6.3 Metabolic Pathways and Enzymes

Reactions do not occur haphazardly in cells; they are usually part of a **metabolic pathway,** a series of linked reactions. Metabolic pathways begin with a particular reactant and terminate with an end product. While it is possible to write an overall equation for a pathway as if the beginning reactant went to the end product in one step, actually many specific steps occur in between. In the pathway, one reaction leads to the next reaction, which leads to the next reaction, and so forth in an organized, highly structured manner. This arrangement makes it possible for one pathway to lead to several others, because various pathways have several molecules in common. Also, metabolic energy is captured and utilized more easily if it is released in small increments rather than all at once.

A metabolic pathway can be represented by the following diagram:

$$A \xrightarrow{E_1} B \xrightarrow{E_2} C \xrightarrow{E_3} D \xrightarrow{E_4} E \xrightarrow{E_5} F \xrightarrow{E_6} G$$

In this diagram, the letters A–F are reactants and the letters B–G are products in the various reactions. In other words, the products from the previous reaction become the reactants of the next reaction. The letters E_1–E_6 are enzymes.

An **enzyme** is a protein molecule that functions as an organic catalyst to speed a chemical reaction. In a crowded ballroom, a mutual friend can cause particular people to interact. In the cell, an enzyme brings together particular molecules and causes them to react with one another.

The reactants in an enzymatic reaction are called the **substrates** for that enzyme. In the first reaction, A is the substrate for E_1, and B is the product. Now B becomes the substrate for E_2, and C is the product. This process continues until the final product "G" forms.

Any one of the molecules (A–G) in this linear pathway could also be a substrate for an enzyme in another pathway. A diagram showing all the possibilities would be highly branched.

Energy of Activation

Molecules frequently do not react with one another unless they are activated in some way. In the lab, for example, in the absence of an enzyme, activation is very often achieved by heating the reaction flask to increase the number of effective collisions between molecules. The energy that must be added to cause molecules to react with one another is called the **energy of activation** (E_a). Figure 6.5 compares E_a when an enzyme is not present to when an enzyme is present, illustrating that enzymes lower the amount of energy required for activation to occur. Nevertheless, the addition of the enzyme does not change ΔG of the reaction.

In baseball, a home-run hitter must not only hit the ball to the fence, but over the fence. When enzymes lower the energy of activation, it is like removing the fence; then it is possible to get a home run by simply hitting the ball as far as the fence was.

Enzyme-Substrate Complexes

The following equation, which is pictorially shown in Figure 6.6, is often used to indicate that an enzyme forms a complex with its substrate:

