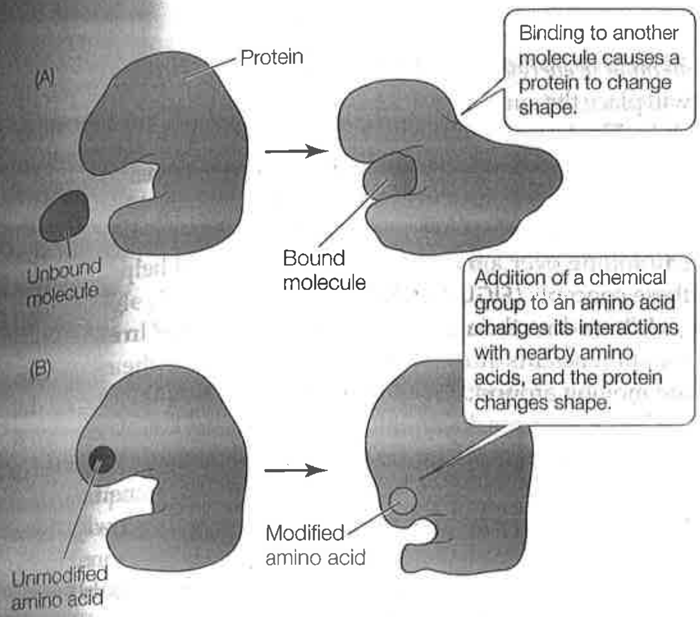


FIGURE 3.11 Protein Structure Can Change Proteins can change their tertiary structure when they bind to other molecules (A) or are modified chemically (B).

CHECKpoint CONCEPT 3.2

- ✓ Sketch the peptide bonding of the two amino acids glycine and leucine (in that order). Now add a third amino acid, alanine, in the position it would have if added within a biological system. What is the directionality of this process?
- ✓ Examine the structure of sucrase (see Figure 3.9). Where in the protein might you expect to find the following amino acids: valine, proline, glutamic acid, and threonine? Explain your answers.
- ✓ Detergents disrupt hydrophobic interactions by coating hydrophobic molecules with a molecule that has a hydrophilic surface. When hemoglobin is treated with a detergent, the four polypeptide chains separate and become random coils. Explain these observations.
- ✓ Several small molecules interact with a protein. The chemical groups on the small molecules interact with specific amino acids as shown in the table below. Fill in the table to show the types of noncovalent interactions that occur between the small molecules and the amino acids.

SMALL MOLECULE CHEMICAL GROUP	AMINO ACID IN PROTEIN	TYPE OF INTERACTION (HYDROGEN BOND; IONIC INTERACTION; HYDROPHOBIC INTERACTION)
$-NH_3^+$	Aspartic acid	
$-CH_3$	Isoleucine	
$-OH$	Glutamine	

We have discussed the remarkable diversity in protein structures. These structures carry functional groups (on exposed amino acid side chains) that can interact with other molecules. In the next section we will see how these interactions can result in catalysis, the speeding up of biochemical reactions.

APPLY THE CONCEPT

Proteins are polymers with important structural and metabolic roles

Biological systems contain "supermolecular complexes" (for example, the ribosome; see Chapter 4), which are composed of individual molecules of RNA and protein that fit together noncovalently. These complexes can be split apart with detergents that disrupt hydrophobic interactions. Based on the concepts discussed in this chapter, fill in the table below to indicate which of the observations are characteristic of RNA, which are characteristic of protein, and which are characteristic of both. Explain your answers.

OBSERVATION	CHARACTERISTIC OF: PROTEIN	RNA
Has three-dimensional (3-D) structure		
3-D structure destroyed by heat		
Monomers connected by N—C bonds		
Contains sulfur atoms		
Contains phosphorus atoms		

CONCEPT 3.3 Some Proteins Act as Enzymes to Speed up Biochemical Reactions

In Chapter 2 we introduced the concepts of biological energetics. We showed that some metabolic reactions are exergonic and some are endergonic, and that biochemistry obeys the laws of thermodynamics (see Figures 2.14 and 2.15). Knowing whether energy is supplied or released in a particular reaction tells us whether the reaction *can* occur in a living system. But it does not tell us *how fast* the reaction will occur.

Living systems depend on reactions that occur spontaneously. But without help, most of these reactions would proceed at such slow rates that an organism could not survive. The role of a **catalyst** is to speed up a reaction without itself being permanently altered. A catalyst does not cause a reaction to occur, but it increases the rate of the reaction. This is an important point: *No catalyst makes a reaction occur that would not proceed without it.*

Biological catalysts are called **enzymes**; for example, the synthesis of prostaglandin (see the opening story) is catalyzed by an enzyme (cyclooxygenase). Most enzymes are proteins, but a few important enzymes are RNA molecules called ribozymes. An enzyme can bind the reactants in a chemical reaction and participate in the reaction itself. However, this participation does not permanently change the enzyme. At the end of the reaction, the enzyme is unchanged and available to catalyze additional, similar reactions.

An energy barrier must be overcome to speed up a reaction

An exergonic reaction releases **free energy** (G), which is the amount of energy in a system that is available to do work. For example, the free energy released in an exergonic reaction can be used by the cell to drive an endergonic reaction, or it can be converted to mechanical energy for movement (see Figure 6.1). But without a catalyst, a reaction will usually take place very slowly. This is because there is an energy barrier between the reactants and the products. Think about the hydrolysis of sucrose, which we described in Concept 2.5.

$$\text{Sucrose} + H_2O \rightarrow \text{glucose} + \text{fructose}$$

In humans, this reaction is part of the process of digestion. The reaction is exergonic, but even if water is abundant, the sucrose molecule will only rarely bind the H atom and the –OH group in the water molecule at the appropriate locations to break the covalent bond between the glucose and fructose—*unless there is an input of energy to initiate the reaction*. Such an input of energy will place the sucrose into a reactive mode called the **transition state**. The energy input required for sucrose to reach this state is called the **activation energy** (E_a). Once the transition state is reached, the reaction can proceed spontaneously with a release of free energy (ΔG is negative) (**FIGURE 3.12A**). The image of a ball rolling over a bump and then down a hill helps illustrate these concepts (**FIGURE 3.12B**).

Where does the activation energy come from? In any collection of reactants at room or body temperature, the molecules are moving around. Recall from Chapter 2 that the energy the molecules possess due to this motion is called kinetic energy. A few molecules are moving fast enough that their kinetic energy can overcome the energy barrier; they enter the transition state and react. So the reaction takes place—but very slowly. If the system is heated, all the reactant molecules have more kinetic energy, and the reaction speeds up. You have probably used this technique in the chemistry laboratory.

Adding enough heat to increase the average kinetic energy of the molecules would not work in a living system, however. Such a nonspecific approach would accelerate all reactions, including destructive ones such as the denaturation of proteins.

