# 19: ENZYMES AND VITAMINS

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# CHAPTER OVERVIEW

## 19: Enzymes and Vitamins

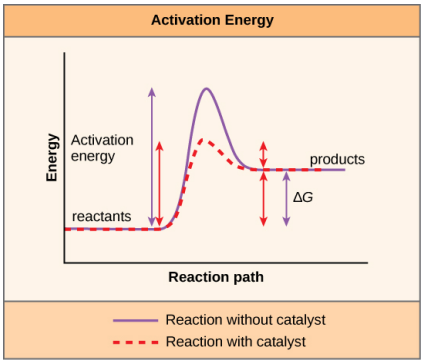


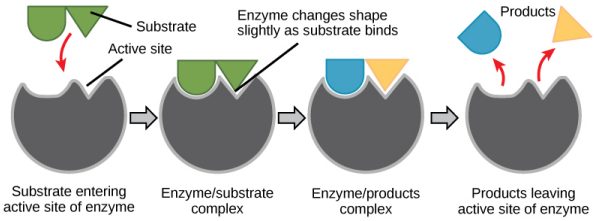
*Thumbnail: Ball-and-stick model of the pyridoxal phosphate molecule, the active form of vitamin $B_6$. (Public Domain)*

# 19.1: Catalysis by Enzymes

## Learning Objectives

- Describe how enzymes catalyze biochemical reactions.



# 19.2: Enzyme Cofactors

**Learning Objectives**

- To explain why vitamins are necessary in the diet.

Many enzymes are simple proteins consisting entirely of one or more amino acid chains. Other enzymes contain a nonprotein component called a **cofactor** that is necessary for the enzyme's proper functioning. There are two types of cofactors: inorganic ions [e.g., zinc or Cu(I) ions] and organic molecules known as coenzymes. Most **coenzymes** are vitamins or are derived from vitamins.

Vitamins are organic compounds that are essential in very small (trace) amounts for the maintenance of normal metabolism. They generally cannot be synthesized at adequate levels by the body and must be obtained from the diet. The absence or shortage of a vitamin may result in a vitamin-deficiency disease. In the first half of the 20th century, a major focus of biochemistry was the identification, isolation, and characterization of vitamins. Despite accumulating evidence that people needed more than just carbohydrates, fats, and proteins in their diets for normal growth and health, it was not until the early 1900s that research established the need for trace nutrients in the diet.

Table 19.2.1: Fat-Soluble Vitamins and Physiological Functions

| Vitamin | Physiological Function | Effect of Deficiency |
|---|---|---|
| vitamin A (retinol) | formation of vision pigments; differentiation of epithelial cells | night blindness; continued deficiency leads to total blindness |
| vitamin D (cholecalciferol) | increases the body's ability to absorb calcium and phosphorus | osteomalacia (softening of the bones); known as rickets in children |
| vitamin E (tocopherol) | fat-soluble antioxidant | damage to cell membranes |
| vitamin K (phylloquinone) | formation of prothrombin, a key enzyme in the blood-clotting process | increases the time required for blood to clot |

Because organisms differ in their synthetic abilities, a substance that is a vitamin for one species may not be so for another. Over the past 100 years, scientists have identified and isolated 13 vitamins required in the human diet and have divided them into two broad categories: the *fat-soluble vitamins*, which include vitamins A, D, E, and K, and the *water-soluble vitamins*, which are the B complex vitamins and vitamin C. All fat-soluble vitamins contain a high proportion of hydrocarbon structural components. There are one or two oxygen atoms present, but the compounds as a whole are nonpolar. In contrast, water-soluble vitamins contain large numbers of electronegative oxygen and nitrogen atoms, which can engage in hydrogen bonding with water. Most water-soluble vitamins act as coenzymes or are required for the synthesis of coenzymes. The fat-soluble vitamins are important for a variety of physiological functions. The key vitamins and their functions are found in Tables 19.2.1 and 19.2.2