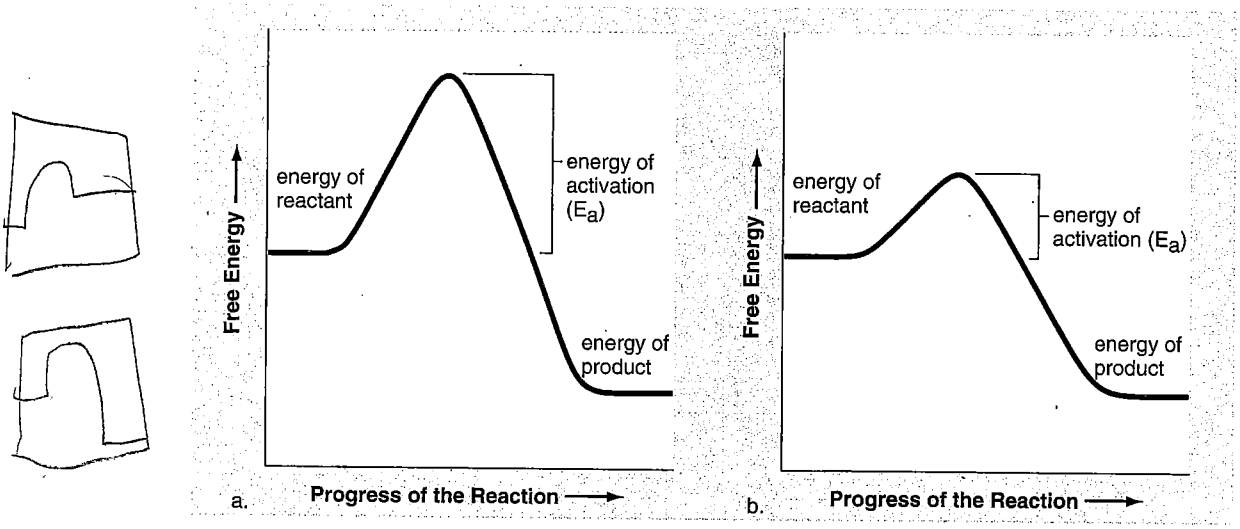


Figure 6.5 Energy of activation (E_a).
Enzymes speed the rate of chemical reactions because they lower the amount of energy required to activate the reactants. **a.** Energy of activation when an enzyme is not present. **b.** Energy of activation when an enzyme is present. Even spontaneous reactions like this one speed up when an enzyme is present.

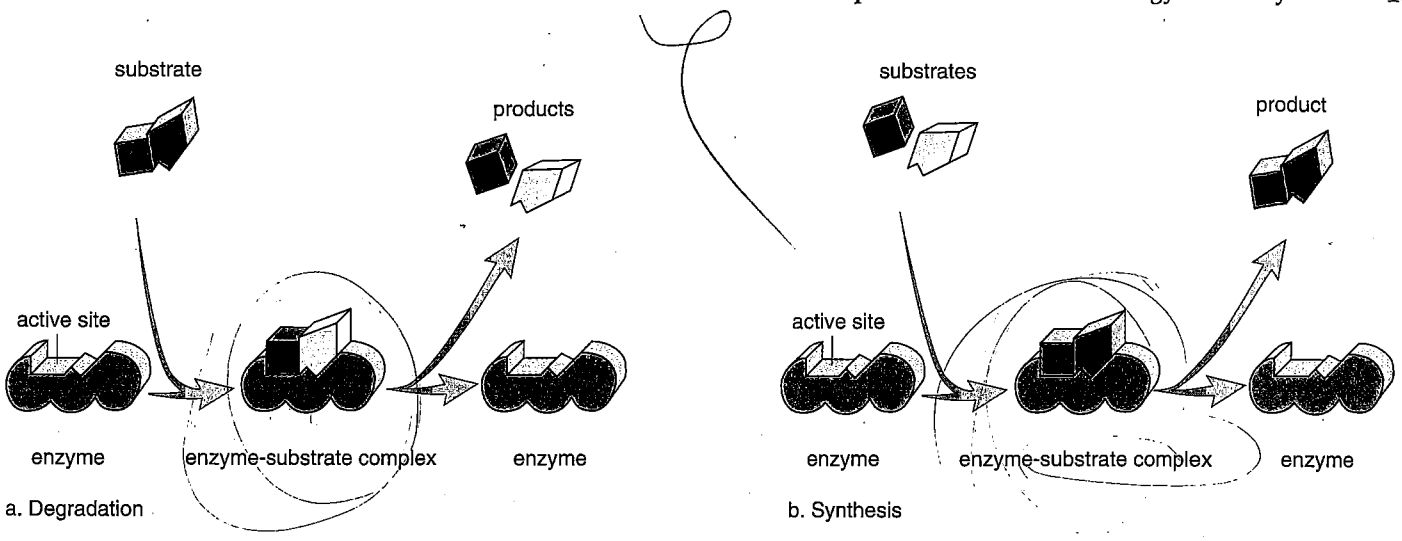


Figure 6.6 Enzymatic action.
An enzyme has an active site, where the substrates and enzyme fit together in such a way that the substrates are oriented to react. Following the reaction, the products are released and the enzyme is free to act again. **a.** Some enzymes carry out degradation; the substrate is broken down to smaller products. **b.** Other enzymes carry out synthesis; the substrates are combined to produce a larger product.

$$\underset{\text{enzyme}}{E} + \underset{\text{substrate}}{S} \rightarrow \underset{\text{enzyme-substrate complex}}{ES} \rightarrow \underset{\text{enzyme}}{E} + \underset{\text{product}}{P}$$

In most instances, only one small part of the enzyme, called the **active site,** complexes with the substrate(s). It is here that the enzyme and substrate fit together, seemingly like a key fits a lock; however, it is now known that the active site undergoes a slight change in shape in order to accommodate the substrate(s). This is called the **induced fit model** because the enzyme is induced to undergo a slight alteration to achieve optimum fit (Fig. 6.7).

