Enzymes as Catalysts

Enzymes are proteins that act as catalysts, compounds that increase the rate of chemical reactions (Fig. 8.1). Enzyme catalysts bind reactants (substrates), convert them to products, and release the products. Although enzymes may be modified during their participation in this reaction sequence, they return to their original form at the end. In addition to increasing the speed of reactions, enzymes provide a means for regulating the rate of metabolic pathways in the body. This chapter describes the properties of enzymes that allow them to function as catalysts. The next chapter explains the mechanisms of enzyme regulation.

**Enzyme binding sites.** An enzyme binds the substrates of the reaction and converts them to products. The substrates are bound to specific substrate binding sites on the enzyme through interactions with the amino acid residues of the enzyme. The spatial geometry required for all the interactions between the substrate and the enzyme makes each enzyme selective for its substrates and ensures that only specific products are formed.

**Active catalytic site.** The substrate binding sites overlap in the active catalytic site of the enzyme, the region of the enzyme where the reaction occurs. Within the catalytic site, functional groups provided by coenzymes, tightly bound metals, and, of course, amino acid residues of the enzyme, participate in catalysis.

**Activation energy and the transition state.** The functional groups in the catalytic site of the enzyme activate the substrates and decrease the energy needed to form the high-energy intermediate stage of the reaction known as the transition state complex. Some of the catalytic strategies employed by enzymes, such as general acid-base catalysis, formation of covalent intermediates, and stabilization of the transition state, are illustrated by chymotrypsin.

**pH and temperature profiles.** Enzymes have a functional pH range determined by the pKₐs of functional groups in the active site and the interactions required for three-dimensional structure. Non-denaturing increases of temperature increase the reaction rate.

**Mechanism-based inhibitors.** The effectiveness of many drugs and toxins depends on their ability to inhibit an enzyme. The strongest inhibitors are covalent inhibitors, compounds that form covalent bonds with a reactive group in the enzyme active site, or transition state analogues that mimic the transition state complex.

**Enzyme names.** Most enzyme names end in “ase.” Enzymes usually have both a common name and a systematic classification that includes a name and an Enzyme Commission (EC) number.

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Fig. 8.1. Catalytic power of enzymes. Many enzymes increase the rate of a chemical reaction by a factor of $10^{11}$ or higher. To appreciate an increase in reaction rate by this order of magnitude, consider a room-sized box of golf balls that “react” by releasing energy and turning blue. The $12$ ft $\times 12$ ft $\times 8$ ft box contains 380,000 golf balls. If the rate of the reaction in the absence of enzyme were 100 golf balls per year, the presence of 1 molecule of enzyme would turn the entire box of golf balls blue in 1 second (assuming a $10^{11}$ increase in reaction rate).
THE WAITING ROOM

A year after recovering from salicylate poisoning (see Chapter 4), Dennis “the Menace” Veere was playing in his grandfather’s basement. Dennis drank an unknown amount of the insecticide malathion, which is sometimes used for killing fruit flies and other insects (Fig. 8.2). Sometime later when he was not feeling well, Dennis told his grandfather what he had done. Mr. Veere retrieved the bottle and rushed Dennis to the emergency room of the local hospital. On the way, Dennis vomited repeatedly and complained of abdominal cramps. At the hospital, he began salivating and had an uncontrollable defecation.

In the emergency room, physicians passed a nasogastric tube for stomach lavage, started intravenous fluids, and recorded vital signs. Dennis’s pulse rate was 48 beats per minute (slow), and his blood pressure was 78/48 mm Hg (low). The physicians noted involuntary twitching of the muscles in his extremities.

Lotta Topaigne was diagnosed with acute gouty arthritis involving her right great toe (see Chapter 5). The presence of insoluble urate crystals within the joint space confirmed the diagnosis. Several weeks after her acute gout attack subsided, Ms. Topaigne was started on allopurinol therapy in an oral dose of 150 mg twice per day.

Al Martini, a 44-year-old man who has been an alcoholic for the past 5 years, had a markedly diminished appetite for food. One weekend he became unusually irritable and confused after drinking two fifths of scotch and eating very little. His landlady convinced him to visit his doctor. Physical examination indicated a heart rate of 104 beats/min. His blood pressure was slightly low, and he was in early congestive heart failure. He was poorly oriented to time, place, and person.

I. THE ENZYME-CATALYZED REACTION

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

1. binding of substrate: \( E + S \leftrightarrow ES \)
2. conversion of bound substrate to bound product: \( ES \leftrightarrow EP \)
3. release of product: \( EP \leftrightarrow E + P \)

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

Enzymes do not invent new reactions; they simply make reactions occur faster. The catalytic power of an enzyme (the rate of the catalyzed reaction divided by the rate of the uncatalyzed reaction) is usually in the range of \( 10^6 \) to \( 10^{14} \). Without the catalytic power of enzymes, reactions such as those involved in nerve conduction, heart contraction, and digestion of food would occur too slowly for life to exist.

Each enzyme usually catalyzes a specific biochemical reaction. The ability of an enzyme to select just one substrate and distinguish this substrate from a group of very similar compounds is referred to as specificity (Fig. 8.3). The enzyme converts...
this substrate to just one product. The specificity, as well as the speed, of enzyme-catalyzed reactions result from the unique sequence of specific amino acids that form the three-dimensional structure of the enzyme.

**A. The Active Site**

To catalyze a chemical reaction, the enzyme forms an enzyme–substrate complex in its active catalytic site (Fig. 8.4). The active site is usually a cleft or crevice in the enzyme formed by one or more regions of the polypeptide chain. Within the active site, cofactors and functional groups from the polypeptide chain participate in transforming the bound substrate molecules into products.

Initially, the substrate molecules bind to their substrate binding sites, also called the substrate recognition sites (see Fig. 8.4B). The three-dimensional arrangement of binding sites in a crevice of the enzyme allows the reacting portions of the substrates to approach each other from the appropriate angles. The proximity of the bound substrate molecules and their precise orientation toward each other contribute to the catalytic power of the enzyme.

The active site also contains functional groups that directly participate in the reaction (see Fig. 8.4C). The functional groups are donated by the polypeptide chain, or by bound cofactors (metals or complex organic molecules called coenzymes). As the substrate binds, it induces conformational changes in the enzyme that promote further interactions between the substrate molecules and the enzyme functional groups. (For example, a coenzyme might form a covalent intermediate with the substrate, or an amino acid side chain might abstract a proton from the reacting substrate.) The activated substrates and the enzyme form a transition state complex, an unstable high-energy complex with a strained electronic configuration that is intermediate between substrate and product. Additional bonds with the enzyme stabilize the transition state complex and decrease the energy required for its formation.

**Fig. 8.3.** Reaction catalyzed by glucokinase, an example of enzyme reaction specificity. Glucokinase catalyzes the transfer of a phosphate from ATP to carbon 6 of glucose. It cannot rapidly transfer a phosphate from other nucleotides to glucose, or from ATP to closely related sugars such as galactose, or from ATP to any other carbon on glucose. The only products formed are glucose 6-phosphate and ADP.