Table 19.2.2: Water-Soluble Vitamins and Physiological Functions

| Vitamin | Coenzyme | Coenzyme Function | Deficiency Disease |
|---|---|---|---|
| vitamin $B_1$ (thiamine) | thiamine pyrophosphate | decarboxylation reactions | beri-beri |
| vitamin $B_2$ (riboflavin) | flavin mononucleotide or flavin adenine dinucleotide | oxidation-reduction reactions involving two hydrogen atoms | — |
| vitamin $B_3$ (niacin) | nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate | oxidation-reduction reactions involving the hydride ion ($H^-$) | pellagra |
| vitamin $B_6$ (pyridoxine) | pyridoxal phosphate | variety of reactions including the transfer of amino groups | — |
| vitamin $B_{12}$ (cyanocobalamin) | methylcobalamin or deoxyadenoxylcobalamin | intramolecular rearrangement reactions | pernicious anemia |
| biotin | biotin | carboxylation reactions | — |
| folic acid | tetrahydrofolate | carrier of one-carbon units such as the formyl group | anemia |

| Vitamin | Coenzyme | Coenzyme Function | Deficiency Disease |
|---|---|---|---|
| pantothenic Acid | coenzyme A | carrier of acyl groups | — |
| vitamin C (ascorbic acid) | none | antioxidant; formation of collagen, a protein found in tendons, ligaments, and bone | scurvy |

Vitamins C and E, as well as the provitamin β-carotene can act as antioxidants in the body. Antioxidants prevent damage from free radicals, which are molecules that are highly reactive because they have unpaired electrons. Free radicals are formed not only through metabolic reactions involving oxygen but also by such environmental factors as radiation and pollution.

> *β-carotene is known as a provitamin because it can be converted to vitamin A in the body.*

Free radicals react most commonly react with lipoproteins and unsaturated fatty acids in cell membranes, removing an electron from those molecules and thus generating a new free radical. The process becomes a chain reaction that finally leads to the oxidative degradation of the affected compounds. Antioxidants react with free radicals to stop these chain reactions by forming a more stable molecule or, in the case of vitamin E, a free radical that is much less reactive (vitamin E is converted back to its original form through interaction with vitamin C).

## Summary

Vitamins are organic compounds that are essential in very small amounts for the maintenance of normal metabolism. Vitamins are divided into two broad categories: fat-soluble vitamins and water-soluble vitamins. Most water-soluble vitamins are needed for the formation of coenzymes, which are organic molecules needed by some enzymes for catalytic activity.

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# 19.3: Enzyme Classification

**Learning Objectives**

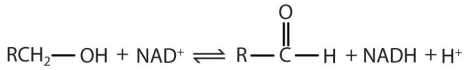
- Objective 1
- Objective 2

Hundreds of enzymes have been purified and studied in an effort to understand how they work so effectively and with such specificity. The resulting knowledge has been used to design drugs that inhibit or activate particular enzymes. An example is the intensive research to improve the treatment of or find a cure for acquired immunodeficiency syndrome (AIDS). AIDS is caused by the human immunodeficiency virus (HIV). Researchers are studying the enzymes produced by this virus and are developing drugs intended to block the action of those enzymes without interfering with enzymes produced by the human body. Several of these drugs have now been approved for use by AIDS patients.

## Enzyme Nomenclature

Most enzymes can be recognized because they have the family name ending *–ase*. However, the first enzymes to be discovered were named according to their source or method of discovery. The enzyme *pepsin*, which aids in the hydrolysis of proteins, is found in the digestive juices of the stomach (Greek *pepsis*, meaning “digestion”). *Papain*, another enzyme that hydrolyzes protein (in fact, it is used in meat tenderizers), is isolated from papayas.