The change in shape of the active site facilitates the reaction that now occurs. After the reaction has been completed, the product(s) is released, and the active site returns to its original state, ready to bind to another substrate molecule. Only a small amount of enzyme is actually needed in a cell because enzymes are not used up by the reaction.

Some enzymes do more than simply complex with their substrate(s); they participate in the reaction. Trypsin digests protein by breaking peptide bonds. The active site of trypsin contains three amino acids with *R* groups that actually interact with members of the peptide bond—first to break the bond and then to introduce the components of water. This illustrates that the formation of the enzyme-substrate complex is very important in speeding up the reaction.

Sometimes it is possible for a particular reactant(s) to produce more than one type of product(s). The presence or absence of an enzyme determines which reaction takes place. If a substance can react to form more than one product, then the enzyme that is present and active determines which product is produced.

Every reaction in a cell requires its specific enzyme. Because enzymes only complex with their substrates, they are named for their substrates, as in the following examples:

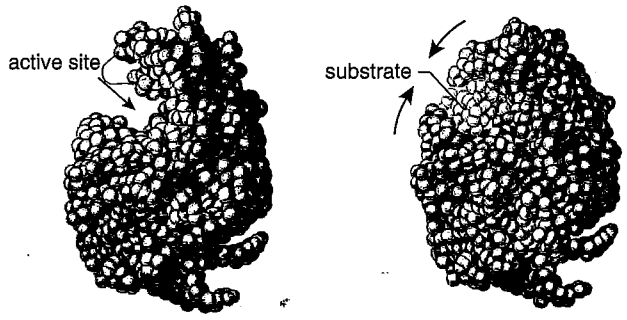


Figure 6.7 Induced fit model.
These computer-generated images show an enzyme called lysozyme that hydrolyzes its substrate, a polysaccharide that makes up bacterial cell walls. **a.** Configuration of enzyme when no substrate is bound to it. **b.** After the substrate binds, the configuration of the enzyme changes so that hydrolysis can better proceed.

Substrate	Enzyme
Lipid	Lipase
Urea	Urease
Maltose	Maltase
Ribonucleic acid	Ribonuclease
Lactose	Lactase

Enzymes are protein molecules that speed chemical reactions by lowering the energy of activation. They do this by forming an enzyme-substrate complex.