An enzyme lowers the activation energy for a reaction by enabling the reactants to come together and react more easily; the reactants need lower amounts of kinetic energy to enter their transition states (**FIGURE 3.13**). In this way, an enzyme can change the rate of a reaction substantially. For example, if a molecule of sucrose just sits in solution, hydrolysis may take hundreds of years. But with the enzyme sucrase present, the same reaction occurs in 1 second! Typically, an enzyme-catalyzed reaction proceeds 10^3 to 10^8 times faster than the uncatalyzed reaction, and the enzyme converts 100 to 1,000 substrate molecules into product per second.

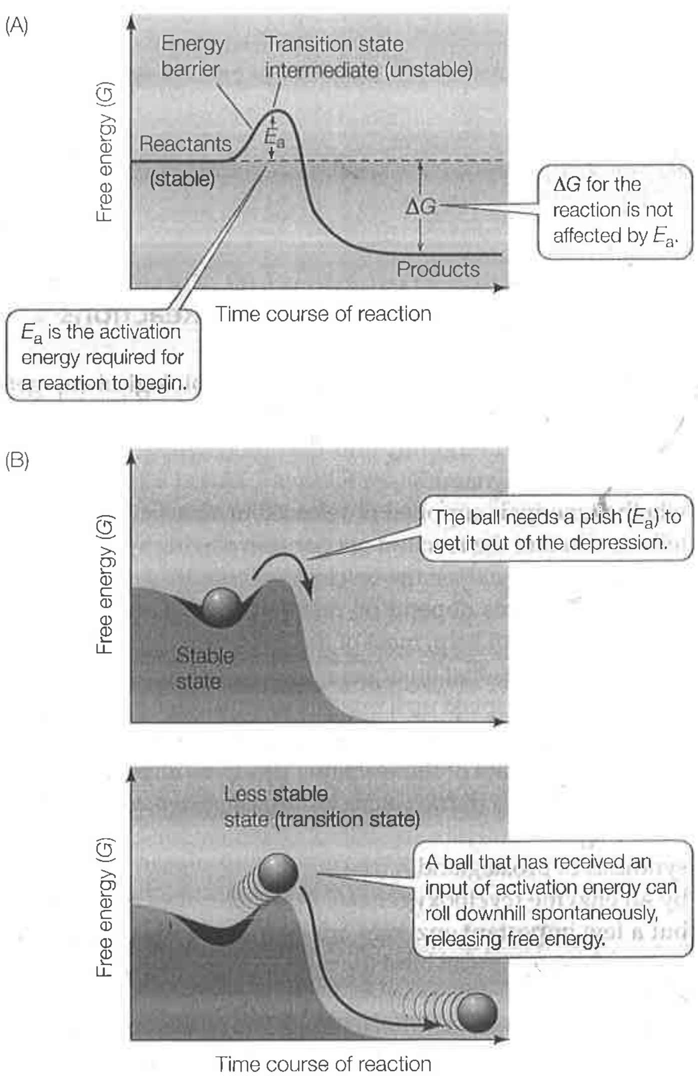


FIGURE 3.12 Activation Energy Initiates Reactions (A) In any chemical reaction, an initial stable state must become less stable before change is possible. (B) A ball on a hillside provides a physical analogy to the biochemical principle graphed in A. Although these graphs show an exergonic reaction, activation energy is needed for endergonic reactions as well.

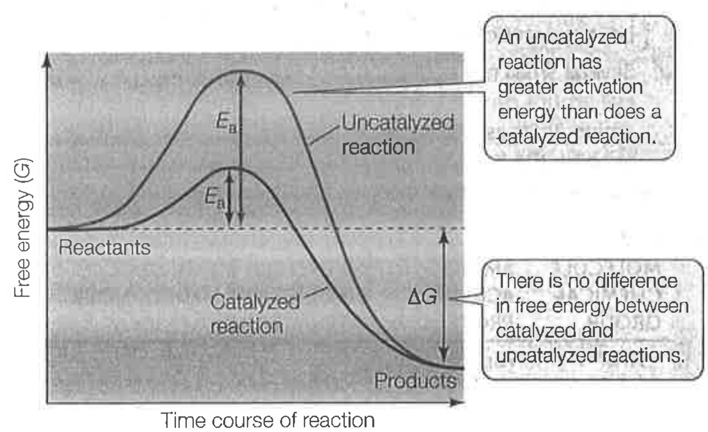


FIGURE 3.13 Enzymes Lower the Energy Barrier The activation energy (E_a) is lower in an enzyme-catalyzed reaction than in an uncatalyzed reaction, but the free energy released is the same with or without catalysis. A lower activation energy means the reaction will take place at a faster rate.

Go to ACTIVITY 3.4 Free Energy Changes
PoL2e.com/ac3.4

Enzymes bind specific reactants at their active sites

Catalysts increase the rates of chemical reactions. Most nonbiological catalysts are nonspecific. For example, powdered platinum catalyzes virtually any reaction in which molecular hydrogen (H_2) is a reactant. In contrast, most biological catalysts are highly specific. An enzyme usually recognizes and binds to only one or a few closely related reactants, and it catalyzes only a single chemical reaction.

In an enzyme-catalyzed reaction, the reactants are called **substrates.** Substrate molecules bind to a particular site on the enzyme, called the **active site**, where catalysis takes place (**FIGURE 3.14**). The specificity of an enzyme results from the exact three-dimensional shape (also called conformation) and chemical properties of its active site. Only a narrow range of substrates, with specific shapes, functional groups, and chemical properties, can fit properly and bind to the active site. The names of enzymes reflect their functions and often end with the suffix "ase." For example, the enzyme sucrase catalyzes the hydrolysis of sucrose, and we write the reaction as follows:

$$\text{Sucrose} + H_2O \xrightarrow{\text{Sucrase}} \text{glucose} + \text{fructose}$$

The binding of a substrate (S) to the active site of an enzyme (E) produces an **enzyme–substrate complex** (ES) that is held together by one or more means, such as hydrogen bonding, ionic attraction, or temporary covalent bonding. The enzyme–substrate complex gives rise to product (P) and free enzyme:

