Enzymes are protein catalysts. A catalyst is a substance that speeds up a chemical reaction without being consumed in the process. In catalyzed reactions, the reactants are converted into products faster than they would be without the catalyst, and, at the end of the process, the catalyst is regenerated intact, ready to catalyze the same reaction again. Chemical change takes place when the bonds between reactant molecules break, and a rearrangement of atoms creates the bonds between the atoms of product molecules. For this to occur, reactant molecules must collide with enough force and with the correct geometric orientation for bond breaking to occur. Only under these conditions can the transition state be reached and product molecules formed. As mentioned earlier, all reactions, whether endergonic or exergonic, possess an activation energy ($E_A$) barrier that must be overcome for reaction to occur (Figure 1). Heat provides the activation energy for many reactions. The spark from a spark plug provides the activation energy for the combustion of gasoline in an internal combustion engine. Without the spark, the gasoline–oxygen collisions are ineffective and do not allow the reactants to reach the transition state.

Although an increase in temperature increases the rate of most reactions, proteins are denatured at high temperatures and lose their function. This could be devastating for the cell. Therefore, living cells cannot rely on high levels of heat as a source of activation energy. Catalysts allow reactions to proceed at suitable rates at moderate temperatures by reducing the activation energy ($E_A$) barrier (Figure 1).

The catalyst does not affect the free energy change ($\Delta G$) of a reaction. It cannot change an endergonic reaction into an exergonic reaction; it can only decrease the potential energy level of the transition state and, thus, allow a greater proportion of colliding reactants to reach the transition state and become products. Catalysts can only speed up reactions that would normally take place anyway. Since a catalyst works by reducing the energy of activation, it speeds up forward and reverse reactions equally. Thus, it cannot affect the position of equilibrium, only the speed in which equilibrium is reached.

The **substrate** is the reactant that an enzyme acts on when it catalyzes a chemical reaction. The substrate binds to a particular site on the enzyme to which it is attracted. Enzymes are proteins in tertiary or quaternary structure with complex conformations. They are very specific for the substrate to which they bind. In most cases, they will not bind isomers of their substrate. The names of enzymes usually end in -ase. Thus, amylase catalyzes the breakdown of amylose into maltose subunits, and maltase catalyzes

![Figure 1](image-url)

**Figure 1**
A catalyst speeds up endergonic and exergonic reactions by lowering the activation energy, but it does not change the value of $\Delta G$.

(a) The effect of a catalyst on an exergonic reaction
(b) The effect of a catalyst on an endergonic reaction
the breakdown of maltose into individual glucose molecules. An enzyme-catalyzed reaction is usually written with the name of the enzyme over the arrow, as follows:

\[
\text{amylase} \quad \text{amylase} \\
\text{maltose} \quad \text{maltose} \\
\text{H}_2\text{O} \quad \text{H}_2\text{O} \\
\text{maltase} \quad \text{maltase} \\
\text{α-glucose} \quad \text{α-glucose}
\]

Notice that reactions are reversible.

**A Model of Enzyme Activity**

In an enzyme-catalyzed reaction, the substrate binds to a very small portion of the enzyme. The location where the substrate binds to the enzyme is called the **active site**, and is usually a pocket or groove in the three-dimensional structure of the protein (Figure 2). The substrate and the active site must possess compatible shapes for binding to occur. As the substrate enters the active site, its functional groups come close to the functional groups of a number of amino acids. The interactions between these chemical groups cause the protein to change its shape, thereby better accommodating the substrate. This is known as the **induced-fit model** of enzyme–substrate interaction. The attachment of the substrate to the enzyme’s active site creates the **enzyme–substrate complex**.

---

**active site** the location where the substrate binds to an enzyme

**induced-fit model** a model of enzyme activity that describes an enzyme as a dynamic protein molecule that changes shape to better accommodate the substrate

**enzyme–substrate complex** an enzyme with its substrate attached to the active site

---

**Figure 2**

This photo illustrates the binding of a substrate to the active site of an enzyme. The action induces a conformational change in the enzyme’s structure that prepares the substrate for reaction.

