and degradation of complex glycosphingolipids found in cellular membranes. Glucocerebrosidase is found in the lysosomes, where it hydrolyzes glucocerebroside into glucose and ceramide.

See A Clinical Perspective: Disorders of Sphingolipid Metabolism in Chapter 18.



In Gaucher's disease, the enzyme is not present and glucocerebroside builds up in macrophages found in the liver, spleen, and bone marrow. These cells become engorged with excess lipid that cannot be metabolized and then displace healthy, normal cells in bone marrow. The symptoms of Gaucher's disease include severe anemia, thrombocytopenia (reduction in the number of platelets), and hepatosplenomegaly (enlargement of the spleen and liver). There can also be skeletal problems including bone deterioration and secondary fractures.

Recombinant DNA technology has been used by the Genzyme Corporation to produce the human lysosomal enzyme β-glucocerebrosidase. Given the trade name *Cerezyme*, the enzyme hydrolyzes glucocerebroside into glucose and ceramide so that the products can be metabolized normally. Patients receive Cerezyme intravenously over the course of one to two hours. The dosage and treatment schedule can be tailored to the individual. The results of testing are very encouraging. Patients experience improved red blood cell and platelet counts and reduced hepatosplenomegaly.

Summary

20.1 Nomenclature and Classification

Enzymes are most frequently named by using the common system of nomenclature. The names are useful because they are often derived from the name of the substrate and/or the reaction of the substrate that is catalyzed by the enzyme. Enzymes are classified according to function. The six general classes are *oxidoreductases*, *transferases*, *hydrolases*, *lyases*, *isomerases*, and *ligases*.

20.2 The Effect of Enzymes on the Activation Energy of a Reaction

Enzymes are the biological catalysts of cells. They lower the activation energies but do not alter the equilibrium constants of the reactions they catalyze.

20.3 The Effect of Substrate Concentration on Enzyme-Catalyzed Reactions

With uncatalyzed reactions, increases in substrate concentration result in an increase in reaction rate. For enzyme-catalyzed reactions, an increase in substrate concentration initially causes an increase in reaction rate, but at a particular concentration the reaction rate reaches a maximum. At this concentration all enzyme active sites are filled with substrate.

20.4 The Enzyme-Substrate Complex

Formation of an *enzyme-substrate complex* is the first step of an enzyme-catalyzed reaction. This involves the binding of the substrate to the active site of the enzyme. The lock-and-key model of substrate binding describes the enzyme as a rigid structure into which the substrate fits precisely. The newer induced fit model describes the enzyme as a flexible molecule. The shape of the active site approximates the shape of the substrate and then "molds" itself around the substrate.

20.5 Specificity of the Enzyme-Substrate Complex

Enzymes are also classified on the basis of their specificity. The four classifications of specificity are *absolute*, *group*, *linkage*, and *stereochemical specificity*. An enzyme with *absolute specificity* catalyzes the reaction of only a single substrate. An enzyme with *group specificity* catalyzes reactions involving similar substrates with the same functional group. An enzyme with *linkage specificity* catalyzes reactions involving a single kind of bond. An enzyme with *stereochemical specificity* catalyzes reactions involving only one enantiomer.

20.6 The Transition State and Product Formation

An enzyme-catalyzed reaction is mediated through an unstable *transition state*. This may involve the enzyme putting "stress" on a bond, bringing reactants into close proximity and in the correct orientation, or altering the local pH.

20.7 Cofactors and Coenzymes

Cofactors are metal ions, organic compounds, or organometallic compounds that bind to an enzyme and help maintain the correct configuration of the active site. The term *coenzyme* refers specifically to an organic group that binds transiently to the enzyme during the reaction. It accepts or donates chemical groups.

20.8 Environmental Effects

Enzymes are sensitive to pH and temperature. High temperatures or extremes of pH rapidly inactivate most enzymes by denaturing them.

20.9 Regulation of Enzyme Activity

Enzymes differ from inorganic catalysts in that they are regulated by the cell. Some of the means of enzyme regulation include allosteric regulation, *feedback inhibition*, production of inactive forms, or *zymogens*, and *protein modification*. *Allosteric enzymes* have an effector binding site, as well as an active site. Effector binding renders the enzyme active (*positive allosterism*) or inactive (*negative allosterism*). In feedback inhibition the product of a biosynthetic pathway turns off the entire pathway via negative allosterism. In protein modification, adding or removing a covalently bound group either activates or inactivates an enzyme.