$$E + S \rightarrow ES \rightarrow E + P$$

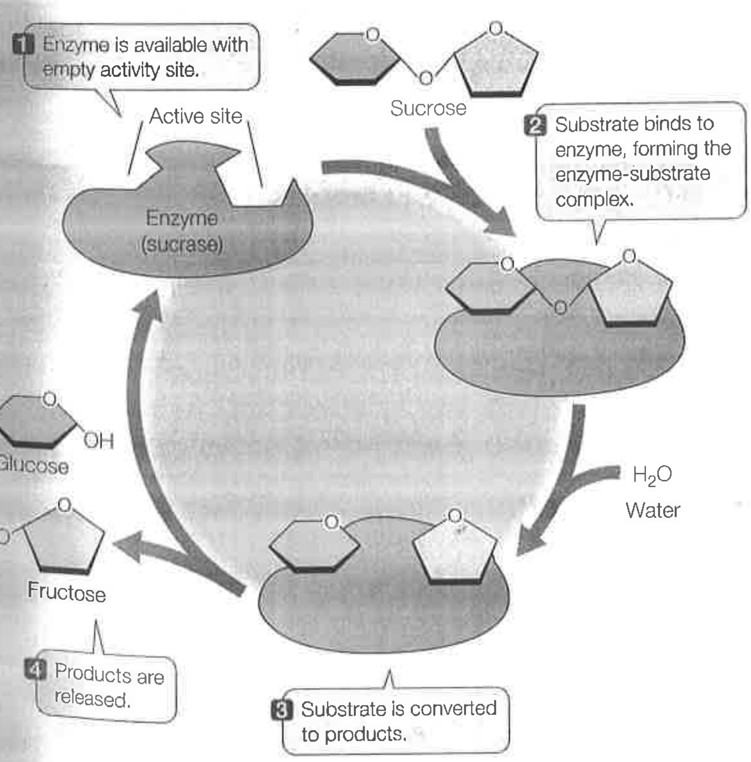
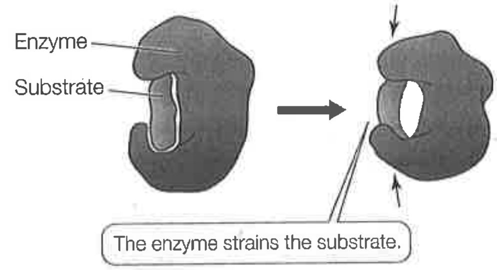


FIGURE 3.14 Enzyme Action Sucrase catalyzes the hydrolysis of sucrose. After the reaction, the enzyme is unchanged and is ready to accept another substrate molecule.

(As we have seen in the case of sucrase, a single enzyme-catalyzed reaction may involve multiple substrates and/or products.) The free enzyme (E) is in the same chemical form at the end of the reaction as at the beginning. While bound to the substrate(s), it may change chemically, but by the end of the reaction it has been restored to its initial form and is ready to catalyze the same reaction again (see Figure 3.14).

HOW ENZYMES WORK During and after the formation of the enzyme–substrate complex, chemical interactions occur. These interactions contribute directly to the breaking of old bonds and the formation of new ones. In catalyzing a reaction, an enzyme may use one or more mechanisms:

- *Inducing strain*: Once the substrate has bound to the active site, the enzyme causes bonds in the substrate to stretch, putting it in an unstable transition state:



- *Substrate orientation:* When free in solution, substrates are moving from place to place randomly while at the same time vibrating, rotating, and tumbling. They only rarely have the proper orientation to react when they collide. The enzyme lowers the activation energy needed to start the reaction, by bringing together specific atoms so that bonds can form.
- *Adding chemical groups:* The side chains (R groups) of an enzyme's amino acids may be directly involved in the reaction. For example, in acid–base catalysis, the acidic or basic side chains of the amino acids in the active site transfer H^+ ions to or from the substrate, destabilizing a covalent bond in the substrate and permitting the bond to break.

The active site is usually only a small part of the enzyme protein. But its three-dimensional structure is so specific that it binds only one or a few related substrates. The binding of the substrate to the active site depends on the same relatively weak forces that maintain the tertiary structure of the enzyme: hydrogen bonds, the attraction and repulsion of charged groups, and hydrophobic interactions. Scientists used to think of substrate binding as being similar to a lock and key fitting together. Actually, for most enzymes and substrates the relationship is more like a baseball and a catcher's mitt: the substrate first binds, and then the active site changes slightly to make the binding tight. **FIGURE 3.15** illustrates this "induced fit" phenomenon. (We introduced the concept of protein structure changes earlier; see Figure 3.11.)

Induced fit at least partly explains why enzymes are so large. The rest of the macromolecule has at least three roles:

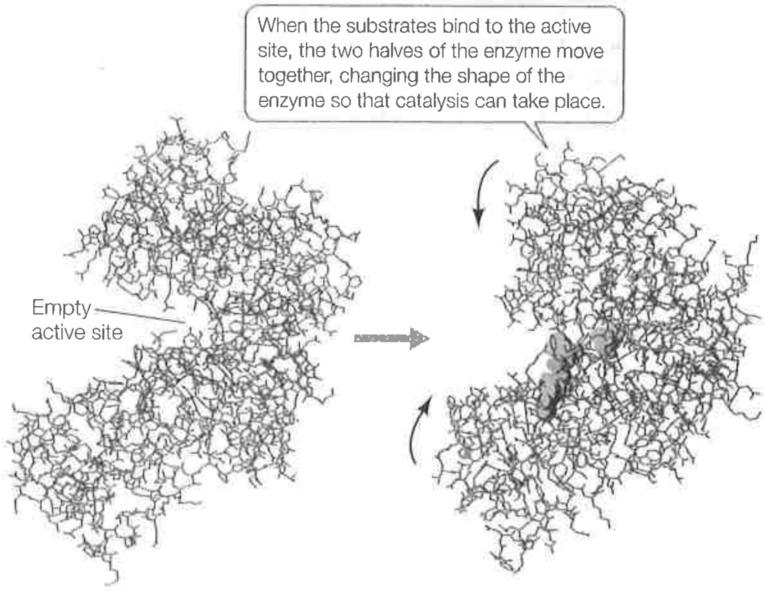


FIGURE 3.15 Some Enzymes Change Shape When Substrate Binds to Them Shape changes result in an induced fit between enzyme and substrate, improving the catalytic ability of the enzyme. Induced fit can be observed in the enzyme hexokinase, seen here with and without its substrates, glucose (green) and ATP (yellow).

- It provides a framework so the amino acids of the active site are properly positioned in relation to the substrate(s).
- It participates in the changes in protein shape and structure that result in induced fit.
- It provides binding sites for regulatory molecules (as we will discuss in Concept 3.4).

NONPROTEIN PARTNERS FOR ENZYMES Some enzymes require ions or other molecules in order to function. These molecules are referred to as **cofactors**, and they can be grouped into three categories (**TABLE 3.3**):

- *Metal ions* such as copper, zinc, and iron bind to certain enzymes and participate in the enzyme-catalyzed reactions. For example, the cofactor zinc binds to the enzyme alcohol dehydrogenase, which catalyzes the breakdown of toxic alcohol.
- A *coenzyme* is a relatively small, carbon-containing (organic) molecule that is required for the action of one or more enzymes. It binds to the active site of the enzyme, adds or removes a chemical group from the substrate, and then separates from the enzyme to participate in other reactions. A coenzyme differs from a substrate in that it can participate in many different reactions with different enzymes.
- *Prosthetic groups* are organic molecules that are permanently bound to their enzymes. An example is a flavin nucleotide, which binds to succinate dehydrogenase, an important enzyme in energy metabolism.

RATE OF REACTION The rate of an uncatalyzed reaction is directly proportional to the concentration of the substrate. The higher the concentration, the more reactions per unit of time.

As we have seen, the addition of the appropriate enzyme speeds up the reaction, but it also changes the shape of the plot of rate versus substrate concentration (**FIGURE 3.16**). For a given concentration of enzyme, the rate of the enzyme-catalyzed reaction initially increases as the substrate concentration increases from zero, but then it levels off.