In addition to the family name, more systematic enzyme names will give two sepcific pieces of information: the first part is the *substrate* upon which the enzyme acts, and the second part is the *type of reaction* it catalyzes. For example, alcohol dehydrogenase (Figure 19.3.1) catalyzes the *oxidation* of an *alcohol* to an aldehyde.



## Enzyme Classification

As more enzymes were discovered, chemists recognized the need for a more systematic and chemically informative identification scheme. In the current numbering and naming scheme, under the oversight of the Nomenclature Commission of the International Union of Biochemistry, enzymes are arranged into *six groups* according to the general type of reaction they catalyze (Table 19.3.1), with subgroups and secondary subgroups that specify the reaction more precisely.

Each enzyme is assigned a four-digit number, preceded by the prefix EC—for enzyme classification—that indicates its group, subgroup, and so forth. This is demonstrated in Table 19.3.2 for alcohol dehydrogenase.

Table 19.3.1: Classes of Enzymes

| Main Class | Type of Reaction Catalyzed | Subclasses | Examples |
|---|---|---|---|
| **Oxidoreductases** | oxidation-reduction reactions | *Dehydrogenases* catalyze oxidation-reduction reactions involving hydrogen. | Alcohol dehydrogenase |
| | | *Oxidases* catalyze oxidation by addition of $O_2$ to a substrate. | |
| | | *Reductases* catalyze reactions in which a substrate is reduced. | |
| **Transferases** | transfer reactions of functional groups | *Transaminases* catalyze the transfer of amino group. | |
| | | *Kinases* catalyze the transfer of a phosphate group. | Phosphofructokinase |
| **Hydrolases** | reactions that use water to break a chemical bond | *Lipases* catalyze the hydrolysis of lipids | |

| Main Class | Type of Reaction Catalyzed | Subclasses | Examples |
|---|---|---|---|
| | | *Proteases* catalyze the hydrolysis of proteins | |
| | | *Amylases* catalyze the hydrolysis of carbohydrates | |
| | | *Nucleases* catalyze the hydrolysis of DNA and RNA | |
| **Lyases** | reactions in which functional groups are added or removed without hydrolysis | *Decarboxylases* catalyze the removal of carboxyl groups. | |
| | | *Deaminases* catalyze the removal of amino groups. | |
| | | *Dehydratases* catalyze the removal of water. | |
| | | *Hydratases* catalyze the addition of water. | Fumarase |
| **Isomerases** | reactions in which a compound is converted to its isomer | *Isomerases* may catalyze the conversion of an aldose to a ketose. | Triose Phosphate Isomerase |
| | | *Mutases* catalyze reactions in which a functional group is transferred from one atom in a substrate to another. | |
| **Ligases** | reactions in which new bonds are formed between carbon and another atom; energy is required | *Synthetases* catalyze reactions in which two smaller molecules are linked to form a larger one. | |
| | | *Carboxylases* catalyze the addition of $CO_2$ using ATP | Pyruvate Carboxylase |

Table 19.3.2: Assignment of an Enzyme Classification Number

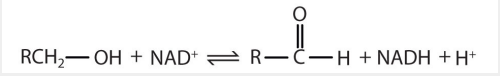
| Alcohol Dehydrogenase: EC 1.1.1.1 | |
|---|---|
| The first digit indicates that this enzyme is an oxidoreductase; that is, an enzyme that catalyzes an oxidation-reduction reaction. | |
| The second digit indicates that this oxidoreductase catalyzes a reaction involving a primary or secondary alcohol. | |
| The third digit indicates that either the coenzyme $NAD^+$ or $NADP^+$ is required for this reaction. | |
| The fourth digit indicates that this was the first enzyme isolated, characterized, and named using this system of nomenclature. | |
| The systematic name for this enzyme is *alcohol:$NAD^+$ oxidoreductase*, while the recommended or common name is alcohol dehydrogenase. | |
| Reaction catalyzed: |  |

***Figure* 19.3.1*:*** *Structure of the alcohol dehydrogenase protein (E.C.1.1.1.1) (EE ISOZYME) complexed wtih nicotinamide adenini dinulceotide (NAD) and zinc (PDB: 1CDO).*

## Summary

An enzyme is a biological catalyst, a substance that increases the rate of a chemical reaction without being changed or consumed in the reaction. A systematic process is used to name and classify enzymes.