**Fig. 8.4.** Reaction in the enzyme active catalytic site. A. The enzyme contains an active catalytic site, shown in dark blue, with a region or domain where the substrate binds. The active site also may contain cofactors, nonprotein components that assist in catalysis. B. The substrate forms bonds with amino acid residues in the substrate binding site, shown in light blue. Substrate binding induces a conformational change in the active site. C. Functional groups of amino acid residues and cofactors in the active site participate in forming the transition state complex, which is stabilized by additional noncovalent bonds with the enzyme, shown in blue. D. As the products of the reaction dissociate, the enzyme returns to its original conformation.
The transition state complex decomposes to products, which dissociate from the enzyme (see Fig. 8.4D). The enzyme generally returns to its original form. The free enzyme then binds another set of substrates, and repeats the process.

B. Substrate Binding Sites

Enzyme specificity (the enzyme’s ability to react with just one substrate) results from the three-dimensional arrangement of specific amino acid residues in the enzyme that form binding sites for the substrates and activate the substrates during the course of the reaction. The “lock-and-key” and the “induced-fit” models for substrate binding describe two aspects of the binding interaction between the enzyme and substrate.

1. LOCK-AND-KEY MODEL FOR SUBSTRATE BINDING

The substrate binding site contains amino acid residues arranged in a complementary three-dimensional surface that “recognizes” the substrate and binds it through multiple hydrophobic interactions, electrostatic interactions, or hydrogen bonds (Fig. 8.5). The amino acid residues that bind the substrate can come from very different parts of the linear amino acid sequence of the enzyme, as seen in glucokinase. The binding of compounds with a structure that differs from the substrate even to a small degree may be prevented by steric hindrance and charge-repulsion. In the lock-and-key model, the complementarity between the substrate and its binding site is compared to that of a key fitting into a rigid lock.

2. “INDUCED FIT” MODEL FOR SUBSTRATE BINDING

Complementarity between the substrate and the binding site is only part of the picture. As the substrate binds, enzymes undergo a conformational change (“induced fit”) that repositions the side chains of the amino acids in the active site and increases the number of binding interactions (see Fig. 8.4). The induced fit model for substrate bind-

[Fig. 8.5. Glucose binding site in glucokinase. A. Glucose, shown in blue, is held in its binding site by multiple hydrogen bonds between each hydroxyl group and polar amino acids from different regions of the enzyme amino acid sequence in the actin fold (see Chapter 7). The position of the amino acid residue in the linear sequence is given by its number. The multiple interactions enable glucose to induce large conformational changes in the enzyme. (Modified from Pilkis SJ, Weber IT, Harisson RW, Bell GI. J Biol Chem. Glucokinase : structural analysis of a protein involved in susceptibility to diabetes 1994;21925–21928.) B. Enzyme specificity is illustrated by the comparison of galactose and glucose. Galactose differs from glucose only in the position of the -OH group shown in blue. However, it is not phosphorylated at a significant rate by the enzyme. Cells therefore require a separate galactokinase for the metabolism of galactose.]
ing recognizes that the substrate binding site is not a rigid “lock” but rather a dynamic surface created by the flexible overall three-dimensional structure of the enzyme.

The function of the conformational change induced by substrate binding, the induced fit, is usually to reposition functional groups in the active site in a way that promotes the reaction, improves the binding site of a cosubstrate, or activates an adjacent subunit through cooperativity. For example, consider the large conformational changes that occur in the actin fold of glucokinase when glucose binds. The induced fit involves changes in the conformation of the whole enzyme that close the cleft of the fold, thereby improving the binding site for ATP, and excluding water (which might interfere with the reaction) from the active site (Fig. 8.6). Thus, the multiple interactions between the substrate and the enzyme in the catalytic site serve both for substrate recognition and for initiating the next stage of the reaction, formation of the transition state complex.

C. The Transition State Complex

For a reaction to occur, the substrates undergoing the reaction need to be activated. If the energy levels of a substrate are plotted as the substrate is progressively converted to product, the curve will show a maximum energy level that is higher than that of either the substrate or the product (Fig. 8.7). This high energy level occurs at the

![Fig. 8.6. Conformational change resulting from the binding of glucose to hexokinase. (The figure is actually yeast hexokinase, which is more similar to human glucokinase than it is to the other human hexokinase isoforms). The shaded and unshaded areas show the two domains (four subdomains) that form the actin fold with its ATP-binding cleft. A. Free enzyme. B. With glucose bound, the cleft closes, forming the ATP binding site. The closure of the cleft when glucose binds to hexokinase (or human glucokinase) is one of the largest “induced fits” known. The combination of secondary structures in the actin fold that give hexokinase the flexibility required for this shift are discussed in Chapter 7, section IV.B.1., “The Actin Fold.” (From Bennett WS, Steitz TA. J Mol Biol. Structure of a complex between yeast hexokinase A and glucose II. Detailed comparisons of conformation and active site configuration with the native hexokinase B. 1980;211–230.)](image)
Because the transition state complex binds more tightly to the enzyme than does the substrate, compounds that resemble its electronic and three-dimensional surface (transition state analogs) are more potent inhibitors of an enzyme than are substrate analogs. Consequently, a drug developed as a transition state analog would be highly specific for the enzyme it is designed to inhibit. However, transition state analogs are highly unstable when not bound to the enzyme, and would have great difficulty making it from the digestive tract or injection site to the site of action. Some of the approaches in drug design that are being used to deal with the instability problem include: designing drugs that are almost transition state analogs but have a stable modification; designing a pro-drug that is converted to a transition state analog at the site of action; using the transition state analog to design a complementary antibody.

Abzymes (catalytic antibodies) are made as antibodies against analogs of the transition state complex. They thus have an arrangement of amino acid side chains in their variable regions that is similar to the active site of the enzyme in the transition state. Consequently, they can act as artificial enzymes. For example, abzymes have been developed against analogs of the transition state complex of cocaine esterase, the enzyme that degrades cocaine in the body. These abzymes have esterase activity, and monthly injections of the abzyme drug can be used to rapidly destroy cocaine in the blood, thereby decreasing the dependence of addicted individuals. (See Chapter 7 for antibody structure.)

Hydrolysis is the use of water to lyse (break) a bond. Proteolysis is the hydrolysis of a peptide bond in a protein, a reaction catalyzed by enzymes called proteases.

II. CATALYTIC MECHANISM OF CHYMOTRYPSIN

The enzyme chymotrypsin provides a good example of the strategies and amino acid side chains used by enzymes to lower the amount of activation energy required. Chymotrypsin is a digestive enzyme released into the intestine that catalyzes the hydrolysis of specific peptide bonds in denatured proteins. It is a member of the serine protease superfamily, enzymes that use a serine in the active site to form a covalent intermediate during proteolysis. In the overall hydrolysis reaction, an OH\(^{-}\) from water is added to the carbonyl carbon of the peptide bond, and an H\(^{+}\) to the N, thereby cleaving the bond (Fig. 8.8). The bond that is cleaved is called the scissile bond.