Why does this happen? The concentration of an enzyme is usually much lower than that of its substrate and does not change as substrate concentration changes. When all the enzyme molecules are bound to substrate molecules, the enzyme is working at its maximum rate. Under these conditions the active sites are said to be saturated.

The maximum rate of a catalyzed reaction can be used to measure how efficient the enzyme is—that is, how many molecules of substrate are converted into product by an individual enzyme molecule per unit of time, when there is an excess of substrate present. This turnover number ranges from 1 molecule every second for sucrase to an amazing 40 million molecules per second for the liver enzyme catalase.

CHECKpoint CONCEPT 3.3

- ✓ Explain how the structure of an enzyme makes that enzyme specific.
- ✓ What is activation energy? How does an enzyme lower the activation energy needed to start a reaction?
- ✓ Compare coenzymes with substrates. How do they work together in enzyme catalysis?
- ✓ Compare the state of an enzyme active site at a low substrate concentration and at a high substrate concentration. How does this affect the rate of the reaction?

TABLE 3.3 Some Examples of Enzyme Cofactors

Type of cofactor	Role in catalyzed reactions
METAL IONS	
Iron (Fe^{2+} or Fe^{3+})	Oxidation/reduction
Copper (Cu^{+} or Cu^{2+})	Oxidation/reduction
Zinc (Zn^{2+})	Helps bind NAD
COENZYMES	
Biotin	Carries $-COO^-$
Coenzyme A	Carries $-CO-CH_3$
NAD	Carries electrons
FAD	Carries electrons
ATP	Provides/extracts energy
PROSTHETIC GROUPS	
Heme	Binds ions, O_2, and electrons; contains iron cofactor
Flavin	Binds electrons
Retinal	Converts light energy

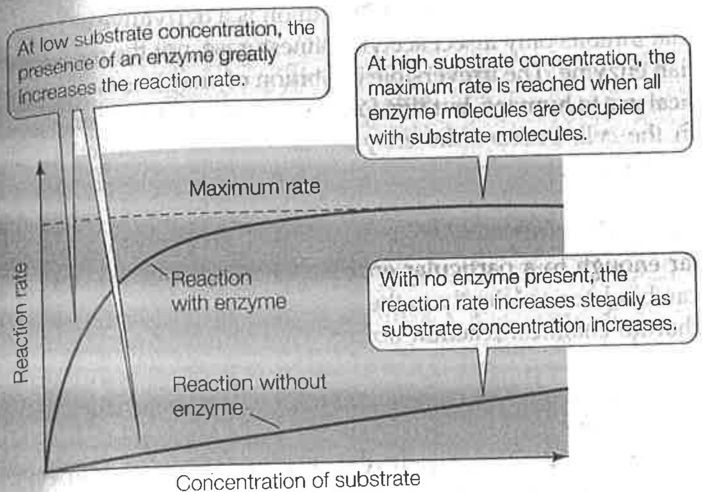


FIGURE 3.16 Catalyzed Reactions Reach a Maximum Rate Because there is usually less enzyme than substrate present, the reaction rate levels off when the enzyme becomes saturated.

Now that you understand more about how enzymes function, let's see how different enzymes work in the metabolism of living organisms.

CONCEPT 3.4 Regulation of Metabolism Occurs by Regulation of Enzymes

The enzyme-catalyzed reactions we have been discussing often operate within **metabolic pathways** in which the product of one reaction is a substrate for the next. For example, the pathway for the catabolism of sucrose begins with sucrase and ends many reactions later with the production of CO_2 and H_2O. Energy is released along the way. Each step of this catabolic pathway is catalyzed by a specific enzyme:

$$\text{Sucrose} + H_2O \xrightarrow{\text{Sucrase}} \text{glucose} + \text{fructose} \longrightarrow$$

$$\xrightarrow{\text{Many enzymes}} \longrightarrow \longrightarrow \longrightarrow \longrightarrow CO_2 + H_2O$$

Other enzymes participate in anabolic pathways, which produce relatively complex molecules from simpler ones. A typical cell contains hundreds of enzymes that participate in many interconnecting metabolic pathways, forming a metabolic system (**FIGURE 3.17**). Consider a single molecule in the midst of this map:

- There may be two or more enzyme-catalyzed reactions affecting it: either making it or metabolizing it.
- Other pathways affect the concentrations of the substrates and products of these reactions.
- Each enzyme-catalyzed reaction has its own rate, depending on these concentrations.

Clearly, every component of this complex system is affected by every other component, making it difficult to predict what would happen if one or more components were altered. In the new field of **systems biology**, scientists describe mathematically the components of metabolic systems—the concentrations of all the reactants and the rates of the reactions—and use computer algorithms to make predictions about what would happen if a component of the system were altered (see Concept 1.2, pp. 8–9).

Cells need to maintain stable internal conditions, including constant levels of certain metabolites. In addition, cells need to regulate their metabolic pathways to respond to changes, either within the organism or in its environment. One way a cell can regulate its metabolism is to control the *amount* of an enzyme. For example, the product of a metabolic pathway may be available from the cell's environment in adequate amounts. In this case, it would be energetically wasteful for the cell to continue making large proteins (as most enzymes are) that it doesn't need. For this reason, cells often have the ability to turn off the synthesis of certain enzymes.

LINK
The amount of an enzyme is controlled by regulating the expression of gene(s), a topic covered in **Chapter 11**

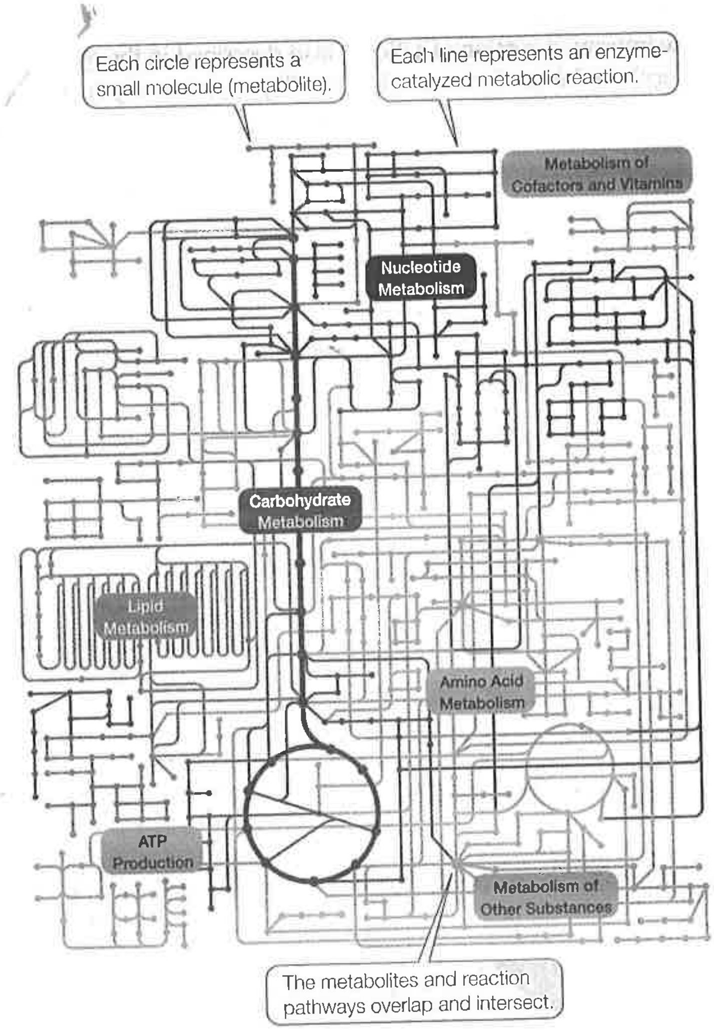


FIGURE 3.17 A Biochemical System The complex interactions of metabolic pathways can be studied using the tools of systems biology. Enzymes are a major element controlling these pathways.