20.10 Inhibition of Enzyme Activity

Enzyme activity can be destroyed by a variety of inhibitors. *Irreversible inhibitors*, or poisons, bind tightly to enzymes and destroy their activity permanently. *Competitive inhibitors*

are generally *structural analogs* of the natural substrate for the enzyme. They compete with the normal substrate for binding to the active site. When the competitive inhibitor is bound by the active site, the reaction cannot occur, and no product is produced.

20.11 Proteolytic Enzymes

Proteolytic enzymes (proteases) catalyze the hydrolysis of peptide bonds. The *pancreatic serine proteases* chymotrypsin, trypsin, and elastase have similar structures and mechanisms of action, but different substrate specificities. It is thought that they evolved from a common ancestral protease.

20.12 Uses of Enzymes in Medicine

Analysis of blood serum for unusually high levels of certain enzymes provides valuable information on a patient's condition. Such analysis is used to diagnose heart attack, liver disease, and pancreatitis. Enzymes are also used as analytical reagents, as in the blood urea nitrogen (BUN) test, and in the treatment of disease.

Key Terms

absolute specificity (20.5)
active site (20.4)
allosteric enzyme (20.9)
apoenzyme (20.7)
coenzyme (20.7)
cofactor (20.7)
competitive inhibitor (20.10)
enzyme (Intro)
enzyme specificity (20.5)
enzyme-substrate complex (20.4)
feedback inhibition (20.9)
group specificity (20.5)
holoenzyme (20.7)
hydrolase (20.1)
induced fit model (20.4)
irreversible enzyme inhibitor (20.10)
isomerase (20.1)
ligase (20.1)
linkage specificity (20.5)
lock-and-key model (20.4)
lyase (20.1)
negative allosterism (20.9)
oxidoreductase (20.1)
pancreatic serine protease (20.11)
pH optimum (20.8)
positive allosterism (20.9)
product (20.2)
protein modification (20.9)
proteolytic enzyme (20.11)
reversible, competitive enzyme inhibitor (20.10)
reversible, noncompetitive enzyme inhibitor (20.10)
stereochemical specificity (20.5)
structural analog (20.10)
substrate (20.1)
temperature optimum (20.8)
transferase (20.1)
transition state (20.6)
vitamin (20.7)
zymogen (20.9)

Questions and Problems

Nomenclature and Classification

20.19 Match each of the following substrates with its corresponding enzyme:

1. Urea	**a.** Lipase
2. Hydrogen peroxide	**b.** Glucose-6-phosphatase
3. Lipid	**c.** Peroxidase
4. Aspartic acid	**d.** Sucrase
5. Glucose-6-phosphate	**e.** Urease
6. Sucrose	**f.** Aspartase

20.20 Give a systematic name for the enzyme that would act on each of the following substrates:
a. Alanine
b. Citrate
c. Ampicillin
d. Ribose
e. Methylamine

20.21 Describe the function implied by the name of each of the following enzymes:
a. Citrate decarboxylase
b. Adenosine diphosphate phosphorylase
c. Oxalate reductase
d. Nitrite oxidase
e. *cis-trans* Isomerase

20.22 List the six classes of enzymes based on the type of reaction catalyzed. Briefly describe the function of each class, and provide an example of each.

The Effect of Enzymes on the Activation Energy of a Reaction

20.23 What is the activation energy of a reaction?
20.24 What is the effect of an enzyme on the activation energy of a reaction?
20.25 Write and explain the equation for the equilibrium constant of an enzyme-mediated reaction. Does the enzyme alter the K_{eq}?
20.26 If an enzyme does not alter the equilibrium constant of a reaction, how does it speed up the reaction?

The Effect of Substrate Concentration on Enzyme-Catalyzed Reactions

20.27 What is the effect of doubling the substrate concentration on the rate of a chemical reaction?
20.28 Why doesn't the rate of an enzyme-catalyzed reaction increase indefinitely when the substrate concentration is made very large?
20.29 Draw a graph that describes the effect of increasing the concentration of the substrate on the rate of an enzyme-catalyzed reaction.
20.30 What does a graph of enzyme activity versus substrate concentration tell us about the nature of enzyme-catalyzed reactions?

The Enzyme-Substrate Complex

20.31 Name three major properties of enzyme active sites.
20.32 If enzyme active sites are small, why are enzymes so large?
20.33 What is the lock-and-key model of enzyme-substrate binding?
20.34 Why is the induced fit model of enzyme-substrate binding a much more accurate model than the lock-and-key model?