A. The Reaction in the Absence of Enzyme

In the reaction carried out in the absence of enzyme, the negatively charged hydroxyl group of water attacks the carbonyl carbon, which carries a partial positive charge. An unstable oxanion tetrahedral transition state complex is formed in which the oxygen atom carries a full negative charge. The rate of the chemical reaction in the absence of chymotrypsin is slow because there are too few OH\(^{-}\)
molecules in H₂O with enough energy to form the transition state complex and too few OH⁻ molecules colliding with the substrate at the right orientation.

B. Catalytic Strategies in the Reaction Catalyzed by Chymotrypsin

In the reaction catalyzed by chymotrypsin, the same oxyanion intermediate is formed by using the hydroxyl group of a serine residue for the attack instead of a free hydroxyl anion. The rate of the chymotrypsin-catalyzed reaction is faster because functional groups in the enzyme active site activate the attacking hydroxyl group, stabilize the oxianion transition state complexes, form a covalent intermediate, and destabilize the leaving group. The reaction takes place in two stages: (a) cleavage of the peptide bond in the denatured substrate protein and formation of a covalent acyl-enzyme intermediate (Fig. 8.9, steps 1–5), and (b) hydrolysis of the acyl-enzyme intermediate to release the remaining portion of the substrate protein (Fig. 8.9, steps 6–9). The names of the catalytic strategies employed in the various steps are in italics in the following paragraphs.

1. Specificity of Binding to Chymotrypsin

Chymotrypsin hydrolyzes the peptide bond on the carbonyl side of a phenylalanine, tyrosine, or tryptophan in a denatured protein. The substrate recognition site consists of a hydrophobic binding pocket that holds the hydrophobic amino acid contributing the carbonyl group of the scissile bond (see Fig. 8.9, Step 1). The substrate protein must be denatured to fit into the pocket and be held rigidly in place by glycines in the enzyme peptide backbone. Scissile bond specificity is also provided by the subsequent steps of the reaction, such as moving serine 195 into attacking position (proximity and orientation).

2. Formation of the Acyl-Enzyme Intermediate in Chymotrypsin

In the first stage of the reaction, the peptide bond of the denatured protein substrate is cleaved as an active site serine hydroxyl group attacks the carbonyl carbon of the scissile bond (nucleophilic catalysis—a nucleophile is a chemical group that is attracted to the positively charged nucleus) (Fig. 8.9, Step 2). Aspartate and histidine cooperate in converting this hydroxyl group (with a partial negative charge on the oxygen) into a better nucleophilic attacking group by giving it a more negative charge. An active site histidine acts as a base and abstracts a proton from the serine hydroxyl (acid-base catalysis). The protonated histidine is stabilized by the negative charge of a nearby aspartate.

The aspartate-histidine-serine combination, referred to as the catalytic triad, is an example of cooperative interactions between amino acid residues in the active site. The strong nucleophilic attacking group created by this charge-relay system has the same general effect on reaction rate as increasing the concentration of hydroxyl ions available for collision in the uncatalyzed reaction.

In the next step of the reaction sequence, an oxyanion tetrahedral transition state complex is formed that is stabilized by hydrogen bonds with –NH groups in the peptide backbone (Fig. 8.9, Step 3). The original view of the way enzymes form transition state complexes was that they stretched the bonds or distorted the bond angles of the reacting substrates. However, most transition state complexes, such as the oxyanion tetrahedral complex, are better described as showing “electronic strain,” an electrostatic surface that would be highly improbable if it were not stabilized by bonds with functional groups on the enzyme. Stabilization of the transition state complex lowers its energy level and increases the number of molecules that reach this energy level.

\[ \text{Acyl-enzyme intermediate} \rightarrow \text{Transition state complex} \rightarrow \text{Hydrolysis} \]

Q: In the stomach, gastric acid decreases the pH to 1 to 2 to denature proteins through disruption of hydrogen bonding. The protease in the stomach, pepsin, is a member of the aspartate protease superfamily, enzymes that use two aspartate residues in the active site for acid-base catalysis of the peptide bond. Why can they not use histidine like chymotrypsin?

Serine proteases in blood coagulation. The use of an active site serine to cleave a peptide bond is common in the proteolytic enzymes of blood coagulation as well as those of digestion. Blood clots are formed from fibrin, a protein present in the blood as the inactive precursor, fibrinogen. The serine protease thrombin cleaves a peptide bond in fibrinogen to form active fibrin. Thrombin has the same aspartate-histidine-serine catalytic triad found in chymotrypsin and works in essentially the same way. Thrombin is also present as an inactive precursor, prothrombin, which is itself activated through proteolytic cleavage by another blood coagulation serine protease.
1. Substrate binding

2. Histidine activates serine for nucleophilic attack

3. The oxyanion tetrahedral intermediate is stabilized by hydrogen bonds

4. Cleavage of the peptide bond

5. The covalent acyl–enzyme intermediate

6. Water attacks the carbonyl carbon

7. Second oxyanion tetrahedral intermediate

8. Acid catalysis breaks the acyl–enzyme covalent bond

9. The product is free to dissociate
Subsequently, the serine in the active site forms a full covalent bond with the carbon of the carbonyl group as the peptide bond is cleaved (covalent catalysis). The formation of a stable covalent intermediate is a catalytic strategy employed by many enzymes and often involves serine or cysteine residues. The covalent intermediate is subsequently hydrolyzed (acid-base catalysis). The dissociating products of an enzyme-catalyzed reaction are often “destabilized” by some degree of charge repulsion in the active site. In the case of chymotrypsin, the amino group formed after peptide bond cleavage is destabilized or “uncomfortable” in the presence of the active site histidine (destabilization of developing product).

3. HYDROLYSIS OF THE ACRYL-CHYMOTRYPSIN INTERMEDIATE

The next sequence of events hydrolyzes the acyl-enzyme intermediate to release the bound carbonyl-side peptide (Fig. 8.9, Steps 6-9). The active site histidine activates water to form an OH− for a nucleophilic attack, resulting in a second oxyanion transition state complex. When the histidine adds the proton back to serine, the reaction is complete, and the product dissociates.

C. Energy Diagram in the Presence of Chymotrypsin

The number of steps in real enzymatic reactions results in a multi-bump energy diagram (Fig. 8.10). At the initial stage of the reaction, a dip occurs because energy is provided by formation of the initial multiple weak bonds between the substrate and enzyme. As the reaction progresses, the curve rises because additional energy is required for formation of the transition state complex. This energy is provided by the subsequent steps in the reaction replacing the initial weak bonds with progressively tighter bonds. Semi-stable covalent intermediates of the reaction have lower energy levels than do the transition state complexes, and are present in the reaction diagram as dips in the energy curve. The final transition state complex has the highest energy level in the reaction and is therefore the most unstable state. It can collapse back to substrates or decompose to form products.

III. FUNCTIONAL GROUPS IN CATALYSIS

The catalytic strategies employed by chymotrypsin to increase the reaction rate are common to many enzymes. One of these catalytic strategies, proximity and orientation, is an intrinsic feature of substrate binding and part of the catalytic mechanism of all enzymes. All enzymes also stabilize the transition state by electrostatic interactions, but not all enzymes form covalent intermediates.

