1.7 Enzymes

enzyme a biological catalyst, usually a protein, that speeds up a chemical reaction

The cellular activity of all living organisms is controlled through the use of enzymes. An **enzyme** is a special type of biological molecule that usually speeds up a chemical reaction without being consumed or changing the products of the reaction. Almost all enzymes are proteins. There are about 4000 different enzymes in a typical living cell. If even one of these enzymes is missing or defective, the results can be disastrous. The enzyme lipase speeds up the hydrolysis of the lipid triglycerides. Sucrase speeds up the hydrolysis of sucrose into glucose and fructose. These are important reactions that a cell may require for energy and survival.

Another term that is used to describe an enzyme is "catalyst." A catalyst is a substance that speeds up a reaction without being consumed by the reaction. In biological systems, specific enzymes catalyze particular cellular reactions. Each enzyme has a unique three-dimensional shape, and this shape determines which reaction it catalyzes. For a chemical reaction to move forward, it must overcome an energy barrier, and this is where enzymes are important. Enzymes bind a specific reactant (or reactants), called a **substrate**; in doing so, they lower the energy barrier so that the reaction proceeds at a faster rate than it would without the enzymes (Section 3.3).

substrate a substance that is recognized by and binds to an enzyme

Enzymes and Substrates

In a reaction that uses an enzyme, the enzyme combines briefly with the substrate(s) and, after releasing the products, is unchanged. For example, hydrogen peroxide is a toxic chemical that occurs in cells as a by-product of metabolism. To prevent cell damage, hydrogen peroxide is broken down by the enzyme catalase:

$$2\ H_2O_2 \xrightarrow{\text{(catalase)}} 2\ H_2O + O_2$$

Each type of enzyme catalyzes the reaction of only one type of molecule or one group of closely related molecules. Enzyme specificity explains why a typical cell needs about 4000 different enzymes to function properly. Enzymes are much larger than the substrate. The substrate interacts with only a very small region of the enzyme called the active site. The **active site** is usually a pocket or groove that forms when the newly synthesized enzyme folds into its correct three-dimensional shape (tertiary structure).

active site a pocket or groove in an enzyme that binds its substrate

In the early twentieth century, biochemists proposed the lock-and-key hypothesis to explain how specific enzymes and substrates interact. The analogy worked well to explain how even similar substrates (the "keys") were unable to bind to the same enzyme (the "lock") and undergo catalysis. However, the more recent introduction of the induced-fit hypothesis better explains the enzyme-substrate relationship.

Induced-Fit Hypothesis

Research by enzymologists shows that enzymes are not rigid objects, like locks, but are flexible. Just prior to substrate binding, the enzyme changes its shape, or what is called its conformation, so that the active site becomes even more precise in its ability to bind to its substrate (**Figure 1**). This is called the **induced-fit model**.

induced-fit model a model of enzyme activity that describes how an enzyme changes shape to better accommodate a substrate

CAREER LINK

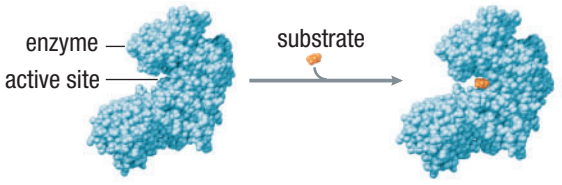


Figure 1 This space-filling model shows the combination of an enzyme, hexokinase (blue), with its substrate glucose (orange). Note that the enzyme undergoes a conformational change, closing the active site more tightly as it binds the substrate.

An enzyme binds to one or more substrates, forming an enzyme-substrate complex. The enzyme then converts the substrate(s) into one or more products. Since enzymes remain unchanged after a reaction, enzyme molecules can rapidly bind to other substrate molecules, catalyzing the same reaction repeatedly. This is the enzyme cycle (**Figure 2**). The rate at which enzymes catalyze reactions varies depending on the enzyme and substrates involved, but typical rates vary between about 100 and 10 million substrate molecules per second!