**Figure 3**

The enzyme maltase catalyzes the hydrolysis of maltose into two separate glucose molecules by breaking the α 1–4 glycosidic linkage.
In Figure 3, the enzyme maltase catalyzes the hydrolysis of maltose into two separate glucose molecules by breaking the α1–4 glycosidic linkage. Figure 3(a) shows the active site ready to receive the substrate. In (b), the substrate enters the active site and forms weak bonds while the enzyme’s conformation changes to better accommodate the substrate (induced fit). In (c), the glycosidic linkage between the two glucose molecules is broken (using a water molecule) while the substrate is in the active site. In (d), breaking the glycosidic linkage changes the conformation of the protein slightly; it loses affinity for the product molecules and releases them. The active site now becomes available for another maltose to attach. The recycling of enzyme molecules causes cells to catalyze many reactions with relatively small numbers of enzymes.

TRYTHIS activity

Synthesizing a Paper Clip Polymer with a Paper Enzyme

Materials: coloured paper clips, 5-cm x 21.5-cm strip of paper

- Prepare the strip of paper as in Figure 4(a).
- Fold the strip of paper as in Figure 4(b).
- Place paper clip substrate 1 on active site 1, spanning back two layers of paper enzyme. Place paper clip substrate 2 on active site 2, spanning front two layers of paper enzyme, as in Figure 4(c).
- Briskly pull the two tabs apart to activate the paper enzyme, as illustrated in Figure 4(d).
  (a) Explain how the action of the paper enzyme relates to a real enzyme-catalyzed condensation reaction.
  (b) Try to produce a “triclipide” or a “tetraclipide” with one pull of the tabs.
  (c) Create a different enzyme simulation, perhaps a simulation of an enzyme-catalyzed hydrolytic process.
Enzymes, such as maltase, decrease the energy of activation by stretching and bending chemical bonds that normally break in the reaction. Heat energy provided by the cell’s internal environment and the action of the enzyme brings the substrate molecule to the transition state. In other cases, the active site may provide an acidic environment in an otherwise neutral part of the cell. Acidic R groups may be prevalent in the area of the active site. This may provide the low pH environment needed for certain reactions to take place.

Enzyme-catalyzed reactions can be saturated. There are a limited number of specific enzyme molecules in a cell at any one time. Since it takes some time for a catalyst to catalyze a particular reaction, the speed at which a catalyzed reaction proceeds cannot increase indefinitely by increasing the concentration of the substrate.

Temperature and pH affect enzyme activity. As with all other reactions, enzyme-catalyzed reactions increase in speed with an increase in temperature, as illustrated in Figure 5(a). However, as the temperature increases beyond a critical point, thermal agitation begins to disrupt protein structure, resulting in denaturation and loss of enzyme function. Every enzyme has an optimal temperature at which it works best. Enzyme activity decreases above and below the optimal temperature. Most human enzymes work best at around 37°C, normal body temperature. There are enzymes in certain species of archaebacteria that work best at or above 100°C. Enzymes also have an optimal pH in which they work best, as Figure 5(b) shows. The digestive enzyme pepsin works best in the acidic environment of the stomach, pH 2. The digestive enzyme trypsin has an optimal pH of 8 and works best in the alkaline environment of the small intestine.

**INVESTIGATION 1.4.1**

**Factors Affecting the Rate of Enzyme Activity (p. 82)**

Enzymes are protein catalysts that, in most cases, catalyze only one chemical reaction. The ability of an enzyme to bind to its substrate and effectively catalyze a reaction is largely determined by its three-dimensional structure and certain environmental conditions. What are the conditions that optimize enzyme activity and how do changes affect enzyme function? In Investigation 1.4.1, you will design controlled experiments to examine the rate of a common enzyme’s activity under various conditions.

**cofactors** nonprotein components, such as dissolved ions, that are needed for some enzymes to function

**coenzymes** organic nonprotein cofactors that are needed for some enzymes to function

Some enzymes require either nonprotein cofactors, such as inorganic substances or organic coenzymes, before they can work properly. These may bind to the active site with covalent bonds, or they may bind weakly with the substrate. Cofactors include zinc ions (Zn²⁺) and manganese ions (Mn²⁺). Coenzymes include the derivatives of many vitamins. One of the most important coenzymes is nicotinamide adenine dinucleotide (NAD⁺), a derivative of vitamin B₃ (niacin). This substance acts as an electron carrier in cellular respiration. A similar compound called nicotinamide adenine dinucleotide phosphate (NADP⁺) performs a similar role in photosynthesis. Many coenzymes shuttle molecules from one enzyme to another. Their activities will be discussed further in Chapter 2.
Enzyme Inhibition