# 19.4: How Enzymes Work

**Learning Objectives**

- To describe the interaction between an enzyme and its substrate.

Enzyme-catalyzed reactions occur in at least two steps. In the first step, an enzyme (E) and the substrate molecule or molecules (S) collide and react to form an intermediate compound called the *enzyme-substrate* (ES) *complex*. (This step is reversible because the complex can break apart into the original substrate or substrates and the free enzyme.) Once the ES complex forms, the enzyme is able to catalyze the formation of product (P), which is then released from the enzyme surface:

$$E + S \leftrightarrow ES \tag{19.4.1}$$

$$ES \rightarrow E + P \tag{19.4.2}$$

Hydrogen bonding and other electrostatic interactions hold the enzyme and substrate together in the complex. The structural features or functional groups on the enzyme that participate in these interactions are located in a cleft or pocket on the enzyme surface. This pocket, where the enzyme combines with the substrate and transforms the substrate to product is called the active site of the enzyme (Figure 19.4.1).

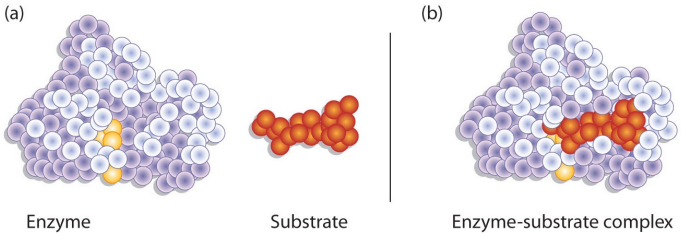



Figure 19.4.1: Substrate Binding to the Active Site of an Enzyme. The enzyme dihydrofolate reductase is shown with one of its substrates: $NADP^+$ (a) unbound and (b) bound. The $NADP^+$ (shown in red) binds to a pocket that is complementary to it in shape and ionic properties.

## Models of Enzyme-Substrate Interaction

The active site of an enzyme possesses a unique conformation (including correctly positioned bonding groups) that is complementary to the structure of the substrate, so that the enzyme and substrate molecules fit together in much the same manner as a key fits into a tumbler lock. In fact, an early model describing the formation of the enzyme-substrate complex was called the **lock-and-key model** (Figure 19.4.2). This model portrayed the enzyme as conformationally rigid and able to bond only to substrates that exactly fit the active site.

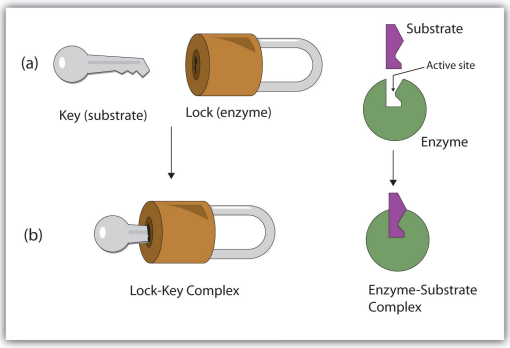



Figure 19.4.2: The Lock-and-Key Model of Enzyme Action. (a) Because the substrate and the active site of the enzyme have complementary structures and bonding groups, they fit together as a key fits a lock. (b) The catalytic reaction occurs while the two are bonded together in the enzyme-substrate complex.

Working out the precise three-dimensional structures of numerous enzymes has enabled chemists to refine the original lock-and-key model of enzyme actions. They discovered that the binding of a substrate often leads to a large conformational change in the enzyme, as well as to changes in the structure of the substrate or substrates. The current theory, known as the **induced-fit model**,

says that enzymes can undergo a change in conformation when they bind substrate molecules, and the active site has a shape complementary to that of the substrate only *after* the substrate is bound, as shown for hexokinase in Figure 19.4.3. After catalysis, the enzyme resumes its original structure.