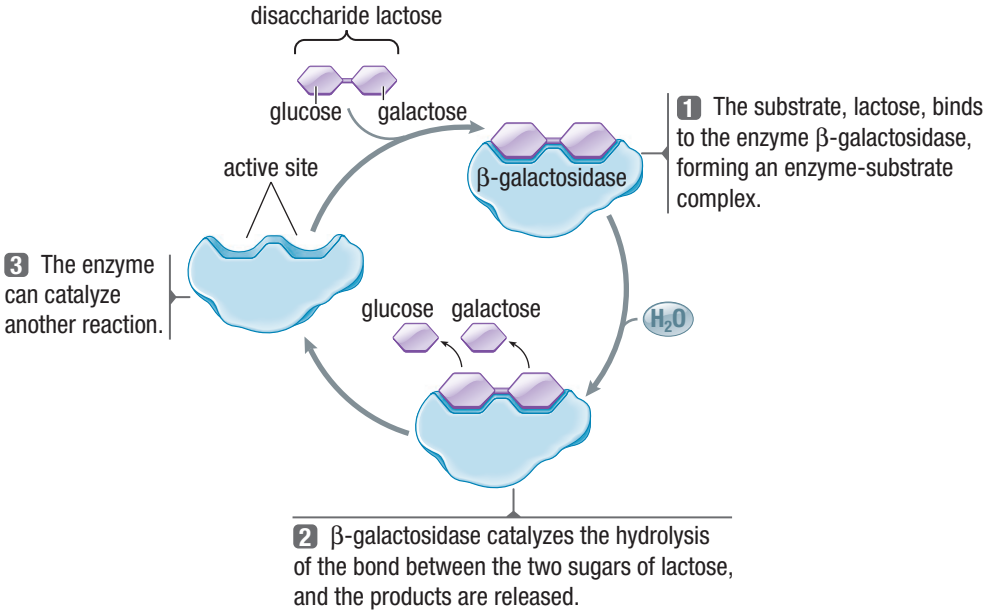


Figure 2 In the catalytic cycle shown, the enzyme β-galactosidase hydrolyzes lactose (a sugar) to produce glucose and galactose. The enzyme is recycled after the reaction is catalyzed. Note that water is a reactant in this reaction.

Mini **Investigation**

Modelling Enzymes and Polymers

Skills: Performing, Analyzing, Evaluating, Communicating

SKILLS HANDBOOK A2.1

In this activity, you will model the interaction of an enzyme and its two substrates.

Equipment and Materials: coloured paper clips; strip of paper, approximately 5 cm × 22 cm

1. Prepare a strip of paper as shown in **Figure 3(a)**.
2. Fold the paper as shown in **Figure 3(b)**.
3. Place paper clip substrate 1 on active site 1, spanning the back two layers of the paper enzyme. Place paper clip substrate 2 on active site 2, spanning two layers of the paper enzyme (**Figure 3(c)**).
4. Briskly pull the two tabs apart to "activate" the enzyme (**Figure 3(d)**).
5. Try to produce a "triclipide" or a "tetraclipide" with one pull of the tabs.

A. Explain how the action of the paper enzyme partly models a real enzyme-catalyzed condensation reaction. T/I

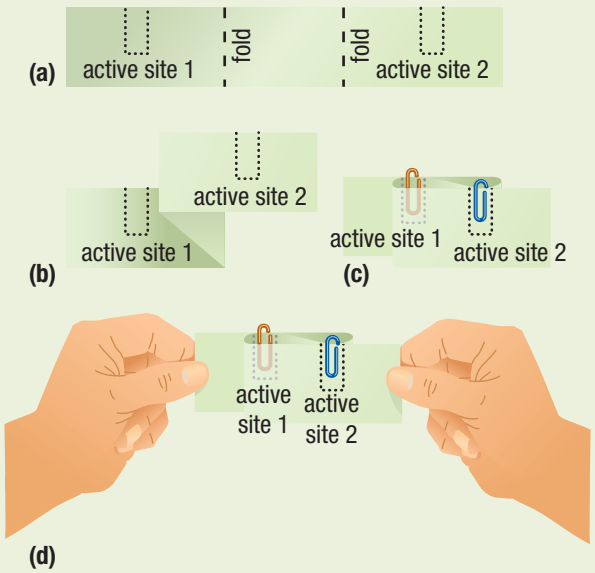


Figure 3

Cofactors and Coenzymes

cofactor a non-protein group that binds to an enzyme and is essential for catalytic activity