A variety of substances inhibit enzyme activity. **Competitive inhibitors** are so similar to the enzyme’s substrate that they can enter the enzyme’s active site and block the normal substrate from binding, as shown in **Figures 6(a)** and **(b)**. This process is reversible and can be overcome by increasing the concentration of the enzyme’s substrate, allowing it to compete favourably with the inhibitor. **Noncompetitive inhibitors** differ from the competitive types. They do not compete with an enzyme’s substrate for the active site. Instead they attach to another site on the enzyme, causing a change in the enzyme’s shape, as illustrated in **Figure 6(c)**. This changes the active site in such a way that it loses affinity for its substrate. Alternatively, the inhibitor may affect those parts of the active site that perform the work of catalysis, resulting in a loss of enzyme activity. An example is DDT, a poison that inhibits enzymes of the nervous system. However, not all enzyme inhibitors are poisons; some are used by the cell to control enzyme activity.

![Enzyme Inhibition Diagram](image)

Allosteric Regulation

Cells must control enzyme activity to coordinate cellular activities. They may accomplish this in two ways: by restricting the production of a particular enzyme, or by inhibiting the action of an enzyme that has already been produced. Some enzymes possess receptor sites, called **allosteric sites**, that are some distance away from the active site. Substances that bind to the allosteric sites may inhibit or stimulate an enzyme’s activity. Allosterically controlled enzymes are usually composed of proteins in quaternary structure having several subunits, each with an active site. Binding an **activator** to an allosteric site stabilizes the protein conformation that keeps all of the active sites available to their substrates (**Figure 7**). Binding an **allosteric inhibitor** stabilizes the inactive form of the enzyme. Noncompetitive inhibitors attach to the allosteric sites of certain enzymes. The binding of an activator or inhibitor to one allosteric site will affect the activity of all the active sites on the enzyme. Allosteric regulators attach to their sites using weak bonds.

![Allosteric Regulation Diagram](image)

**Competitive inhibitors** substances that compete with the substrate for an enzyme’s active site

**Noncompetitive inhibitors** substances that attach to a binding site on an enzyme other than the active site, causing a change in the enzyme’s shape and a loss of affinity for its substrate

**Allosteric sites** receptor sites, some distance from the active site of certain enzymes, that bind substances that may inhibit or stimulate an enzyme’s activity

**Activator** a substance that binds to an allosteric site on an enzyme and stabilizes the protein conformation that keeps all the active sites available to their substrates

**Allosteric inhibitor** a substance that binds to an allosteric site on an enzyme and stabilizes the inactive form of the enzyme

![Allosteric Regulation Diagram](image)
Feedback Inhibition

Feedback inhibition is a method used by cells to control metabolic pathways involving a series of sequential reactions, each catalyzed by a specific enzyme. In this case, a product formed later in the sequence of reaction steps allosterically inhibits an enzyme that catalyzes a reaction occurring earlier in the process (Figure 8). This effectively reduces the production of the inhibitor, which is, at the same time, a product of the process. The inhibitor binds to the allosteric site of the enzyme using weak bonds. As the product is used up over time, its concentration decreases. This causes the enzyme to be in the active form more often and the production of the inhibitor product increases. As this occurs, inhibition of the enzyme increases again and the production of the inhibitor is reduced once again. Thus, the amount of product is kept tightly controlled by the feedback inhibition process. Feedback inhibition is one of the most common control mechanisms used in metabolism.

![Figure 8](image)

The production of the amino acid isoleucine is regulated by feedback inhibition. In this case, the end product, isoleucine, allosterically inhibits enzyme 1, threonine deaminase.

Finally, cells control metabolic processes by restricting the location of enzymes and enzyme complexes to certain locations within the cell. In some cases, enzymes are incorporated into specific membranes or fluid-filled spaces in the cell. Some of the enzymes for cellular respiration are inside the mitochondria, whereas others are dissolved in the cell’s cytoplasm. Thus, the rate of the inner mitochondrial reactions can be controlled by restricting the movement into the mitochondrion of intermediates that are formed in the cytoplasm.
Commercial and Industrial Uses of Enzymes