Great variety occurs in the functional groups employed by different enzymes to carry out these catalytic strategies. Some enzymes, such as chymotrypsin, rely on

Fig. 8.9. Catalytic mechanism of chymotrypsin. The substrate (a denatured protein) is in the shaded area. 1. As the substrate protein binds to the active site, serine-195 and a histidine (his57) are moved closer together and at the right orientation for the nitrogen electrons on histidine to attract the hydrogen of serine. Without this change of conformation on substrate binding, the catalytic triad cannot form. 2. Histidine serves as a general base catalyst as it abstracts a proton from the serine, increasing the nucleophilicity of the serine-oxygen, which attacks the carbonyl carbon. 3. The electrons of the carbonyl group form the oxyanion tetrahedral intermediate. The oxyanion is stabilized by the N-H groups of serine-195 and glycine in the chymotrypsin peptide backbone. 4. The amide nitrogen in the peptide bond is stabilized by interaction with the histidine proton. Here the histidine acts as a general acid catalyst. As the electrons of the carbon-nitrogen peptide bond withdraw into the nitrogen, the electrons of the carboxyanion return to the substrate carbonyl carbon, resulting in cleavage of the peptide bond. 5. The cleavage of the peptide bond results in formation of the covalent acyl-enzyme intermediate, and the amide half of the cleaved protein dissociates. 6. The nucleophilic attack by H2O on the carbonyl carbon is activated by histidine, whose nitrogen electrons attract a proton from water. 7. The second tetrahedral oxyanion intermediate (the transition state complex) is formed. It is again stabilized by hydrogen bonds with the peptide backbone bonds of glycine and serine. 8. As the histidine proton is donated to the electrons of the bond between the serine oxygen and the substrate carbonyl group, the electrons from the oxyanion return to the substrate carbon to form the carboxylic acid, and the acyl-enzyme bond is broken. 9. The enzyme, as it releases substrate, returns to its original state.
Because most vitamins function as coenzymes, the symptoms of vitamin deficiencies reflect the loss of specific enzyme activities dependent on the coenzyme form of the vitamin. Thus, drugs and toxins that inhibit proteins required for coenzyme synthesis (e.g., vitamin transport proteins or biosynthetic enzymes) can cause the symptoms of a vitamin deficiency. This type of deficiency is called a functional deficiency, whereas an inadequate intake is called a dietary deficiency.

Most coenzymes are tightly bound to their enzymes and do not dissociate during the course of the reaction. However, a functional or dietary vitamin deficiency that decreases the level of a coenzyme will result in the presence of the apoenzyme in cells (an enzyme devoid of cofactor).

Ethanol is an “antivitamin” that decreases the cellular content of almost every coenzyme. For example, ethanol inhibits the absorption of thiamine, and acetaldehyde produced from ethanol oxidation displaces pyridoxal phosphate from its protein binding sites, thereby accelerating its degradation.

A. Functional Groups on Amino Acid Side Chains

Almost all of the polar amino acids participate directly in catalysis in one or more enzymes (Table 8.1). Serine, cysteine, lysine, and histidine can participate in covalent

Table 8.1. Some Functional Groups in the Active Site

<table>
<thead>
<tr>
<th>Function of Amino Acid</th>
<th>Enzyme Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>CovaIent intermediates</td>
<td>Glyceraldehyde 3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>Cysteine–SH</td>
<td>Acetlycholinesterase</td>
</tr>
<tr>
<td>Serine–OH</td>
<td>Aldolase</td>
</tr>
<tr>
<td>Lysine–NH$_2$</td>
<td>Phosphoglucomutase</td>
</tr>
<tr>
<td>Histidine–NH</td>
<td></td>
</tr>
<tr>
<td>Acid–base catalysis</td>
<td>Chymotrypsin</td>
</tr>
<tr>
<td>Histidine–NH</td>
<td>Pepsin</td>
</tr>
<tr>
<td>Aspartate–COOH</td>
<td>Lysozyme</td>
</tr>
<tr>
<td>Stabilization of anion formed during the reaction</td>
<td></td>
</tr>
<tr>
<td>Peptide backbone–NH</td>
<td>Chymotrypsin</td>
</tr>
<tr>
<td>Arginine–NH</td>
<td>Carboxypeptidase A</td>
</tr>
<tr>
<td>Serine–OH</td>
<td>Alcohol dehydrogenase</td>
</tr>
<tr>
<td>Stabilization of cation formed during the reaction</td>
<td></td>
</tr>
<tr>
<td>Aspartate–COO</td>
<td>Lysozyme</td>
</tr>
</tbody>
</table>
catalysis. Histidine, because it has a pK\textsubscript{a} that can donate and accept a proton at neutral pH, often participates in acid-base catalysis. Most of the polar amino acid side chains are nucleophilic and participate in nucleophilic catalysis by stabilizing more positively charged groups that develop during the reaction.

### B. Coenzymes in Catalysis

Coenzymes are complex nonprotein organic molecules that participate in catalysis by providing functional groups, much like the amino acid side chains. In the human, they are usually (but not always) synthesized from vitamins. Each coenzyme is involved in catalyzing a specific type of reaction for a class of substrates with certain structural features. Coenzymes can be divided into two general classes: activation-transfer coenzymes and oxidation-reduction coenzymes.

#### 1. ACTIVATION-TRANSFER COENZYMES

The activation-transfer coenzymes usually participate directly in catalysis by forming a covalent bond with a portion of the substrate; the tightly held substrate moiety is then activated for transfer, addition of water, or some other reaction. The portion of the coenzyme that forms a covalent bond with the substrate is its functional group. A separate portion of the coenzyme binds tightly to the enzyme.

Thiamine pyrophosphate provides a good illustration of the manner in which coenzymes participate in catalysis (Fig. 8.11). It is synthesized in human cells from the vitamin thiamine by the addition of a pyrophosphate. This pyrophosphate provides negatively charged oxygen atoms that chelate Mg\textsuperscript{2+}, which then binds tightly to the enzyme. The functional group that extends into the active site is the reactive carbon atom with a dissociable proton (see Fig. 8.11). In all of the enzymes that use thiamine pyrophosphate, this reactive thiamine carbon forms a covalent bond with a substrate keto group while cleaving the adjacent carbon–carbon bond. However, each thiamine-containing enzyme catalyzes the cleavage of a different substrate (or group of substrates with very closely related structures).

Coenzymes have very little activity in the absence of the enzyme and very little specificity. The enzyme provides specificity, proximity, and orientation in the substrate recognition site, as well as other functional groups for stabilization of the transition state, acid-base catalysis, etc. For example, thiamine is made into a better nucleophilic attacking group by a basic amino acid residue in the enzyme that removes the dissociable proton (EnzB: in Fig. 8.11), thereby generating a negatively charged thiamine carbon anion. Later in the reaction, the enzyme returns the proton.