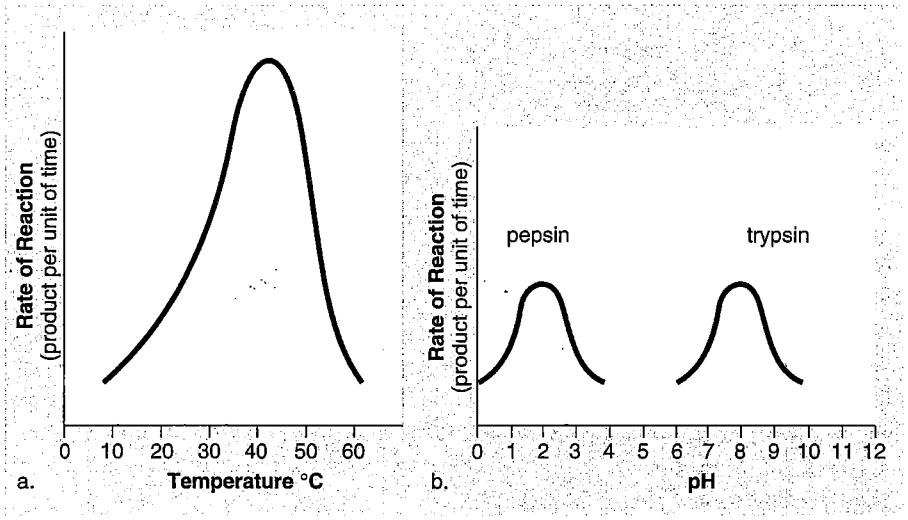


Figure 6.8 **Rate of an enzymatic reaction as a function of temperature and pH.**
a. At first, as with most chemical reactions, the rate of an enzymatic reaction doubles with every 10°C rise in temperature. In this graph, the rate of reaction is maximum at about 40°C; then it decreases until the reaction stops altogether, because the enzyme has become denatured. **b.** Pepsin, an enzyme found in the stomach, acts best at a pH of about 2, while trypsin, an enzyme found in the small intestine, performs optimally at a pH of about 8. The shape that enables these proteins to bind with their substrates is not properly maintained at other pHs.

Factors Affecting Enzymatic Speed

Enzymatic reactions proceed quite rapidly. Consider, for example, the breakdown of hydrogen peroxide (H_2O_2) as catalyzed by the enzyme catalase: $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$. The breakdown of hydrogen peroxide can occur 600,000 times a second when catalase is present. To achieve maximum product per unit time, there should be enough substrate to fill active sites most of the time. Temperature and optimal pH also increase the rate of an enzymatic reaction.

Substrate Concentration

Generally, enzyme activity increases as substrate concentration increases because there are more collisions between substrate molecules and the enzyme. As more substrate molecules fill active sites, more product results per unit time. But when the enzyme's active sites are filled almost continuously with substrate, the enzyme's rate of activity cannot increase any more. Maximum rate has been reached.

Temperature and pH

As the temperature rises, enzyme activity increases (Fig. 6.8*a*). This occurs because higher temperatures cause more effective collisions between enzyme and substrate. However, if the temperature rises beyond a certain point, enzyme activity eventually levels out and then declines rapidly because the enzyme is **denatured.** An enzyme's shape changes during denaturation, and then it can no longer bind its substrate(s) efficiently.

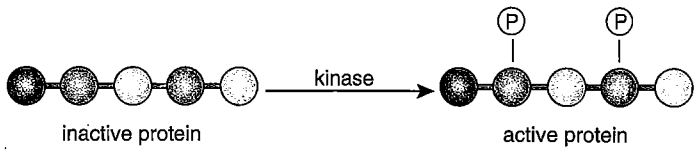
Each enzyme also has an optimal pH at which the rate of the reaction is highest. Figure 6.8*b* shows the optimal pH for the enzymes pepsin and trypsin. At this pH value, these enzymes have their normal configurations. The globular structure of an enzyme is dependent on interactions, such as hydrogen bonding, between *R* groups. A change in pH can alter the ionization of these side chains and disrupt normal interactions, and under extreme conditions of pH, denaturation eventually occurs. Again, the enzyme has an altered shape and is then unable to combine efficiently with its substrate.

Enzyme Concentration

Since enzymes are specific, a cell regulates which enzymes are present and/or active at any one time. Otherwise, enzymes may be present that are not needed, or one pathway may negate the work of another pathway.

Genes must be turned on to increase the concentration of an enzyme and turned off to decrease the concentration of an enzyme.

Another way to control enzyme activity is to activate or deactivate the enzyme. Phosphorylation is one way to activate an enzyme. Molecules received by membrane receptors often turn on kinases, which then activate enzymes by phosphorylating them:



Enzyme Inhibition

Enzyme inhibition occurs when an active enzyme is prevented from combining with its substrate. The activity of almost every enzyme in a cell is regulated by feedback inhibition. In the simplest case, when a product is in abundance, it binds competitively with its enzyme's active site. As the product is used up, inhibition is reduced, and more product can be produced. In this way, the concentration of the product is always kept within a certain range.

Most metabolic pathways are regulated by a more complicated type of feedback inhibition (Fig. 6.9). In these instances, the end product of the pathway binds to an allosteric site, which is a site other than the active site of an enzyme. The binding shuts down the pathway, and no more product is produced.

Poisons are often enzyme inhibitors. Cyanide is an inhibitor for an essential enzyme (cytochrome *c* oxidase) in all cells, which accounts for its lethal effect on humans. Penicillin blocks the active site of an enzyme unique to bacteria. Therefore, penicillin is a poison for bacteria. When penicillin is administered, bacteria die, but humans are unaffected.