Many enzymes require a **cofactor**, which is a non-protein group that binds very precisely to an enzyme. Cofactors are often metals, such as iron, copper, zinc, and manganese. Although your body may need only very small amounts of some of these metals, they are absolutely essential for the catalytic activity of the enzyme to which they bind. For example, an enzyme that is essential for providing one of the key components of the chemical pathway within mitochondria for the production of energy requires a magnesium cofactor to function properly.

coenzyme an organic molecule that acts as a cofactor of an enzyme

Organic cofactors called **coenzymes** play similar roles and are often derived from water-soluble vitamins. Many coenzymes shuttle molecules from one enzyme to another. One of the most important coenzymes is nicotinamide adenine dinucleotide (NAD^+), a derivative of vitamin B_3 (niacin). NAD^+ acts as an electron carrier during a number of biochemical pathways.

Conditions and Factors That Affect Enzyme Activity

Several conditions can alter enzyme activity, including enzyme and substrate concentration, temperature, and pH. In addition, several control mechanisms modify enzyme activity. These control mechanisms adjust reaction rates to meet a cell's requirements for chemical products.

Enzyme and Substrate Concentration

The concentration of both the enzyme and the substrate will influence the rate of a catalysis reaction. If there is excess substrate present, then the rate of reaction is proportional to the enzyme concentration (**Figure 4(a)**). This occurs because the amount of enzyme limits the rate of reaction. If, however, the amount of enzyme is at a constant intermediate concentration, then increasing the substrate concentration will increase the rate of reaction up to a point, called the saturation level (**Figure 4(b)**). The rate of the reaction increases as collisions become more frequent. However, as the enzyme molecules approach the maximum rate at which they combine with the substrate, increasing the substrate concentration has a reduced effect. Eventually, the rate of reaction levels off. At this point, the enzyme molecules are saturated with substrate.

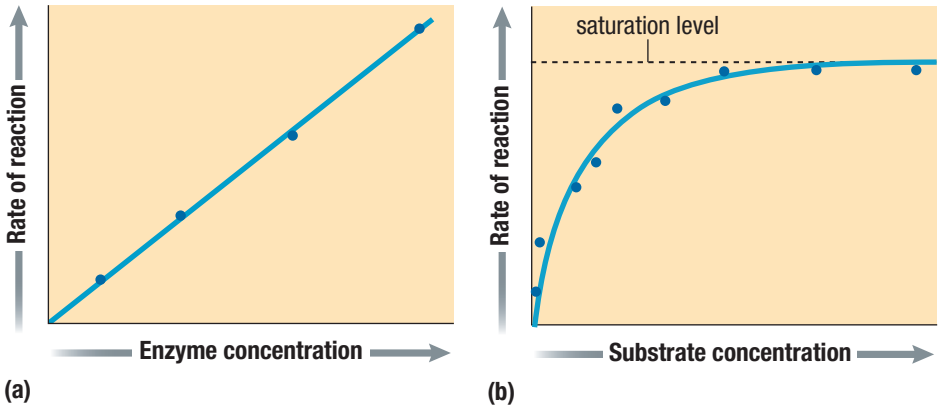


Figure 4 (a) The rate of a reaction (usually measured as the rate of product formation) is proportional to the enzyme concentration if the concentration of the substrate is constant and at a high level. (b) The rate of a reaction as a function of increasing substrate concentration is a curve if the amount of enzyme is kept constant.

Enzyme Inhibitors

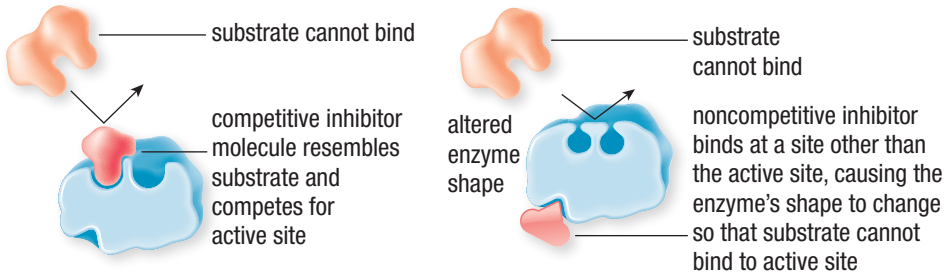
Enzyme inhibitors lower the rate at which an enzyme catalyzes a reaction. Inhibitors are molecules that bind to an enzyme and decrease its activity. Some inhibitors work by binding to the active site of an enzyme, while other inhibitors bind to critical sites located elsewhere in the structure of the enzyme.