Coenzyme A (CoA), biotin, and pyridoxal phosphate are also activation-transfer coenzymes synthesized from vitamins. CoA (CoASH), which is synthesized from the vitamin pantothenate, contains an adenosine 3′, 5′-bisphosphate which binds reversibly, but tightly, to a site on an enzyme (Fig. 8.12A). Its functional group, a sulfhydryl group at the other end of the molecule, is a nucleophile that always

Nucleophiles carry full or partial negative charges (like the oxygen atom in the serine -OH) or have a nitrogen that can act as an electron-donating group by virtue of its two unpaired electrons. Covalent catalysis and acid-base catalysis are carried out by nucleophilic groups. Electrophiles carry full or partial positive charges (e.g., the peptide backbone-NH was used as an electrophilic group in chymotrypsin). In general, nucleophilic and electrophilic catalysis occur when the respective nucleophilic or electrophilic groups on the enzyme stabilize substrate groups of the opposite polarity that develop during the reaction.

**Q:** Although coenzymes look as though they should be able to catalyze reactions autonomously (on their own), they have almost no catalytic power when not bound to the enzyme. Why?

Many alcoholics such as Al Martini develop thiamine deficiency because alcohol inhibits the transport of thiamine through the intestinal mucosal cells. In the body, thiamine is converted to thiamine pyrophosphate (TPP). TPP acts as a coenzyme in the decarboxylation of α-keto acids such as pyruvate and α-ketoglutarate (see Fig. 8.11) and in the utilization of pentose phosphates in the pentose phosphate pathway. As a result of thiamine deficiency, the oxidation of α-keto acids is impaired. Dysfunction occurs in the central and peripheral nervous system, the cardiovascular system, and other organs.

Most coenzymes, such as functional groups on the enzyme amino acids, are regenerated during the course of the reaction. However, CoASH and a few of the oxidation-reduction coenzymes are transformed during the reaction into products that dissociate from the enzyme at the end of the reaction (e.g., CoASH is converted to an acyl CoA derivative, and NAD\textsuperscript{+} is reduced to NADH). These dissociating coenzymes are nonetheless classified as coenzymes rather than substrates because they are common to so many reactions, the original form is regenerated by subsequent reactions in a metabolic pathway, they are synthesized from vitamins, and the amount of coenzyme in the cell is nearly constant.
For a substrate to react with a coenzyme, it must collide with a coenzyme at exactly the right angle. The probability of the substrate and coenzyme in free solution colliding in exactly the right place at the exactly right angle is very small. In addition to providing this proximity and orientation, enzymes contribute in other ways, such as activating the coenzyme by abstracting a proton (e.g., thiamine-pyrophosphate and coenzyme A) or polarizing the substrate to make it more susceptible to nucleophilic attack.

attacks carbonyl groups and forms acyl thioesters (in fact, the “A” in CoA stands for the acyl group that becomes attached).

Biotin, which does not contain a phosphate group, is covalently bound to a lysine in enzymes called carboxylases (see Fig. 8.12B). Its functional group is a nitrogen atom that covalently binds a CO₂ group in an energy-requiring reaction. This bound CO₂ group is activated for addition to another molecule. In the human, biotin functions only in carboxylation reactions.

Pyridoxal phosphate is synthesized from the vitamin pyridoxine, which is also called vitamin B₆ (Fig. 8.13). The reactive aldehyde group usually functions in enzyme-catalyzed reactions by forming a covalent bond with the amino groups on amino acids. The positively charged ring nitrogen withdraws electrons from a bond in the bound amino acid, resulting in cleavage of that bond. The enzyme participates by removing protons from the substrate and by keeping the amino acid and the pyridoxal group in a single plane to facilitate shuttling of electrons.

Fig. 8.11. The role of the functional group of thiamine pyrophosphate (the reactive carbon shown in blue) in formation of a covalent intermediate. A. A base on the enzyme (B) abstracts a proton from thiamine, creating a carbanion (general acid-base catalysis). B. The carbanion is a strong nucleophile and attacks the partially positively charged keto group on the substrate. C. A covalent intermediate is formed, which is stabilized by resonance forms. The uncharged intermediate is the stabilized transition state complex.
These coenzymes illustrate three features all activation-transfer coenzymes have in common: (1) a specific chemical group involved in binding to the enzyme, (2) a separate and different functional or reactive group that participates directly in the catalysis of one type of reaction by forming a covalent bond with the substrate, and (3) dependence on the enzyme for additional specificity of substrate and additional catalytic power.

2. OXIDATION-REDUCTION CCOENZYMES

A large number of coenzymes are involved in oxidation-reduction reactions catalyzed by enzymes categorized as oxidoreductases. Some coenzymes, such as
When a compound is oxidized, it loses electrons. As a result, the oxidized carbon has fewer H atoms or gains an O atom. The reduction of a compound is the gain of electrons, which shows in its structure as the gain of H, or loss of O. In the oxidation of lacatate to pyruvate (see Fig. 8.14), lactate loses two electrons as a hydride ion, and a proton (H\(^+\)) is released; NAD\(^+\), which accepts the hydride ion, is reduced to NADH. The carbon atom with the keto group is now at a higher oxidation state because both of the electrons in bonds between carbon and oxygen are counted as belonging to oxygen, whereas the two electrons in the C-H bond are shared equally between carbon and hydrogen.

The catalysis of oxidation-reduction reactions is carried out by a class of enzymes called oxidoreductases. A subclass of oxidoreductases is given the common name dehydrogenases (such as lactate dehydrogenase), because they transfer hydrogen (hydrogen atoms or hydride atoms) from the substrate to an electron-accepting coenzyme, such as NAD\(^+\).

nicotinamide adenine dinucleotide (NAD\(^+\)) and flavin adenine dinucleotide (FAD), can transfer electrons together with hydrogen and have unique roles in the generation of ATP from the oxidation of fuels. Other oxidation-reduction coenzymes work with metals to transfer single electrons to oxygen. Vitamin E and vitamin C (ascorbic acid) are oxidation-reduction coenzymes that can act as antioxidants and protect against oxygen free radical injury. The different functions of oxidation-reduction coenzymes in metabolic pathways are explained in Chapters 19 through 22.

Oxidation-reduction coenzymes follow the same principles as activation-transfer coenzymes, except that they do not form covalent bonds with the substrate. Each coenzyme has a unique functional group that accepts and donates electrons and is specific for the form of electrons it transfers (e.g., hydride ions, hydrogen atoms, oxygen). A different portion of the coenzyme binds the enzyme. Like activation-transfer coenzymes, oxidation-reduction coenzymes are not good catalysts without participation from amino acid side chains on the enzyme.