The consequences of *too little* enzyme can be significant. For example, in humans sucrase is important in digestion. In rare cases, infants are born with a congenital sucrase deficiency and the pathway that begins with sucrose is essentially blocked. If these infants ingest foods containing sucrose, the sucrose accumulates rather than being catabolized, and the infant gets diarrhea and stomach cramps. In some cases this leads to slower growth. This deficiency can be treated by limiting sucrose consumption or taking tablets that contain sucrase at every meal.

Cells can also maintain stable internal conditions by regulating the *activity* of enzymes. An enzyme protein may be present continuously, but it may be active or inactive depending on the needs of the cell. Synthesizing and breaking down enzymes takes time, whereas regulating enzyme activity allows cells to fine-tune metabolism relatively quickly in response to changes in the environment. In this section, we will describe how enzyme regulation occurs.

Enzymes can be regulated by inhibitors

Various chemical inhibitors can bind to enzymes, slowing down the rates of the reactions they catalyze. Some inhibitors occur naturally in cells; others can be made in laboratories. Naturally occurring inhibitors regulate metabolism; artificial ones (such as the improved version of salicylic acid described in the opening story) can be used to treat disease, kill pests, or study how enzymes work. In some cases the inhibitor binds the enzyme irreversibly, and the enzyme becomes permanently inactivated. In other cases the inhibitor has reversible effects; it can separate from the enzyme, allowing the enzyme to function fully as before.

IRREVERSIBLE INHIBITION If an inhibitor covalently binds to an amino acid side chain at the active site of an enzyme, the enzyme is permanently inactivated because it cannot interact with its substrate. An example of an irreversible inhibitor is DIPF (diisopropyl phosphorofluoridate), which irreversibly inhibits acetylcholinesterase, an important enzyme that functions in the nervous system. DIPF does so by reacting with a hydroxyl group on a serine in the active site (**FIGURE 3.18**).

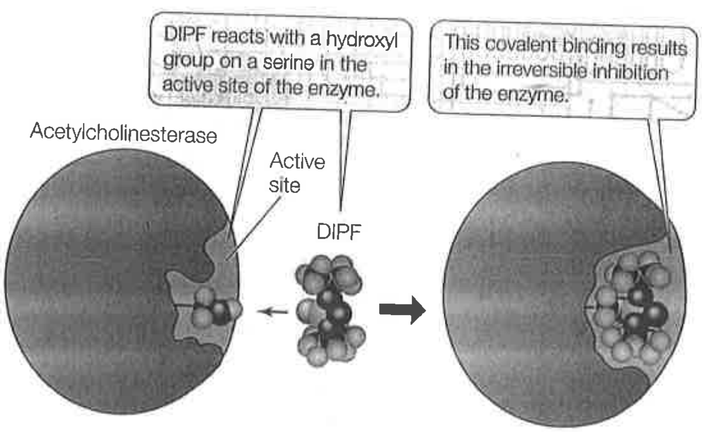


FIGURE 3.18 Irreversible Inhibition DIPF forms a stable covalent bond with the amino acid serine at the active site of the enzyme acetylcholinesterase, thus irreversibly disabling the enzyme.

The widely used insecticide malathion is a derivative of DIPF that inhibits only insect acetylcholinesterase, not the mammalian enzyme. The irreversible inhibition of enzymes is of practical use to humans, but this form of regulation is not common in the cell, because the enzyme is permanently inactivated and cannot be recycled. Instead, cells use reversible inhibition.

REVERSIBLE INHIBITION In some cases, an inhibitor is similar enough to a particular enzyme's natural substrate that it can bind noncovalently to the active site, yet different enough that no chemical reaction occurs. This is analogous to a key that inserts into a lock but does not turn it. When such a molecule is bound to the enzyme, the natural substrate cannot enter the active site and the enzyme is unable to function. Such a molecule is called a **competitive inhibitor** because it competes with the natural substrate for the active site (**FIGURE 3.19A**). Many drugs are competitive inhibitors of enzyme targets. For example, methotrexate is a drug designed with a structure similar to the metabolite dihydrofolate. The latter is converted by an enzyme to a substance essential to cell division. Acting as a competitive inhibitor of the enzyme, methotrexate blocks cell division and is used in cancer therapy. Competitive inhibition is reversible. When the concentration of the competitive inhibitor is reduced, the active site is less likely to be occupied by the inhibitor, and the enzyme regains activity.

A **noncompetitive inhibitor** binds to an enzyme at a site distinct from the active site. This binding causes a change in the shape (the conformation) of the enzyme, altering its activity (**FIGURE 3.19B**). The active site may no longer bind the substrate, or if it does, the rate of product formation may be reduced. Like competitive inhibitors, noncompetitive inhibitors can become unbound, so their effects are reversible.

An allosteric enzyme is regulated by changes in its shape

Noncompetitive inhibition is an example of allostery (*allo*, "different"; *stereos*, "shape"). **Allosteric regulation** occurs when a non-substrate molecule binds or modifies a site other than the active site of an enzyme. The site bound by the non-substrate molecule is called the allosteric site. This binding induces the enzyme to change its conformation, altering the chemical attraction (affinity) of the active site for the substrate. As a result, the rate of the reaction is changed.