Inhibitors that combine with the active site have shapes that resemble the normal substrate closely enough to fit into and occupy the active site. When binding to the active site, inhibitors block access to the normal substrate and slow the rate of the reaction. If the concentration of the inhibitor is high enough, the reaction stops completely. This type of inhibition is called **competitive inhibition** because the inhibitor actually competes with the normal substrate for access to the active site of the enzyme (**Figure 5(a)**).

competitive inhibition a situation in which a competitor substance binds to a normal substrate binding site to block enzyme activity

In **noncompetitive inhibition**, specific molecules inhibit enzyme activity, but they do not compete with substrate molecules for binding to the active site (**Figure 5(b)**). Instead, noncompetitive inhibitors bind to an enzyme at a location other than the active site. This changes the shape of the enzyme, reducing the ability of the substrate to bind efficiently.

noncompetitive inhibition a situation in which molecules bind to an enzyme at a site that is not the active site, thus blocking enzyme activity



(a) competitive inhibition **(b)** noncompetitive inhibition

Figure 5 Actions of enzyme inhibitors: (a) competitive inhibition and (b) noncompetitive inhibition

Inhibitors differ in how strongly they bind to enzymes. In reversible inhibition, the binding of the inhibitor to the enzyme is weak and readily reversible. Enzyme activity returns to normal following the release of the inhibitor. By contrast, some inhibitors bind so strongly to the enzyme through the formation of covalent bonds that they completely disable the enzyme. This is irreversible inhibition.

Not surprisingly, many irreversible inhibitors that act on critical enzymes are highly toxic to the cell. Irreversible inhibitors include a wide variety of drugs and pesticides. Cyanide is a potent poison because it binds strongly to and inhibits cytochrome oxidase, the enzyme that catalyzes a key step in cellular respiration. Many antibiotics are toxic to bacteria and work by inhibiting enzyme activity in the bacteria. Irreversible inhibition can be overcome only by the cell synthesizing more of the enzyme. The antibiotic penicillin acts by inhibiting the synthesis of peptidoglycan, a key component of the bacterial cell wall. The enzyme transpeptidase catalyzes the formation of a peptide bond between the two amino acids that are responsible for linking two parts of peptidoglycan. The structure of penicillin mimics the structure of the two amino acids that are normally brought together by the active site. Penicillin binds irreversibly to the active site of transpeptidase, effectively destroying the molecule.

ALLOSTERIC CONTROL OF ENZYME ACTIVITY

Molecules that naturally regulate enzyme activity in a cell often behave somewhat like a noncompetitive reversible inhibitor. These regulatory molecules bind to an enzyme on a site that is not its active site, called the **allosteric site**, and cause a change in the shape of the enzyme, thus affecting the active site. This type of regulation, in which a protein's function at one site is affected by a molecule binding to a separate site, is called **allosteric regulation**. Allosteric regulation may either inhibit or stimulate enzyme activity.

allosteric site a binding site on an enzyme that binds regulatory molecules

allosteric regulation the regulation of one site of a protein by binding to another site on the same protein

Binding of an allosteric activator molecule stabilizes the enzyme in a shape that causes its active site to have a high affinity for its substrate (**Figure 6(a)**, next page). In this high-affinity state, the enzyme binds its substrate. Conversely, binding of an allosteric inhibitor stabilizes an inactive form of the enzyme. The inhibitor molecule changes the shape of the enzyme in such a way that the substrate is released from the active site (**Figure 6(b)**, next page).