The enzyme lactate dehydrogenase, which catalyzes the transfer of electrons from lactate to NAD\(^+\), illustrates these principles (Fig. 8.14). The coenzyme nicotinamide adenine dinucleotide (NAD\(^+\)) is synthesized from the vitamin niacin (which forms the nicotinamide ring), and from ATP (which contributes an AMP). The ADP portion of the molecule binds tightly to the enzyme and causes conformational

![Fig. 8.14](image-url) The coenzyme NAD\(^+\) accepting a hydride ion, shown in blue, from lactate. NAD\(^+\)-dependent dehydrogenases catalyze the transfer of a hydride ion (H\(_2\)) from a carbon to NAD\(^+\) in oxidation reactions such as the oxidation of alcohols to ketones or aldehydes to acids. The positively charged pyridine ring nitrogen of NAD\(^+\) increases the electrophilicity of the carbon opposite it in the ring. This carbon then accepts the negatively charged hydride ion. The proton from the alcohol group is released into water. NADP functions by the same mechanism, but it is usually involved in pathways of reductive synthesis.
changes in the enzyme. The functional group of NAD\(^+\) is the carbon on the nicotinamide ring opposite the positively charged nitrogen. This carbon atom accepts the hydride ion (a hydrogen atom that has two electrons) transferred from a specific carbon atom on the substrate. The H\(^+\) from the substrate alcohol (OH) group then dissociates, and a keto group (C = O) is formed. One of the roles of the enzyme is to contribute a histidine nitrogen that can bind the dissociable proton on lactate, thereby making it easier for NAD\(^+\) to pull off the other hydrogen with both electrons. Finally, NADH dissociates.

**C. Metal Ions in Catalysis**

Metal ions, which have a positive charge, contribute to the catalytic process by acting as electrophiles (electron-attracting groups). They assist in binding of the substrate, or they stabilize developing anions in the reaction. They can also accept and donate electrons in oxidation-reduction reactions.

The ability of certain metals to bind multiple ligands in their coordination sphere enables them to participate in binding substrates or coenzymes to enzymes. For example, Mg\(^{2+}\) plays a role in the binding of the negatively charged phosphate groups of thiamine pyrophosphate to anionic or basic amino acids in the enzyme (see Fig. 8.11). The phosphate groups of ATP are usually bound to enzymes through Mg\(^{2+}\) chelation.

The metals of some enzymes bind anionic substrates or intermediates of the reaction to alter their charge distribution, thereby contributing to catalytic power. The enzyme alcohol dehydrogenase, which transfers electrons from ethanol to NAD\(^+\) to generate acetaldehyde and NADH, illustrates this role (Fig. 8.15). In the active site of alcohol dehydrogenase, an activated serine pulls a proton off the ethanol –OH group, leaving a negative charge on the oxygen that is stabilized by zinc. This electronic configuration allows the transfer of a hydride ion to NAD\(^+\). Zinc is essentially fulfilling the same function in alcohol dehydrogenase that histidine fulfills in lactate dehydrogenase.

**D. Noncatalytic Roles of Cofactors**

Cofactors sometimes play a noncatalytic structural role in certain enzymes, binding different regions of the enzyme together to form the tertiary structure. They also can serve as substrates that are cleaved during the reaction.

**IV. OPTIMAL pH AND TEMPERATURE**

If the activity of most enzymes is plotted as a function of the pH of the reaction, an increase of reaction rate is usually observed as the pH goes from a very acidic level to the physiologic range; a decrease of reaction rate occurs as the pH goes from the physiologic range to a very basic range (Fig. 8.16). The shape of this curve in the acid region usually reflects the ionization of specific functional groups in the active site (or in the substrate) by the increase of pH, and the more general formation of hydrogen bonds important for the overall conformation of the enzyme. The loss of activity on the basic side usually reflects the inappropriate ionization of amino acid residues in the enzyme.

In humans, most of ingested ethanol is oxidized to acetaldehyde in the liver by alcohol dehydrogenase (ADH):

\[
\text{Ethanol} + \text{NAD}^+ \leftrightarrow \text{Acetaldehyde} + \text{NADH} + \text{H}^+
\]

ADH is active as a dimer, with an active site containing zinc present in each subunit. The human has at least seven genes that encode isoforms of ADH, each with a slightly different range of specificities for the alcohols it oxidizes.

The acetaldehyde produced from ethanol is highly reactive, toxic, and immunogenic. In Al Martini and other patients with chronic alcoholism, acetaldehyde is responsible for much of the liver injury associated with chronic alcoholism.

**In the liver by alcohol dehydrogenase (ADH):**

The parietal cells of the stomach secrete HCl into the lumen of the stomach, resulting in a pH between 1 and 2. This strongly acidic environment is capable of irreversibly denaturing most proteins by protonating amino acids, thereby preventing the hydrogen bond formation necessary for tertiary structure. Many of the peptide bonds in proteins would not be accessible to digestive proteases unless the protein was denatured. Pepsin, a digestive protease present in the stomach, is an exceptional enzyme because its pH optimum is approximately 1.6 and it is active in the acidic environment of the stomach. As denatured dietary proteins pass into the intestinal lumen, the pH of the gastric juice is raised above 6 by secretion of bicarbonate from the exocrine pancreas. At this higher pH, chymotrypsin and other proteases from the pancreas can act on the denatured proteins.
Most human enzymes function optimally at a temperature of approximately 37°C. An increase of temperature from 0°C to 37°C increases the rate of the reaction by increasing the vibrational energy of the substrates. The maximum activity for most human enzymes occurs near 37°C because denaturation (loss of secondary and tertiary structure) occurs at higher temperatures.

V. MECHANISM-BASED INHIBITORS

Inhibitors are compounds that decrease the rate of an enzymatic reaction. Mechanism-based inhibitors mimic or participate in an intermediate step of the catalytic reaction. The term includes transition state analogs and compounds that can react irreversibly with functional groups in the active site.

A. Covalent Inhibitors

Covalent inhibitors form covalent or extremely tight bonds with functional groups in the active catalytic site. These functional groups are activated by their interactions with other amino acid residues, and are therefore far more likely to be targeted by drugs and toxins than amino acid residues outside the active site.

The lethal compound diisopropyl phosphofluoridate (DFP, or diisopropylfluorophosphate) is an organophosphorus compound that served as a prototype for the development of the nerve gas Sarin and other organophosphorus toxins, such as the insecticides malathion and parathion (Fig. 8.17). DFP exerts its toxic effect by forming a covalent intermediate in the active site of acetylcholinesterase, thereby preventing the enzyme from degrading the neurotransmitter acetylcholine. Once the covalent bond is formed, the inhibition by DFP is essentially irreversible, and activity can only be recovered as new enzyme is synthesized. DFP also inhibits many other enzymes that use serine for hydrolytic cleavage, but the inhibition is not as lethal.

Aspirin (acetylsalicylic acid) provides an example of a pharmacologic drug that exerts its effect through the covalent acetylation of an active site serine in the enzyme prostaglandin endoperoxide synthase (cyclooxygenase). Aspirin resembles a portion of the prostaglandin precursor that is a physiologic substrate for the enzyme. The symptoms experienced by Dennis Veere resulted from inhibition of acetylcholinesterase. Acetylcholinesterase cleaves the neurotransmitter acetylcholine to acetate and choline in the postsynaptic terminal, thereby terminating the transmission of the neural signal (see Fig. 8.17). Malathion is metabolized in the liver to a toxic derivative (malaoxon) that binds to the active site serine in acetylcholinesterase and other enzymes, an action similar to that of diisopropylfluorophosphate. As a result, acetylcholine accumulates and overstimulates the autonomic nervous system (the involuntary nervous system, including heart, blood vessels, glands), thereby accounting for Dennis’s vomiting, abdominal cramps, salivation, and sweating. Acetylcholine is also a neurotransmitter for the somatic motor nervous system, where its accumulation resulted in Dennis’s involuntary muscle twitching (muscle fasciculations).
B. Transition State Analogs and Compounds that Resemble Intermediate Stages of the Reaction

Transition state analogs are extremely potent and specific inhibitors of enzymes because they bind so much more tightly to the enzyme than do substrates or products. Drugs cannot be designed that precisely mimic the transition state because of its highly unstable structure. However, substrates undergo progressive changes in their overall electrostatic structure during the formation of a transition state complex, and effective drugs often resemble an intermediate stage of the reaction more closely than they resemble the substrate. Medical literature often refers to such compounds as substrate analogs, even though they bind more tightly than substrates.