Specificity of the Enzyme-Substrate Complex

20.35 List and define four classes of enzyme specificities.
20.36 Give an example of an enzyme that is representative of each class of enzyme specificity.

The Transition State and Product Formation

20.37 Outline the four general stages in an enzyme-catalyzed reaction.
20.38 Describe the transition state.

20.39 What types of transition states might be envisioned that would decrease the energy of activation of an enzyme?
20.40 If an enzyme catalyzed a reaction by modifying the local pH, what kind of amino acid R groups would you expect to find in the active site?

Cofactors and Coenzymes

20.41 What is the role of a cofactor in enzyme activity?
20.42 How does a coenzyme function in an enzyme-catalyzed reaction?
20.43 What is the function of NAD^+? What class of enzymes would require a coenzyme of this sort?
20.44 What is the function of FAD? What class of enzymes would require this coenzyme?

Environmental Effects

20.45 List the factors that affect enzyme activity.
20.46 How will each of the following changes in conditions alter the rate of an enzyme-catalyzed reaction?
a. Decreasing the temperature from 37°C to 10°C
b. Increasing the pH of the solution from 7 to 11
c. Heating the enzyme from 37°C to 100°C
20.47 Why does an enzyme lose activity when the pH is drastically changed from optimum pH?
20.48 Define the optimum pH for enzyme activity.
20.49 High temperature is an effective mechanism for killing bacteria on surgical instruments. How does high temperature result in cellular death?
20.50 An increase in temperature will increase the rate of a reaction if a nonenzymatic catalyst is used; however, an increase in temperature will eventually *decrease* the rate of a reaction when an enzyme catalyst is used. Explain the apparent contradiction of these two statements.
20.51 What is a lysosome?
20.52 Of what significance is it that lysosomal enzymes have a pH optimum of 4.8?
20.53 Why are enzymes that are used for clinical assays in hospitals stored in refrigerators?
20.54 Why do extremes of pH inactivate enzymes?

Regulation of Enzyme Activity

20.55 **a.** Why is it important for cells to regulate the level of enzyme activity?
b. Why must synthesis of digestive enzymes be carefully controlled?
20.56 What is an allosteric enzyme?
20.57 What is the difference between positive and negative allosterism?
20.58 **a.** Define feedback inhibition.
b. Describe the role of allosteric enzymes in feedback inhibition.
c. Is this positive or negative allosterism?
20.59 What is a zymogen?
20.60 Three zymogens that are involved in digestion of proteins in the stomach and intestines are pepsinogen, chymotrypsinogen, and trypsinogen. What is the advantage of producing these enzymes as inactive peptides?

Inhibition of Enzyme Activity

20.61 Define *competitive enzyme inhibition.*
20.62 How do the sulfa drugs selectively kill bacteria while causing no harm to humans?
20.63 Describe the structure of a structural analog.
20.64 How can structural analogs serve as enzyme inhibitors?
20.65 Define *irreversible enzyme inhibition.*
20.66 Why are irreversible enzyme inhibitors often called *poisons?*
20.67 Suppose that a certain drug company manufactured a compound that had nearly the same structure as a substrate for a certain enzyme but that could not be acted upon chemically by the enzyme. What type of interaction would the compound have with the enzyme?
20.68 The addition of phenylthiourea to a preparation of the enzyme polyphenoloxidase completely inhibits the activity of the enzyme.
a. Knowing that phenylthiourea binds all copper ions, what conclusion can you draw about whether polyphenoloxidase requires a cofactor?
b. What kind of inhibitor is phenylthiourea?

Proteolytic Enzymes

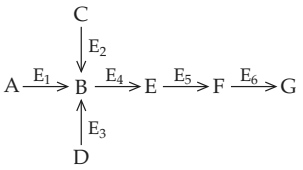
20.69 What do the similar structures of chymotrypsin, trypsin, and elastase suggest about their evolutionary relationship?
20.70 What properties are shared by chymotrypsin, trypsin, and elastase?
20.71 Draw the complete structural formula for the peptide tyr-lys-ala-phe. Show which bond would be broken when this peptide is reacted with chymotrypsin.
20.72 Repeat Question 20.71 for the peptide trp-pro-gly-tyr.
20.73 The sequence of a peptide that contains ten amino acid residues is as follows:
ala-gly-val-leu-trp-lys-ser-phe-arg-pro
Indicate with arrows and label the peptide bond(s) that are cleaved by elastase, trypsin, and chymotrypsin.
20.74 What structural features of trypsin, chymotrypsin, and elastase account for their different specificities?