FEEDBACK INHIBITION

Allosteric regulators are important molecules, functioning to control chemical activity in a cell. Frequently, an allosteric inhibitor is a product of the biochemical

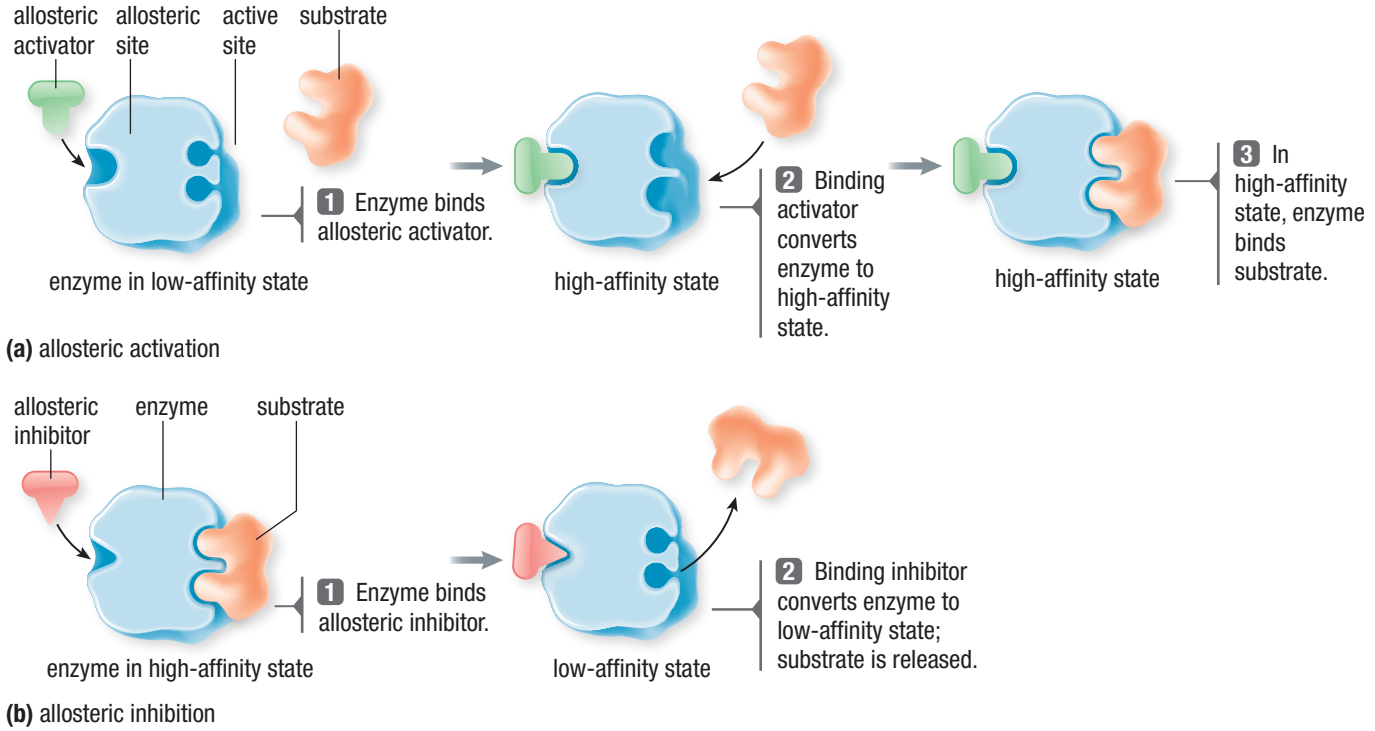


Figure 6 Allosteric regulation includes (a) an allosteric activator, which causes an enzyme to have a high affinity for the substrates it binds, and (b) an allosteric inhibitor, which causes an enzyme to have a low affinity for a substrate, causing its release.

feedback inhibition the regulation of a pathway by one of the products of this pathway

pathway that it regulates. If the product accumulates in excess, its effect as an inhibitor automatically slows or stops the enzymatic reaction that produces it. Usually, it inhibits the enzyme that catalyzes the first reaction of the pathway. If the product is scarce, the inhibition is reduced, and the rate of the reaction increases. Regulation of this type, in which the product of a reaction acts as a regulator of the reaction, is called **feedback inhibition**. Feedback inhibition prevents cellular resources from being wasted in the synthesis of molecules at intermediate steps in the pathway.