1. PENICILLIN

The antibiotic penicillin is a transition state analog that binds very tightly to glycopeptidyl transferase, an enzyme required by bacteria for synthesis of the cell wall (Fig. 8.18). Glycopeptidyl transferase catalyzes a partial reaction with penicillin that covalently attaches penicillin to its own active site serine. The reaction is favored by the strong resemblance between the peptide bond in the β-lactam ring of penicillin and the transition state complex of the natural transpeptidation reaction. Active site inhibitors such as penicillin that undergo partial reaction to form irreversible inhibitors in the active site are sometimes termed “suicide inhibitors.”

2. ALLOPURINOL

Allopurinol, a drug used to treat gout, decreases urate production by inhibiting xanthine oxidase. This inhibition provides an example of an enzyme that commits suicide by converting a drug to a transition state analog. The normal physiologic
The function of xanthine oxidase is the oxidation of hypoxanthine to xanthine and xanthine to uric acid (urate) in the pathway for degradation of purines (Fig. 8.19). The enzyme contains a molybdenum–sulfide (Mo-S) complex that binds the substrates and transfers the electrons required for the oxidation reactions. Xanthine oxidase oxidizes the drug allopurinol to oxypurinol, a compound that binds very tightly to a molybdenum–sulfide complex in the active site. As a result, the enzyme has committed suicide and is unable to carry out its normal function, the generation of uric acid (urate).

D. Heavy Metals

Heavy metal toxicity is caused by tight binding of a metal such as mercury (Hg), lead (Pb), aluminum (Al), or iron (Fe), to a functional group in an enzyme. Heavy metals are relatively nonspecific for the enzymes they inhibit, particularly if the metal is associated with high dose toxicity. Mercury, for example, binds to so many enzymes, often at reactive sulphydryl groups in the active site, that it has been difficult to determine which of the inhibited enzymes is responsible for mercury toxicity. Lead provides an example of a metal that inhibits through replacing the normal functional metal in an enzyme. Its developmental and neurologic toxicity may be caused by its
Once ingested, the liver converts malathion to the toxic reactive compound, malaoxon, by replacing the sulfur with an oxygen. Malaoxon then binds to the active site of acetylcholinesterase and reacts to form the covalent intermediate. Unlike the complex formed between diisopropylfluorophosphate and acetylcholinesterase, this initial acylenzyme intermediate is reversible. However, with time, the enzyme-inhibitor complex “ages” (dealkylation of the inhibitor and enzyme modification) to form an irreversible complex.

ability to replace \( \text{Ca}^{2+} \) in two regulatory proteins important in the central nervous system and other tissues, \( \text{Ca}^{2+} \)-calmodulin and protein kinase C.

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**CLINICAL COMMENTS**

**Dennis Veere.** Dennis Veere survived his malathion intoxication because he had ingested only a small amount of the chemical, vomited shortly after the agent was ingested, and was rapidly treated in the emergency room. Lethal doses of oral malathion are estimated at 1 g/kg of body weight for humans. Emergency room physicians used a drug (oxime) to reactivate the acetylcholinesterase in Dennis before the aged complex formed. They also used intravenous atropine, an anticholinergic (antimuscarinic) agent, to antagonize the action of the excessive amounts of acetylcholine accumulating in cholinergic receptors throughout his body.

After several days of intravenous therapy, the signs and symptoms of acetylcholine excess abated, and therapy was slowly withdrawn. Dennis made an uneventful recovery.
At low concentrations of ethanol, liver alcohol dehydrogenase is the major route of ethanol oxidation to acetaldehyde, a highly toxic chemical. Acetaldehyde not only damages the liver, it can enter the blood and potentially damage the heart and other tissues. At low ethanol intakes, much of the acetaldehyde produced is safely oxidized to acetate in the liver by acetaldehyde dehydrogenases.

**Lotta Topaigne.** Within several days of starting allopurinol therapy, Ms. Topaigne’s serum uric acid level began to decrease. Several weeks later, the level in her blood was normal. However, while Lotta was adapting to allopurinol therapy, she experienced a mild gout attack, which was treated with a low dose of colchicine (see Chapter 10).

**Al Martini.** Al Martini was admitted to the hospital after intravenous thiamine was initiated at a dose of 100 mg/day (compared with an RDA of 1.4 mg/day). His congestive heart failure was believed to be the result, in part, of the cardiomyopathy (heart muscle dysfunction) of acute thiamine deficiency known as beriberi heart disease. This nutritional cardiac disorder and the peripheral nerve dysfunction usually respond to thiamine replacement. However, an alcoholic cardiomyopathy can also occur in well-nourished patients with adequate thiamine levels. Exactly how ethanol, or its toxic metabolite acetaldehyde, causes alcoholic cardiomyopathy in the absence of thiamine deficiency is unknown.

**BIOCHEMICAL COMMENTS**

**Basic Reactions and Classes of Enzymes.** In the following chapters of the text, students will be introduced to a wide variety of reaction pathways and enzyme names. Although it may seem that the number of reactions is infinite, many of these reactions are similar and occur frequently in different pathways. Recognition of the type of reaction can aid in remembering the pathways and enzyme names, thereby reducing the amount of memorization required. You may wish to use this section for reference as you go through your first biochemical pathways.

The Enzyme Commission has divided the basic reaction types and the enzymes catalyzing them into six broad numbered classes: (1) oxidoreductases, (2) transferases, (3) hydrolases, (4) lyases, (5) isomerases, and (6) ligases. Each broad class of enzymes includes subsets of enzymes with a systematic name and a common name (e.g., dehydrogenases and kinases).

**Oxidoreductases.** Oxidation-reduction reactions are very common in biochemical pathways and are catalyzed by a broad class of enzymes called oxidoreductases. Whenever an oxidation-reduction reaction occurs, at least one substrate gains electrons and becomes reduced, and another substrate loses electrons and becomes oxidized. One subset of reactions is catalyzed by dehydrogenases, which accept and donate electrons in the form of hydride ions (H:−) or hydrogen atoms. Usually an electron-transferring coenzyme, such as NAD+/NADH, acts as an electron donor or acceptor (e.g., see Fig. 8.14 and Fig. 8.15).

In another subset of reactions, O2 donates either one or both of its oxygen atoms to an acceptor (for example, see xanthine oxidase, Fig. 8.19). When this occurs, O2 becomes reduced, and an electron donor is oxidized. Enzymes participating in reactions with O2 are called hydroxylases and oxidases when one oxygen atom is incorporated into a substrate and the other oxygen atom into water, or both atoms are incorporated into water. They are called oxygenases when both atoms of oxygen are incorporated into the acceptor. Most hydroxylases and oxidases require metal ions, such as Fe2+, for electron transfer.