An allosteric site may be modified by either noncovalent or covalent binding:

- *Noncovalent binding*: A regulatory molecule may bind noncovalently to an allosteric site, causing the enzyme to change shape. This noncovalent binding is reversible, and may result in the inactivation of an enzyme (see Figure 3.19B) or the activation of a formerly inactive enzyme (**FIGURE 3.20A**).
- *Covalent binding*: Some allosteric sites can be modified by the covalent binding of a molecule or chemical group. For example, an amino acid residue can be covalently modified by the addition of a phosphate group, in a process called phosphorylation (**FIGURE 3.20B**). If this occurs in a hydro-

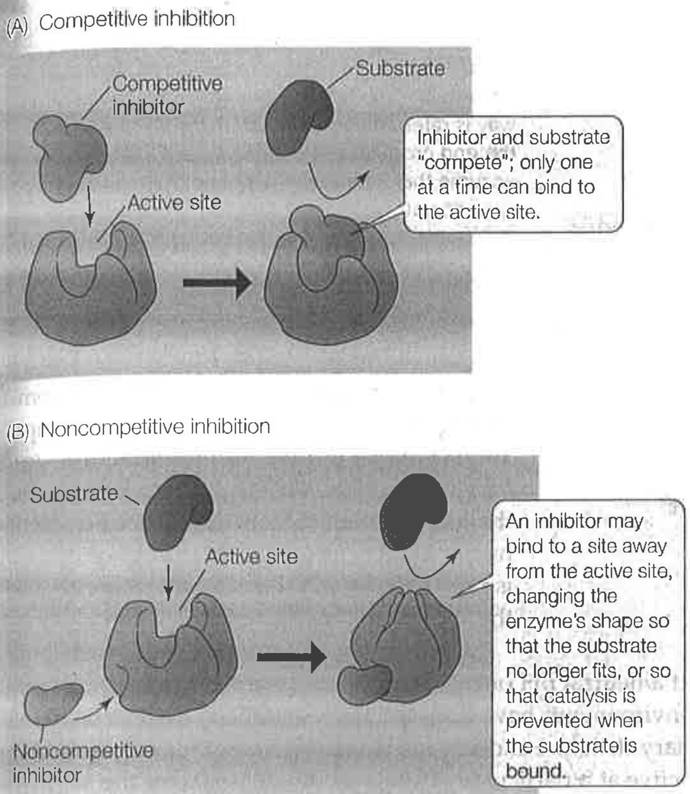


FIGURE 3.19 Reversible Inhibition (A) A competitive inhibitor binds temporarily to the active site of an enzyme. (B) A noncompetitive inhibitor binds temporarily to the enzyme at a site away from the active site. In both cases, the enzyme's function is disabled for only as long as the inhibitor remains bound.

Go to ANIMATED TUTORIAL 3.2
Enzyme Catalysis
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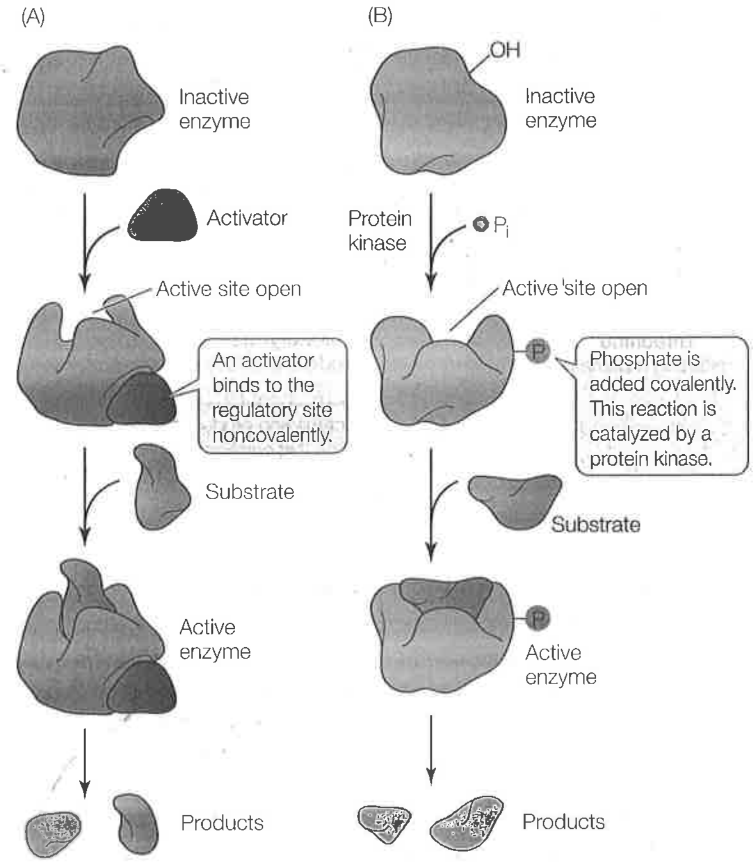

FIGURE 3.20 Allosteric Regulation of Enzyme Activity
(A) Noncovalent binding of a regulator (in this case an activator) can cause an enzyme to change shape and expose an active site. B) Enzymes can also be activated by covalent modification, in this case phosphorylation. Note that allosteric regulation can be negative as well, with the active site becoming hidden.

Go to ANIMATED TUTORIAL 3.3
Allosteric Regulation of Enzymes
PoL2e.com/at3.3

phobic region of the enzyme, it makes that region hydrophilic, because phosphate carries a negative charge. The protein twists, and this can expose or hide the active site. Protein phosphorylation is an extremely important mechanism by which cells regulate many different enzymes and other proteins. It is a reversible process: a class of enzymes called protein kinases catalyze the addition of phosphate groups to proteins, whereas protein phosphatases remove phosphate groups from proteins. Humans have hundreds of different protein kinases and phosphatases. We will return to the exact functions of these proteins many times in this book.

LINK

Protein kinases are of particular importance in intracellular signaling pathways (see Concepts 5.5 and 5.6) and in the control of cell reproduction (see Concept 7.3)

Some metabolic pathways can be controlled by feedback inhibition

A metabolic pathway typically involves a starting material, various intermediate products, and an end product that is used for some purpose by the cell. In each pathway there are a number of reactions, each forming an intermediate product and each catalyzed by a different enzyme. In many pathways the first step is the commitment step, meaning that once this enzyme-catalyzed reaction occurs, the "ball is rolling," and the other reactions happen in sequence, leading to the end product. But as we pointed out earlier, it is energetically wasteful for the cell to make something it does not need.

One way to regulate a metabolic pathway is by having the final product inhibit the enzyme that catalyzes the commitment step (**FIGURE 3.21**). When the end product is present at a high concentration, some of it binds to a site on the commitment step enzyme, thereby causing it to become inactive. The end product may bind to the active site on the enzyme (as a competitive inhibitor) or an allosteric site (as a noncompetitive inhibitor). This mechanism is known as **feedback inhibition** or end-product inhibition. We will describe many other examples of such inhibition in later chapters.

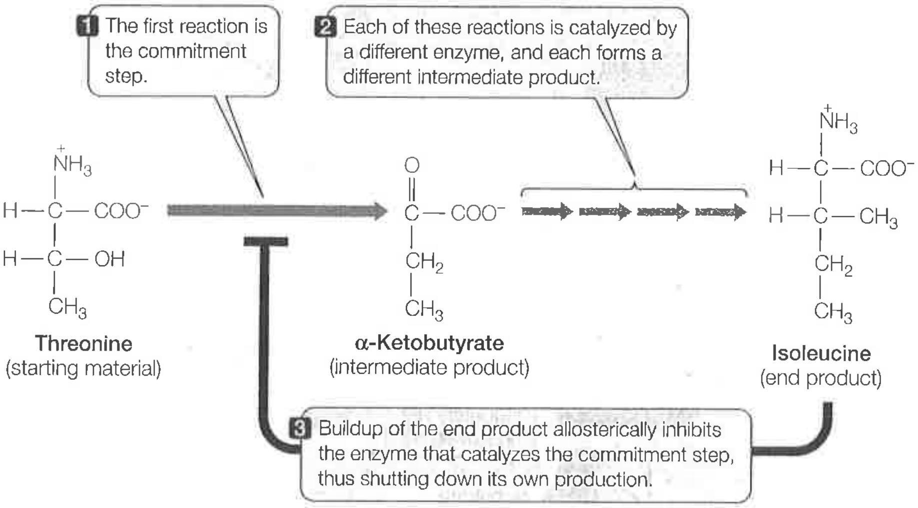

FIGURE 3.21 Feedback Inhibition of Metabolic Pathways The first reaction in a metabolic pathway is referred to as the commitment step. Often the end product of the pathway can inhibit the enzyme that catalyzes the commitment step. The specific pathway shown here is the synthesis of isoleucine from threonine in bacteria. It is typical of many enzyme-catalyzed biosynthetic pathways.