Uses of Enzymes in Medicine

20.75 List the enzymes whose levels are elevated in blood serum following a myocardial infarction.
20.76 List the enzymes whose levels are elevated as a result of hepatitis or cirrhosis of the liver.

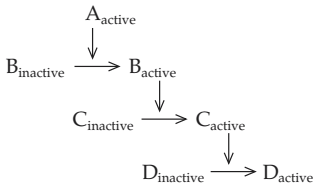
Critical Thinking Problems

1. Ethylene glycol is a poison that causes about fifty deaths a year in the United States. Treating people who have drunk ethylene glycol with massive doses of ethanol can save their lives. Suggest a reason for the effect of ethanol.
2. Generally speaking, feedback inhibition involves regulation of the first step in a pathway. Consider the following hypothetical pathway:



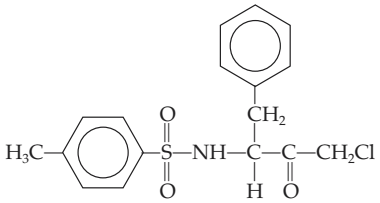
Which step in this pathway do you think should be regulated? Explain your reasoning.

3. In an amplification cascade, each step greatly increases the amount of substrate available for the next step, so that a very large amount of the final product is made. Consider the following hypothetical amplification cascade:



If each active enzyme in the pathway converts one hundred molecules of its substrate to active form, how many molecules of D will be produced if the pathway begins with one molecule of A?

4. L-1-(*p*-toluenesulfonyl)-amido-2-phenylethylchloromethyl ketone (TPCK, shown below) inhibits chymotrypsin, but not trypsin. Propose a hypothesis to explain this observation.



5. A graduate student is trying to make a "map" of a short peptide so that she can eventually determine the amino acid sequence. She digested the peptide with several proteases and determined the sizes of the resultant digestion products.

Enzyme	**M.W. of Digestion Products**
Trypsin	2000, 3000
Chymotrypsin	500, 1000, 3500
Elastase	500, 1000, 1500, 2000

Suggest experiments that would allow the student to map the order of the enzyme digestion sites along the peptide.

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Some familiar fermentation products.

21

Carbohydrate Metabolism

Learning Goals

1. Discuss the importance of ATP in cellular energy transfer processes.
2. Describe the three stages of catabolism of dietary proteins, carbohydrates, and lipids.
3. Discuss glycolysis in terms of its two major segments.
4. Looking at an equation representing any of the chemical reactions that occur in glycolysis, describe the kind of reaction that is occurring and the significance of that reaction to the pathway.
5. Describe the mechanism of regulation of the rate of glycolysis. Discuss particular examples of that regulation.
6. Discuss the practical and metabolic roles of fermentation reactions.
7. List several products of the pentose phosphate pathway that are required for biosynthesis.
8. Compare glycolysis and gluconeogenesis.
9. Summarize the regulation of blood glucose levels by glycogenesis and glycogenolysis.

Outline

Chemistry Connection: *The Man Who Got Tipsy from Eating Pasta*

21.1 ATP: The Cellular Energy Currency

21.2 Overview of Catabolic Processes
- Stage I: Hydrolysis of Dietary Macromolecules into Small Subunits
- Stage II: Conversion of Monomers into a Form That Can Be Completely Oxidized
- Stage III: The Complete Oxidation of Nutrients and the Production of ATP

21.3 Glycolysis

A Medical Perspective: *Genetic Disorders of Glycolysis*
- An Overview
- Reactions of Glycolysis
- Regulation of Glycolysis

21.4 Fermentations

A Human Perspective: *Fermentations: The Good, the Bad, and the Ugly*
- Lactate Fermentation
- Alcohol Fermentation

21.5 The Pentose Phosphate Pathway

21.6 Gluconeogenesis: The Synthesis of Glucose

21.7 Glycogen Synthesis and Degradation
- The Structure of Glycogen
- Glycogenolysis: Glycogen Degradation
- Glycogenesis: Glycogen Synthesis

A Clinical Perspective: *Diagnosing Diabetes*
- Compatibility of Glycogenesis and Glycogenolysis

A Human Perspective: *Glycogen Storage Diseases*

Summary

Key Terms

Questions and Problems

Critical Thinking Problems

BIOCHEMISTRY