The biochemical pathway that makes the amino acid isoleucine from threonine is an example of feedback inhibition. The pathway proceeds in five steps, each catalyzed by a different enzyme (**Figure 7**). The end product of the pathway, isoleucine, is an allosteric inhibitor of the first enzyme in the pathway, threonine deaminase. If the cell makes more isoleucine than it needs, isoleucine combines reversibly with threonine deaminase at the allosteric site. Threonine deaminase is then converted to the low-affinity state, which inhibits its ability to combine with threonine, the substrate for the first reaction in the pathway. If isoleucine levels drop too low, the allosteric site of threonine deaminase is vacated, threonine deaminase converts to the high-affinity state, and isoleucine production increases.

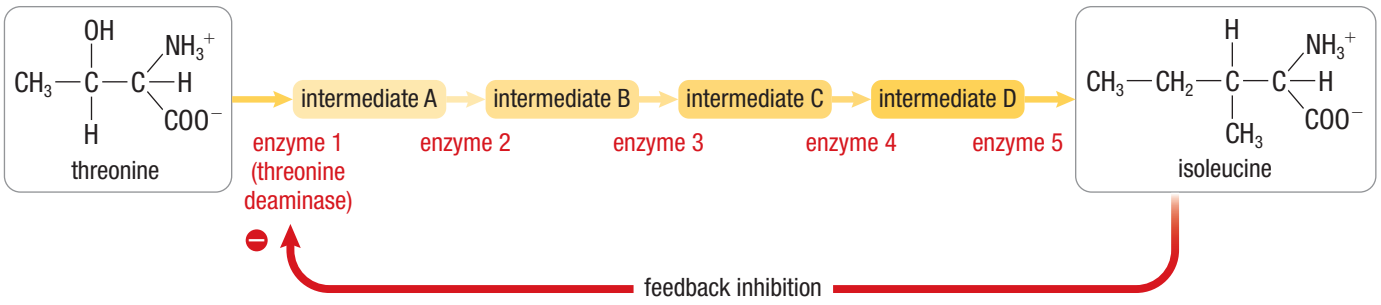


Figure 7 This diagram shows feedback inhibition in the pathway that produces isoleucine from threonine. If the product of the pathway, isoleucine, accumulates in excess, it slows or stops the pathway by acting as an allosteric inhibitor of the enzyme that catalyzes the first step in the pathway.

pH and Temperature Effects on Enzyme Activity

Changes in pH and temperature strongly affect the activity of most enzymes. Enzymes usually reach maximal activity within a narrow range of temperatures and pH values. At levels outside this range, enzyme activity drops off. This usually produces a peaked curve when enzyme activity is plotted, with the peak where temperature and pH produce maximal activity.

Typically, each enzyme has an optimal pH where it operates at its highest efficiency. As the pH either increases or decreases away from its optimal value, the rate of the catalyzed reaction decreases (**Figure 8**). The more the pH value deviates from the optimal value, the more extreme the effects on the structure and function of the active site of the enzyme become, until the rate of the reaction falls to zero. Most enzymes have a pH optimum that is near the pH of their cellular contents, about pH 7. Enzymes that are secreted from cells may have more variable pH optima. Pepsin, for example, a protein-digesting enzyme that is secreted into the stomach, has a pH optimum of 1.5, which is close to the acidity of stomach contents. Similarly, trypsin, a protein-digesting enzyme in the intestine, has a pH optimum of about 8. This allows it to function well in the mildly alkaline contents of the intestine.

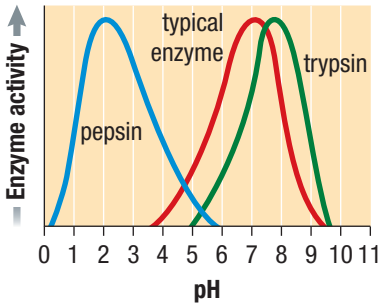


Figure 8 Typical enzymes have an optimal pH value of about 7, but pepsin and trypsin have optimal pH values outside this range.

The effects of temperature changes on enzyme activity reflect two distinct processes. First, temperature has a general effect on all kinds of chemical reactions. As the temperature rises, the rate of a chemical reaction usually increases. This effect reflects increases in the kinetic motion of the molecules. As the temperature rises, there are more frequent and stronger collisions. Second, temperature has an effect on all proteins, including enzymes. As the temperature rises, the kinetic motions of the amino acid chains of an enzyme increase. At the same time, the strength and frequency of the collisions between the enzyme molecules and any surrounding molecules also increases. In the range of 0 to about 40 °C, the reaction rate doubles for every 10 °C increase in temperature (**Figure 9**).