Enzymes are affected by their environment

As we have seen, the specificity and activity of an enzyme depend on its three-dimensional structure, and this in turn depends on weak forces such as hydrogen bonds (see Figure 3.7). In living systems, two environmental factors can change protein structure and thereby enzyme activity.

pH We introduced the concept of acids and bases when we discussed amino acids. Some amino acids have side chains that are acidic or basic (see Table 3.2). That is, they either generate H^+ and become anions, or attract H^+ and become cations. These reactions are often reversible. For example:

$$\text{Glutamic acid—COOH} \rightleftharpoons \text{glutamic acid—COO}^- + H^+$$

The ionic form of this amino acid (right) is far more hydrophilic than the nonionized form (left).

From your studies of chemistry, you may recall the law of mass action or Le Chatelier's principle. In this case the law implies that the higher the H^+ concentration in the solution, the more the reaction will be driven to the left (forming more of the nonionized form of glutamic acid). Therefore changes in the H^+ concentration can alter how hydrophobic some regions of a protein are and thus affect its shape. To generalize, protein tertiary structure, and therefore enzyme activity, is very sensitive to the concentration of H^+ in the aqueous environment. You may also recall that H^+ concentration is measured by pH (the negative logarithm of the H^+ concentration).

Although the water inside cells is generally at a neutral pH of 7, this can change, and different biological environments have different pH values. Each enzyme has a tertiary structure and amino acid sequence that make it optimally active at a particular pH. Its activity decreases as the solution is made more acidic or more basic than this ideal (optimal) pH (**FIGURE 3.22A**). As an example, consider the human digestive system (see Concept 30.4). The pH inside the human stomach is highly acidic, about pH 1.5. Pepsin, an enzyme that is active in the stomach, has a pH optimum near 2. Many enzymes that hydrolyze macromolecules in the intestine, such as proteases, have pH optima in the neutral range. So when food enters the small intestine, a buffer (bicarbonate) is secreted into the intestine to raise the pH to 6.5. This allows the hydrolytic enzymes to be active and digest the food.

TEMPERATURE In general, warming increases the rate of a chemical reaction because a greater proportion of the reactant molecules have enough kinetic energy to provide the activation energy for the reaction. Enzyme-catalyzed reactions are no different (**FIGURE 3.22B**). However, temperatures that are

APPLY THE CONCEPT

Regulation of metabolism occurs by regulation of enzymes

The concept of enzymes as biological catalysts has many applications. In a pile of clothes in your garage, you notice there are bacteria growing on some socks made of this synthetic polymer:

$$[\text{—CO—(CH}_2)_4\text{—CO—NH—(CH}_2)_4\text{—NH—}]_n$$

You make a protein extract from the bacteria and isolate what you think is an enzyme that can cleave the monomers from the polymer. You also synthesize the dipeptide glycine-glycine (see Table 3.2) to test as a possible inhibitor of the enzyme. The table shows the results from several of your experiments.

EXPERIMENT	CONDITION	RATE OF POLYMER CLEAVAGE
1	No enzyme	0.505
2	Enzyme	825.0
3	Enzyme pre-boiled at 100°C	0.520
4	Enzyme + dipeptide	0.495
5	Enzyme + RNA	799.0

1. Explain the results of each experiment.
2. How might the dipeptide work? How would you test your hypothesis?

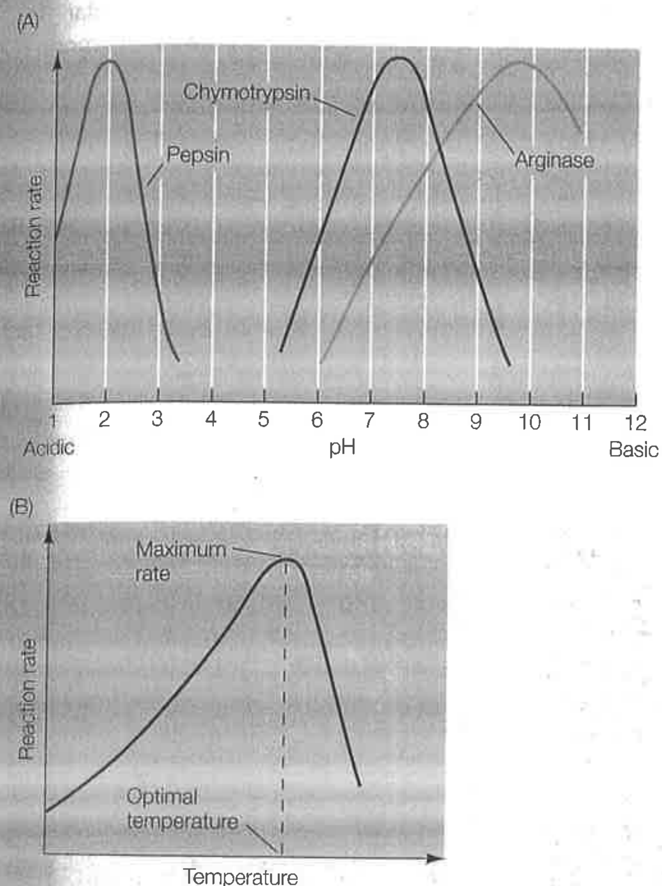

FIGURE 3.22 Enzyme Activity Is Affected by the Environment (A) The activity curve for each enzyme peaks at its optimal pH. For example, pepsin is active in the acidic environment of the stomach, whereas chymotrypsin is active in the neutral environment of the small intestine, and arginase is active in a basic environment. (B) Similarly, there is an optimal temperature for each enzyme. At higher temperatures the enzyme becomes denatured and inactive; this explains why the activity curve falls off abruptly at temperatures that are above optimal.

too high inactivate enzymes, because at high temperatures the polypetides vibrate and twist so rapidly that some of their noncovalent bonds break. When an enzyme's tertiary structure is changed by heat, the enzyme can no longer function. Some enzymes denature at temperatures only slightly above that of the human body, but a few are stable even at the boiling point (or freezing point) of water. All enzymes have an optimal temperature for activity.