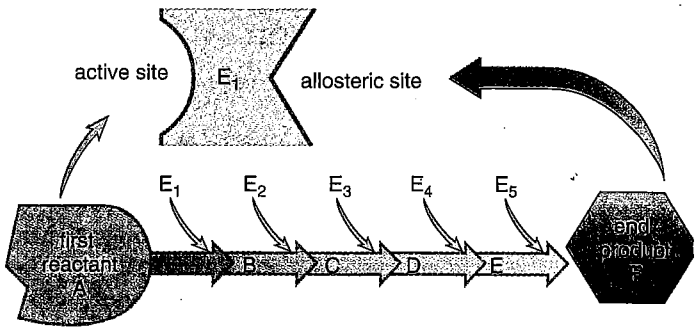
Enzyme Cofactors

Many enzymes require an inorganic ion or organic but nonprotein molecule to function properly; these necessary ions or molecules are called **cofactors.** The inorganic ions are metals such as copper, zinc, or iron. The organic, nonprotein molecules are called **coenzymes.** These cofactors assist the enzyme and may even accept or contribute atoms to the reactions.

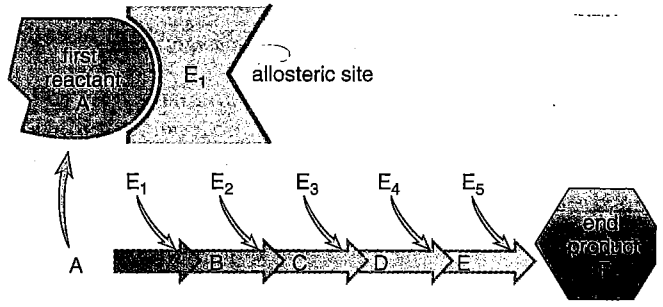
It is interesting that vitamins are often components of coenzymes. **Vitamins** are relatively small organic molecules that are required in trace amounts in our diet and in the diets of other animals for synthesis of coenzymes that affect health and physical fitness. The vitamin becomes a part of the coenzyme's molecular structure. For example, the vitamin niacin is part of the coenzyme NAD, and B_{12} is part of the coenzyme FAD.

A deficiency of any one of these vitamins results in a lack of the coenzyme listed and therefore a lack of certain enzymatic actions. In humans, this eventually results in vitamin-deficiency symptoms: niacin deficiency results in a skin disease called pellagra, and riboflavin deficiency results in cracks at the corners of the mouth.

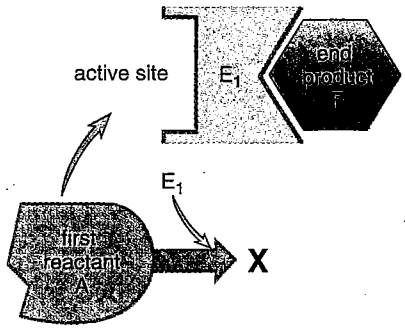
Various factors affect enzymatic speed, including substrate concentration, temperature, pH, enzyme concentration, inhibition, or necessary cofactors.



Pathway. E_1 has two sites: the active site where reactant A binds and an allosteric site where end product F binds.



Active Pathway. Reactant A binds to the active site of E_1; therefore, the pathway is active and the end product is produced.



Inhibited Pathway. When there is sufficient end product F, some binds to the allosteric site of E_1. Now a change of shape prevents reactant A from binding to the active site of E_1, and the end product is no longer produced.

Figure 6.9 Feedback inhibition.
Feedback inhibition occurs when the end product of a metabolic pathway binds to the first enzyme of the pathway, preventing the reactant from binding and the reaction from occurring.

6.4 Oxidation-Reduction and the Flow of Energy

When oxygen (O) combines with a metal such as iron or magnesium (Mg), oxygen receives electrons and forms ions that are negatively charged; the metal loses electrons and forms ions that are positively charged. When magnesium oxide (MgO) forms, it is obviously appropriate to say that magnesium has been oxidized and that because of oxidation, it has lost electrons. On the other hand, oxygen has been reduced because it has gained negative charges (i.e., electrons).