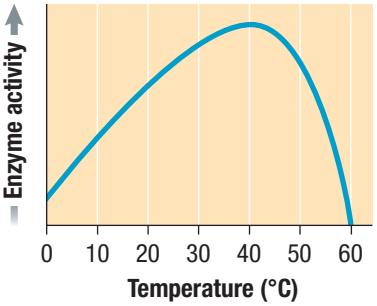


Figure 9 As the temperature increases, the rate of the enzyme-catalyzed reaction increases until the enzyme begins to denature. At this point, the rate of the reaction drops off steeply to zero.

Above 40 °C, the increasing kinetic motion begins to unravel, or denature, an enzyme. The hydrogen bonds and other forces that hold together the enzyme's three-dimensional structure break. As this happens, the enzyme loses its ability to function. The two effects of temperature work in opposition to each other to produce changes in the rate of enzyme activity. The denaturation process reduces the rate of increase in enzyme activity. At some point, as the temperature rises, the reaction rate reaches a peak. Further increases cause unfolding and the reaction rate decreases rapidly to zero.

For most enzymes, the peak in activity lies between 40 °C and 50 °C. The drop-off becomes steep at 55 °C and falls to zero by about 60 °C. The rate of an enzyme-catalyzed reaction peaks at a temperature at which kinetic motion is the greatest but no significant unfolding of the enzyme has occurred. Some enzymes have activity peaks below or above this 40 to 50 °C temperature range. For example, the enzymes of corn pollen function best near 30 °C, and there is a steep reduction in activity above 32 °C. As a result, environmental temperatures above 32 °C inhibit the fertilization of corn crops. Many animals living in frigid regions have enzymes with much lower temperature optima than average. For example, the enzymes of fish in Antarctica are most active near 0 °C. At the other extreme are the enzymes of the single-celled archaea, which live in hot springs. These enzymes are so resistant to denaturation that they remain active at temperatures of 85 °C or more.

Investigation **1.7.1**

Investigating Factors That Affect Enzyme Activity (p. 61)
Certain factors in a reaction's surrounding environment can affect enzyme activity. In this investigation, you will examine the effects of pH and temperature on enzyme activity.

Applications of Enzymes

Milk and other dairy products are recognized as highly nutritious food sources. However, many people suffer from lactose intolerance: the inability to properly break down lactose, the primary disaccharide in milk. For people with lactose intolerance, the problem begins once the lactose passes from the stomach into the small intestine. To absorb the lactose, the digestive cells need to secrete an enzyme called lactase. Lactase catalyzes the efficient breakdown of lactose in the monosaccharides glucose and galactose. People with lactose intolerance do not produce enough lactase, and therefore lactose is not digested or absorbed properly. The lactose is then consumed by bacteria

living in the gut, which leads to symptoms including nausea, cramps, and abdominal bloating. Many people overcome this complication by consuming commercial lactase enzymes when they eat dairy products that contain lactose.

Another dairy product, cheese, relies on the enzyme chymosin for its production. Chymosin was originally obtained from the stomach of calves, where it aids in the digestion of milk proteins. This enzyme is now genetically engineered for use in the cheese-making industry. When making cheese, bacteria are added to milk to aid the curdling process. The bacteria feed on the milk and produce lactic acid as a waste product. As the lactic acid is produced, the pH of the milk is lowered and the milk proteins begin to denature. The enzyme chymosin is then added to hydrolyze the most abundant milk protein, casein. The hydrolysis of casein causes the milk to coagulate into semisolid cheese curds. Different types of cheese result from further processing the milk curd. Fat-hydrolyzing enzymes produce cheeses with stronger flavours, such as the Italian cheese Romano.