Individual organisms adapt to changes in the environment in many ways, one of which is based on groups of enzymes called isozymes, which catalyze the same reaction but have different chemical compositions and physical properties. Different isozymes within a given group may have different optimal temperatures. The rainbow trout, for example, has several isozymes of the enzyme acetylcholinesterase. If a rainbow trout is transferred from warm water to near-freezing water (2°C), the fish produces a different isozyme of acetylcholinesterase. The new isozyme has a lower optimal temperature, allowing the fish's nervous system to perform normally in the colder water.

In general, enzymes adapted to warm temperatures do not denature at those temperatures because their tertiary structures are held together largely by covalent bonds such as disulfide bridges, instead of the more heat-sensitive weak chemical interactions.

CHECKpoint CONCEPT 3.4

- ✓ Explain and give examples of irreversible and reversible enzyme inhibitors.
- ✓ The amino acid glutamic acid (see Table 3.2) is at the active site of an enzyme. Normally the enzyme is active at pH 7. At pH 4 (higher concentration of H^+), the enzyme is inactive. Explain these observations.
- ✓ An enzyme is subject to allosteric regulation. How would you design an inhibitor of the enzyme that was competitive? Noncompetitive? Irreversible?
- ✓ Some organisms thrive at pH 2; other organisms thrive at a temperature of 65°C. Yet mammals cannot tolerate either environment in their tissues. Explain.

Q How does an understanding of proteins and enzymes help explain how aspirin works?

ANSWER The mechanism by which aspirin works exemplifies many of the concepts introduced in this chapter. Robert Vane showed that aspirin binds to a protein with a specific three-dimensional structure (Concept 3.2). This protein is cyclooxygenase, an enzyme (Concept 3.3) that catalyzes the commitment step in a metabolic pathway (Concept 3.4). Aspirin acts as an irreversible inhibitor of cyclooxygenase (Concept 3.4). Follow the description below carefully, as it illustrates these important concepts.

Cyclooxygenase catalyzes the conversion of a fatty acid with 20 carbon atoms, arachidonic acid, to a structure with a ring (thus the "cyclo" in the name of the enzyme). O_2 is a substrate (thus the "oxygen"; **FIGURE 3.23**). The product of

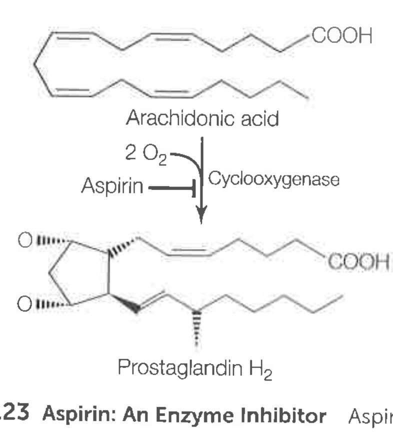

FIGURE 3.23 Aspirin: An Enzyme Inhibitor Aspirin inhibits a key enzyme in the metabolic pathways leading to inflammation and blood clotting.

this reaction (prostaglandin H_2) is the starting material for biochemical pathways that produce two types of molecules:

- prostaglandins, which are involved in inflammation and pain, and
- thromboxanes, which stimulate blood clotting and constriction of blood vessels.

Aspirin binds and reacts with a serine residue within the active site of cyclooxygenase. As a result of this binding, an acetyl group is transferred to the exposed hydroxyl group of the serine residue (**FIGURE 3.24**):

$$\text{(Cyclooxygenase)–serine–OH} \longrightarrow \text{(cyclooxygenase)–serine–O–}CH_2\text{–}CH_3$$

This covalent modification changes the exposed, polar serine to a less polar molecule, and it becomes slightly more hydrophobic. The conformation of the active site changes and becomes inaccessible to the substrate, arachidonic acid. The enzyme is inhibited, and the pathways leading to prostaglandins and thromboxanes are shut down. Less pain, inflammation, and blood clotting are the result. Small wonder that aspirin is taken as a pain reliever and a preventive medicine for heart attacks and strokes. It has come a long way from Edward Stone's walk in the woods

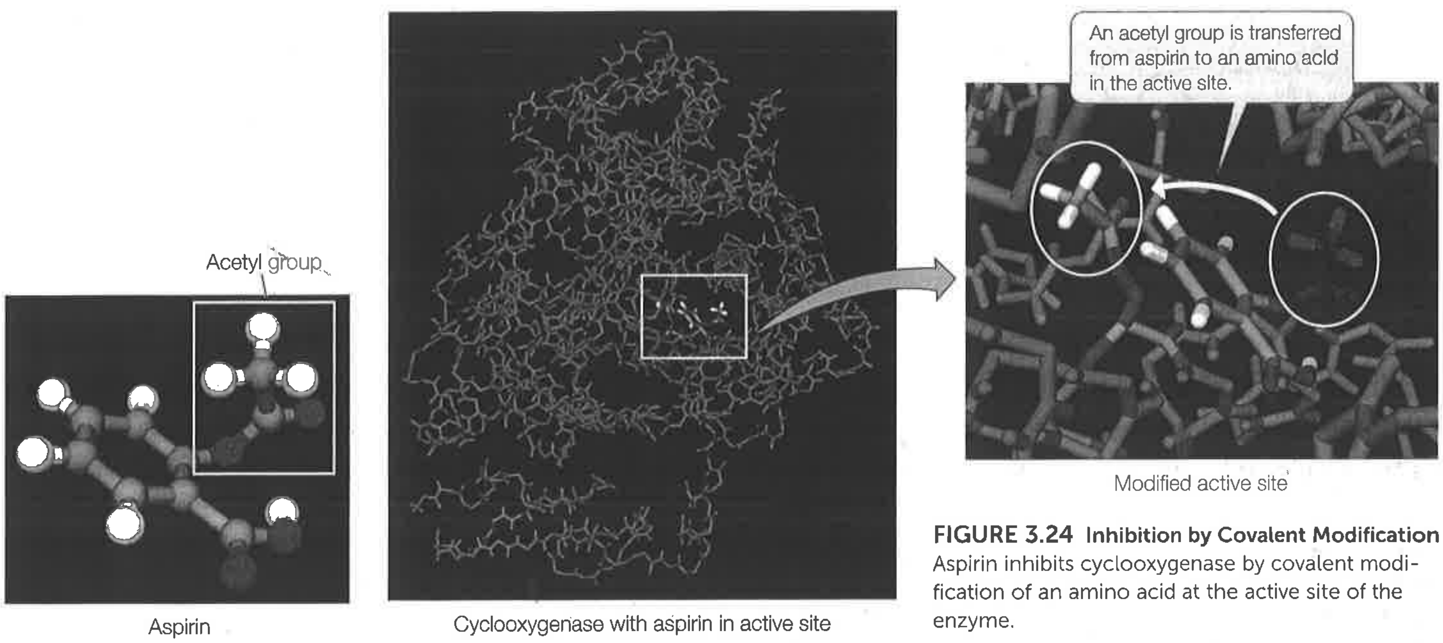

FIGURE 3.24 Inhibition by Covalent Modification Aspirin inhibits cyclooxygenase by covalent modification of an amino acid at the active site of the enzyme.