Today, the terms oxidation and reduction are applied to many reactions, whether or not oxygen is involved. Very simply, **oxidation** is the loss of electrons and **reduction** is the gain of electrons. In the ionic reaction $Na + Cl \rightarrow NaCl$, sodium has been oxidized (loss of electron) and chlorine has been reduced (gain of electron). Because oxidation and reduction go hand-in-hand, the entire reaction is called a **redox reaction.**

The terms oxidation and reduction also apply to covalent reactions in cells. In this case, however, oxidation is the loss of hydrogen atoms ($e^- + H^+$), and reduction is the gain of hydrogen atoms. A hydrogen atom contains one proton and one electron; therefore, when a molecule loses a hydrogen atom, it has lost an electron, and when a molecule gains a hydrogen atom, it has gained an electron. This form of oxidation-reduction is exemplified by the overall reactions for photosynthesis and cellular respiration, pathways that permit a flow of energy from the sun through all living things.

Photosynthesis

The overall reaction for photosynthesis can be written like this:

$$\text{energy} + 6\,CO_2 + 6\,H_2O \rightarrow C_6H_{12}O_6 + 6\,O_2$$

carbon dioxide; water; glucose; oxygen

This equation shows that during photosynthesis, hydrogen atoms are transferred from water to carbon dioxide, and glucose is formed. Therefore, water has been oxidized and carbon dioxide has been reduced. It takes energy to form glucose, and this energy is supplied by solar energy. Chloroplasts are able to capture solar energy and convert it to chemical energy of ATP molecules that are used along with hydrogen atoms to reduce carbon dioxide.

The reduction of carbon dioxide to form a mole of glucose stores 686 kcal in the chemical bonds of glucose. This is the energy that living things use to support themselves. But glucose must be broken down during cellular respiration for this energy to be made available. Only in this way is the energy stored in glucose made available to cells.

Cellular Respiration

The overall equation for cellular respiration is the opposite of the one we used to represent photosynthesis:

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O + \text{energy}$$

glucose; oxygen; carbon dioxide; water

In this reaction, glucose has lost hydrogen atoms (been oxidized) and oxygen has gained hydrogen atoms (been reduced). When oxygen gains electrons, it becomes water. The complete oxidation of a mole of glucose releases 686 kcal of energy, and this energy is used to synthesize ATP molecules. The amount of energy in one ATP molecule is sufficient for most reactions in cells.

If the energy within glucose were released all at once, most of it would be dissipated as heat. Instead, cells oxidize glucose slowly, and gradually use the energy to synthesize ATP molecules.

Organelles and the Flow of Energy

During photosynthesis, the chloroplasts in plants capture solar energy and use it to convert water and carbon dioxide into carbohydrates, which serve as food for all living things. Oxygen is one by-product of photosynthesis (Fig. 6.10).

Mitochondria, present in both plants and animals, complete the breakdown of carbohydrates and use the released energy to build ATP molecules. Cellular respiration consumes oxygen and produces carbon dioxide and water, the very molecules taken up by chloroplasts.

It is actually the cycling of molecules between chloroplasts and mitochondria that allows a flow of energy from the sun through all living things. As mentioned at the beginning of the chapter, this flow of energy maintains the levels of biological organization from molecules to ecosystems. In keeping with the laws of thermodynamics, energy is dissipated with each chemical transformation, and eventually the solar energy captured by plants is lost in the form of heat. Therefore, most living things are dependent upon an input of solar energy.

Human beings are also involved in the cycling of molecules between plants and animals and in the flow of energy from the sun. We inhale oxygen and eat plants and their stored carbohydrates, or we eat other animals that have eaten plants. Oxygen and nutrient molecules enter our mitochondria, which produce ATP and release carbon dioxide and water, the molecules used by plants to produce carbohydrates. Without a supply of energy-rich molecules produced by plants, we could not produce the ATP molecules needed to maintain our bodies.

The oxidation-reduction pathways of photosynthesis in chloroplasts and cellular respiration in mitochondria permit a flow of energy from the sun through all living things.