**Transferases.** Transferases catalyze group transfer reactions—the transfer of a functional group from one molecule to another. If the transferred group is a high-energy phosphate (as shown in Fig. 8.3), the enzyme is a kinase; if the transferred group is a carbohydrate residue, the enzyme is a...
glycosyltransferase; if it is a fatty acyl group, the enzyme is an acyltransferase. A common feature of these reactions is that the group being transferred exists as a good leaving group on the donor molecule.

Another subset of group transfer reactions consists of transaminations (Fig. 8.20). In this type of reaction, the nitrogen group from an amino acid is donated to an \( \alpha \)-keto acid, forming a new amino acid and the \( \alpha \)-keto acid corresponding to the donor amino acid. Enzymes catalyzing this last type of reaction are called transaminases or aminotransferases. The coenzyme pyridoxal phosphate is required for all transaminases (see Fig. 8.13).

When the physiologically important aspect of the reaction is the compound synthesized, the transferase may be called a synthase. For example, the enzyme commonly called glycogen synthase transfers a glucosyl residue from UDP-glucose to the end of a glycogen molecule. Its systematic name is UDP-glucose-glycogen glycosyltransferase.

**Hydrolases.** In hydrolysis reactions, C-O, C-N, or C-S bonds are cleaved by the addition of \( \text{H}_2\text{O} \) in the form of \( \text{OH}^- \) and \( \text{H}^+ \) to the atoms forming the bond (see, for example, Fig. 8.8). The enzyme class names specify the group being cleaved (e.g., the enzyme commonly named chymotrypsin is a protease, a hydrolase that cleaves peptide bonds in proteins).

**Lyases.** The lyase class of enzymes consists of a diverse group of enzymes cleaving C-C, C-O, and C-N bonds by means other than hydrolysis or oxidation. Some of the enzymes catalyzing C-C bond cleavage are called aldolases, decarboxylases (when carbon dioxide is released from a substrate), and thiolases (when the sulfur-containing nucleophile of cysteine or CoASH is used to break a carbon-carbon bond) (Fig. 8.21). The structures amenable to carbon–carbon bond cleavage usually require a carbonyl carbon that can act as an electron sink to stabilize the carbanion transiently formed when the carbon–carbon bond breaks.

This broad class of enzymes also includes dehydratases and many synthases. Dehydratases remove the elements of water from two adjacent carbon–carbon bonds to form a double bond. Certain enzymes in this group, such as certain group transferases, are commonly called synthases when the physiologically important direction of the reaction favors the formation of a carbon–carbon bond (e.g., citrate synthase).

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**Fig. 8.20.** Transamination reactions. Pyridoxal phosphate (PLP) on aspartate aminotransferase transfers an amino group from aspartate to the \( \alpha \)-keto acid (\( \alpha \)-ketoglutarate) to form a new amino acid (glutamate). The enzyme used to be called glutamate-oxaloacetate transaminase.

**Fig. 8.21.** Aldolases catalyze carbon–carbon cleavage in reactions that are usually reversible. In glycolysis, the enzyme fructose 1,6-bisphosphate aldolase cleaves a carbon–carbon bond in fructose 1,6-bisphosphate. Aldolases have a lysine epsilon amino group in the active site that participates in the reaction.
Isomerases. Many biochemical reactions simply rearrange the existing atoms of a molecule, that is, create isomers of the starting material (Fig. 8.22). Enzymes rearranging the bond structure of a compound are called isomerases, whereas enzymes catalyzing movement of a phosphate from one atom to another are called mutases.

Ligases. Ligases synthesize C-C, C-S, C-O, and C-N bonds in reactions coupled to the cleavage of a high-energy phosphate bond in ATP or another nucleotide. Carboxylases, for example, add CO$_2$ to another compound in a reaction requiring ATP cleavage to provide energy (see Fig. 8.12B). Most carboxylases require the coenzyme biotin. Other ligases are named synthetases (e.g., fatty acyl CoA synthetase). Synthetases differ from the synthases mentioned under “lyases” and “group transferases” in that synthetases derive the energy for new bond formation from cleavage of high-energy phosphate bonds, and synthases use a different source of energy.

Suggested References


EPA has a good website for information on organophosphate compounds at www.epa.gov/pesticides.

The ENZYME database (http://www.expasy.ch/enzyme/) provides basic information about specific enzymes. It is based on the recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology. In this database, you can obtain the EC number of any enzyme, its recommended name, alternative names, cofactors, and human diseases associated with the enzyme. You can trace the enzyme from its name to the reaction it catalyses—to the metabolic pathway(s) in which it participates—to the large and intricate Boehringer Mannheim Biochemical Pathways Wallchart. You also can search the database by metabolites or pathways.

REVIEW QUESTIONS—CHAPTER 8

Questions below cover material from Chapters 6 and 7, as well as Chapter 8 (including Biochemical Comments).

1. A patient was born with a congenital mutation in an enzyme, severely affecting its ability to bind an activation-transfer coenzyme. As a consequence,

   (A) the enzyme would be unable to bind the substrate of the reaction.
   (B) the enzyme would be unable to form the transition state complex.
   (C) the enzyme would normally use a different activation-transfer coenzyme.
   (D) the enzyme would normally substitute the functional group of an active site amino acid residue for the coenzyme.
   (E) the reaction could be carried out by the free coenzyme, provided the diet carried an adequate amount of its vitamin precursor.

2. An individual had a congenital mutation in glucokinase in which a proline was substituted for a leucine on a surface helix far from the active site, but within the hinge region of the actin fold. This mutation would be expected to

   (A) have no effect on the rate of the reaction because it is not in the active site.
   (B) have no effect on the rate of the reaction because proline and leucine are both nonpolar amino acids.
   (C) have no effect on the number of substrate molecules reaching the transition state.
   (D) probably affect the binding of ATP or a subsequent step in the reaction sequence.
   (E) probably cause the reaction to proceed through an alternate mechanism.
3. A patient developed a bacterial overgrowth in his intestine that decreased the pH of the luminal contents from their normal pH of approximately 6.5 down to 5.5. This decrease of pH is likely to

(A) denature proteins reaching the intestine with their native structure intact.
(B) disrupt hydrogen bonding essential for maintenance of tertiary structure.
(C) inhibit intestinal enzymes dependent on histidine for acid-base catalysis.
(D) inhibit intestinal enzymes dependent on an active site lysine for binding substrate.
(E) have little effect on hydrolases.

Questions 4 and 5 refer to the reaction shown below:

4. The type of reaction shown above fits into which of the following classifications?

(A) Group transfer
(B) Isomerization
(C) Carbon–carbon bond breaking
(D) Carbon–carbon bond formation
(E) Oxidation-reduction

5. The type of enzyme catalyzing this reaction is a

(A) kinase
(B) dehydrogenase
(C) glycosyltransferase
(D) transaminase
(E) isomerase