Industrial food production relies on many types of enzymes. The starch-producing industry is one of the largest users of enzymes. Enzymes break down starch into glucose syrup. Glucose syrup sweetens many foods, medicines, and vitamins. The cleaning industry also relies on enzymes. There are other industrial uses for enzymes. For example, enzymes are added to laundry detergents to improve stain removal. Enzymes are more effective at removing stains such as blood, grass, milk, and perspiration than non-biological chemicals used as cleaning agents. Adding enzymes to detergents removes stains at a lower temperature and with less agitation in a washing machine. Other industrial uses of enzymes are summarized in **Table 1**. CAREER LINK

Table 1 Additional Uses of Enzymes

Product or process	Effects of enzymes
animal feed	degradation of the components of feed to improve nutrient digestion and uses of the feed
brewing	faster maturation of beer; removal of carbohydrates in light beer
dairy	cheese making; removal or conversion of lactose in milk
detergent	breakdown of starch and fatty stains as an active biological component of powder and liquid detergents; colour brightening and softening of cotton garments
leather	unhairing, batting, and defatting; soaking to soften hides and skins
starch	production of glucose, dextrose, fructose, and special syrups for baking and soft-drink production
wine and juice	degradation of the protein pectin for clarification and increase in juice yield

Research This

Researching Enzymes in Industry

Skills: Researching, Evaluating, Communicating

SKILLS HANDBOOK A4.1

There are many commercial applications of enzymes. In this activity, you will research one enzyme that has a commercial application.

1. Choose one of the products listed in Table 1.
2. Research and answer the following questions:

A. What enzymes are used to make this product? T/I

B. What type of reactions do the enzymes catalyze, and why is this of value? T/I

C. What are the sources of the enzymes? T/I

D. Have the enzymes been modified for the process? T/I

E. How are the enzymes regulated? T/I

F. Why are the enzymes not used in the organism or cell where they are naturally found? T/I

G. What careers are associated with the industry that produces the product you chose? T/I

WEB LINK

1.7 Review

Summary

- An enzyme is a biological catalyst with a specific three-dimensional shape, which is necessary for its function. The active site of an enzyme is specific to a particular substrate(s).
- Enzyme activity is affected by substrate and enzyme concentrations, temperature, and pH.
- Competitive inhibitors enter an enzyme's active site to block the binding of the substrate. Noncompetitive inhibitors attach to another site on the enzyme, which changes the shape of the enzyme and its affinity for the substrate.
- Allosteric regulation of enzymes can inhibit or stimulate enzyme activity by altering the affinity of the active site for the substrate.
- Biochemical pathways often use feedback inhibition as a mechanism for regulating the pathway. In negative feedback inhibition, an enzyme involved at the beginning of the pathway is inhibited by a product in the pathway.
- There are many industrial and commercial uses of enzymes.

Questions

1. (a) What is a substrate? What is an active site? How are they related?
   (b) Why is an enzyme considered a biological catalyst? K/U
2. Describe the induced-fit hypothesis of an enzyme-substrate interaction. K/U
3. What is the functional role of a coenzyme or a cofactor in an enzyme-induced reaction? Give an example of an enzyme that requires a cofactor or a coenzyme. K/U
4. Vitamins and their derivatives are important for enzymatic activity and cell metabolism. Research the effects of the absence or overconsumption of one water-soluble vitamin in a diet. Prepare a short report. T/I
5. How does the rate of a reaction change as a result of each of the following factors? Support your answer with a graphic representation of the enzymatic rates. K/U
   (a) enzyme concentration
   (b) substrate concentration
   (c) temperature
   (d) pH
6. Describe noncompetitive enzyme inhibition. Provide an example to support your answer. K/U
7. Malonate is a competitive inhibitor of an enzyme called succinate dehydrogenase. Research this interaction and describe how malonate prevents the enzyme from acting on its substrate, succinate. K/U
8. Describe the different effects of an activator and an inhibitor on an allosterically regulated enzyme. K/U
9. Describe how feedback inhibition reduces the waste of cellular resources. K/U T/I
10. Why is it important for the human body to maintain a proper temperature and a proper pH at all times? K/U
11. You are making a gelatin dessert, but the directions tell you not to use fresh pineapple because the gelatin will not solidify. Gelatin is a structural protein made from collagen. Pineapple contains an enzyme, bromelain, which is a protease. T/I
    (a) What effect does a protease have on a protein like collagen?
    (b) Could you use cooked or canned pineapple instead? Explain your answer.
12. Humans produce enzymes in the mouth, stomach, and small intestine that aid in the process of digestion. As we age, we tend to produce less of these enzymes. What effect could this have on digestion and nutrition? T/I A
13. Digestion cannot take place unless water is present. Explain this statement. T/I

WEB LINK