Many enzymes are used in industrial processes and consumer products. One of the largest industrial users of enzymes is the starch-processing industry. Starch from corn, wheat, and other grains is a plentiful carbohydrate resource, consisting of amylose and amylopectin. Starch can be converted to glucose syrups by hydrolysis, and the syrups find many applications as sweeteners in foods, such as candy, biscuits, jams and jellies, and pharmaceuticals like cough syrups, tonics, and vitamin preparations. Hydrolysis can be accomplished without enzymes, but this method can generate distasteful flavours and unsightly colours. Enzymatic hydrolysis offers a cleaner and more efficient hydrolysis. In a common process, \( \alpha \)-amylose, produced by the bacterium *Bacillus licheniformis*, hydrolyzes starch to maltose. Then the enzyme glucoamylase, produced by moulds such as *Aspergillus* and *Rhizopus*, is added to hydrolyze maltose into individual glucose molecules.

\[
\text{amylose and amylopectin} \rightarrow \text{maltose} \rightarrow \alpha\text{-glucose}
\]

The most frequently used enzymes in the dairy industry are the protein-hydrolyzing enzymes, called proteases. They are most commonly used to coagulate milk for the manufacture of cheese. The cheese industry often uses the term rennet to refer to an enzyme preparation that coagulates milk. Historically, rennet was obtained from the stomach of calves. The primary enzyme obtained from this source is chymosin, which normally aids calves in the digestion of milk proteins. Since around 1990, genetic engineering has made it possible to produce chymosin from microbial sources. The gene for chymosin was removed from the DNA of a calf stomach cell and inserted into the DNA of certain bacteria and yeast cells (genetic engineering is discussed further in Chapter 6). The microbes reproduce rapidly in large vats containing a nutrient broth and secrete exact copies of calf chymosin. When making cheese, bacteria are added to milk to begin the curdling process. The bacteria feed on lactose in the milk and produce lactic acid as a waste product. The lactic acid lowers the pH and begins to denature milk proteins. Chymosin is then added to hydrolyze casein, a protein that makes up about 85% of the total protein in milk, causing it to coagulate into semisolid cheese curd. The curd shrinks and separates from a greenish liquid called whey. Whey is removed from the mixture and the curd is further processed according to the type of cheese being produced.

Many dairy products are made from cow's milk. Lactose, a disaccharide composed of glucose and galactose, accounts for 40% of the solids in cow's milk. Lactose is removed from some milk products because many people are intolerant to it and suffer gastrointestinal distress when exposed to it in their diet. Lactose-intolerant individuals may easily absorb and metabolize glucose and galactose. Lactose is hydrolyzed into glucose and galactose by the enzyme \( \alpha \)-galactosidase (lactase), which may be obtained from several microorganisms, including *Aspergillus niger* and *Saccharomyces lactis*.

\[
\text{lactose (milk sugar)} \rightarrow \text{lactose} \rightarrow \text{glucose + galactose}
\]

In addition to causing illness in people who are intolerant, lactose causes severe problems in cheese making. Large quantities of whey are made as a byproduct of cheese production. Whey contains the lactose present in the original milk. Problems arise because lactose is not particularly soluble and tends to crystallize out of solution. This leads to an undesirable grainy texture in some cheeses and ice creams. The enzyme \( \alpha \)-galactosidase produced by *S. lactis* is used to solve this problem because it is active at 4°C, the temperature at which milk is normally stored. This means that the enzyme can be added to the milk during the overnight cold storage period used in dairies.

DID YOU KNOW??

**Blueing in Cheese**

The blueing in blue cheeses, such as Gorgonzola, Roquefort, and Stilton, is produced by *Penicillium roqueforti* fungus, which occurs naturally or is added to the cheese during the ripening stage.
Fat-hydrolyzing enzymes called lipases are used by the dairy industry to develop characteristic cheeses, especially in Italian cheeses and other varieties with strong flavours. Although mozzarella receives most of its flavour from the proteases, lipases produce the strong-flavoured cheeses like Romano. These enzymes hydrolyze the fat in milk to produce free fatty acids. The type and concentration of fatty acids give the cheeses their distinct flavours.

In addition to the food industry, enzymes are also used in the cleaning industry. Dirt commonly found on clothing includes proteins, starch, and lipids. Although it is possible to remove these stains with soaps and detergents, enzymes allow stains to be removed at lower temperatures and with less mechanical agitation in a washing machine. Enzymes are also more effective than nonbiological cleaning agents at removing stains such as blood, grass, milk, and perspiration. The cleaning industry commonly adds proteases and amylases to detergents to help remove protein and carbohydrate stains from clothing and other fabrics. Additional uses of enzymes are outlined in Table 1.

### Table 1 Additional Uses of Enzymes

<table>
<thead>
<tr>
<th>Product/Process</th>
<th>Effects of enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ethanol</td>
<td>enzymes convert starch into sugars, which are then converted into ethanol</td>
</tr>
<tr>
<td>animal feed</td>
<td>degradation of feed components for improved feed utilization and nutrient digestion</td>
</tr>
<tr>
<td>baking</td>
<td>modification of flour for improved baking properties</td>
</tr>
<tr>
<td>brewing</td>
<td>faster maturation of beer, chill-proofing, removal of carbohydrates for light beers</td>
</tr>
<tr>
<td>dairy</td>
<td>enzymes used in cheese making, removal/conversion of lactose in milk</td>
</tr>
<tr>
<td>detergent</td>
<td>active biological component of washing powders or liquids; breakdown of starch and fatty stains by proteases, amylases, and lipases; colour brightening and softening of cotton garments by cellulases</td>
</tr>
<tr>
<td>leather</td>
<td>soaking of hides and skins; unhairing, batting, and defatting</td>
</tr>
<tr>
<td>protein</td>
<td>improvement of nutritional and functional properties of animal and vegetable proteins; development of flavour bases from proteins</td>
</tr>
<tr>
<td>pulp and paper</td>
<td>pulp bleaching, viscosity control in starch-based coatings, de-inking for recycling programs</td>
</tr>
<tr>
<td>starch</td>
<td>production of dextrose, fructose, and special syrups for the baking and soft-drink industries</td>
</tr>
<tr>
<td>textiles</td>
<td>stone washing of denim (in combination with pumice stones), bio-polishing and softening of cotton, bleaching, cleanup, removal of starch from woven materials</td>
</tr>
<tr>
<td>wine and juice</td>
<td>degradation of pectin for clarification and increase in juice yields</td>
</tr>
</tbody>
</table>

### SUMMARY

- An enzyme is a biological protein catalyst. The substrate is the reactant that an enzyme acts on when it catalyzes a chemical reaction. The active site is the location where the substrate binds to an enzyme.
- The induced-fit model of enzyme–substrate interaction describes a protein as a dynamic molecule that changes its shape to better accommodate the substrate.
1. Define catalyst.

2. Draw a labelled free-energy diagram to illustrate the effect of an enzyme on the activation energy of a hypothetical reaction. (Assume it is an exergonic reaction.)

3. What is meant by the statement, “an enzyme cannot affect the free-energy change of a reaction”?

4. Describe the induced-fit model of enzyme activity. Use diagrams if necessary.

5. How does an enzyme lower the activation energy of a biochemical reaction?

6. How do competitive enzyme inhibition and noncompetitive enzyme inhibition differ?

7. Enzymes in the testicles of males are responsible for both sperm and hormone (testosterone) production. Some of these enzymes have an optimal temperature of 33°C (4°C lower than normal body temperature). If this temperature is elevated or lowered, sperm and testosterone production are adversely affected.
   (a) What anatomical features help the testicles maintain this temperature?
   (b) Describe two lifestyle choices that could affect sperm and hormone production.

8. What happens to an enzyme after it has catalyzed a reaction?

9. The browning reaction that occurs when the flesh of fresh fruit comes into contact with air is caused by the naturally occurring enzyme polyphenol oxidase. Suggest a hypothesis for controlling the formation of the brown product and design a controlled experiment to test your hypothesis.

10. Papain and bromelain are the two most commonly used enzymes in commercial meat tenderizers. Conduct library or Internet research to answer the following questions regarding these two enzymes.
   (a) What are meat tenderizers? What are they used for?
   (b) What type of enzyme are papain and bromelain? (What is their substrate?)
   (c) What is the source of the papain and bromelain found in commercial meat tenderizer preparations?
   (d) What is the Milk Clot Assay (MSA), and how is it used in the meat processing industry?
   (e) The antemortem method of tenderizing meat involves the physical injection of solution of papain or bromelain into the living animal. The enzyme tenderizes muscle tissue while the animal is alive. Discuss this method with fellow classmates and write a brief position statement on the ethics of this procedure.

11. Recent advances in wound treatment include the use of enzymatic debridement preparations. Conduct library or Internet research to answer the following questions regarding novel debridement procedures.
   (a) What is debridement?
   (b) Why is debridement performed?
   (c) What debridement methods are available to the physician treating a wound?
   (d) What types of enzymes are used in chemical debridement procedures?
   (e) What are the benefits and drawbacks of enzymatic debridement?