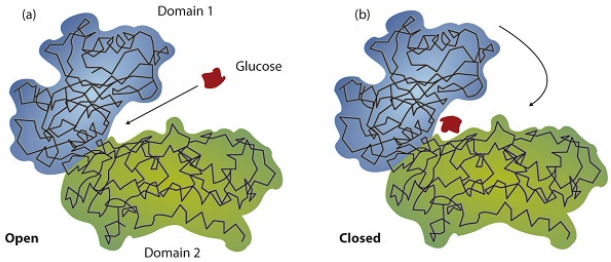



Figure 19.4.3: The Induced-Fit Model of Enzyme Action. (a) The enzyme hexokinase without its substrate (glucose, shown in red) is bound to the active site. (b) The enzyme conformation changes dramatically when the substrate binds to it, resulting in additional interactions between hexokinase and glucose.

The structural changes that occur when an enzyme and a substrate join together bring specific parts of a substrate into alignment with specific parts of the enzyme's active site. Amino acid side chains in or near the binding site can then act as acid or base catalysts, provide binding sites for the transfer of functional groups from one substrate to another or aid in the rearrangement of a substrate. The participating amino acids, which are usually widely separated in the primary sequence of the protein, are brought close together in the active site as a result of the folding and bending of the polypeptide chain or chains when the protein acquires its tertiary and quaternary structure. Binding to enzymes brings reactants close to each other and aligns them properly, which has the same effect as increasing the concentration of the reacting compounds.

### ✔ Example 19.4.1

a. What type of interaction would occur between an OH group present on a substrate molecule and a functional group in the active site of an enzyme?
b. Suggest an amino acid whose side chain might be in the active site of an enzyme and form the type of interaction you just identified.

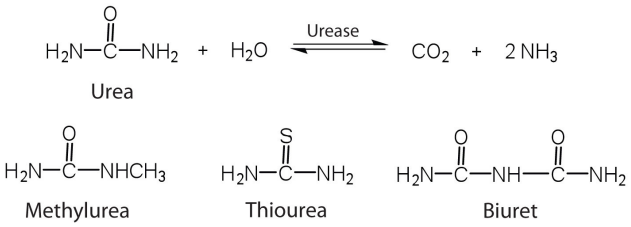
#### Solution

a. An OH group would most likely engage in hydrogen bonding with an appropriate functional group present in the active site of an enzyme.
b. Several amino acid side chains would be able to engage in hydrogen bonding with an OH group. One example would be asparagine, which has an amide functional group.

### ? Exercise 19.4.1

a. What type of interaction would occur between an $COO^-$ group present on a substrate molecule and a functional group in the active site of an enzyme?
b. Suggest an amino acid whose side chain might be in the active site of an enzyme and form the type of interaction you just identified.

One characteristic that distinguishes an enzyme from all other types of catalysts is its **substrate specificity**. An inorganic acid such as sulfuric acid can be used to increase the reaction rates of many different reactions, such as the hydrolysis of disaccharides, polysaccharides, lipids, and proteins, with complete impartiality. In contrast, enzymes are much more specific. Some enzymes act on a single substrate, while other enzymes act on any of a group of related molecules containing a similar functional group or chemical bond. Some enzymes even distinguish between D- and L-stereoisomers, binding one stereoisomer but not the other. Urease, for example, is an enzyme that catalyzes the hydrolysis of a single substrate—urea—but not the closely related compounds methyl urea, thiourea, or biuret. The enzyme carboxypeptidase, on the other hand, is far less specific. It catalyzes the removal of nearly any amino acid from the carboxyl end of any peptide or protein.




Enzyme specificity results from the uniqueness of the active site in each different enzyme because of the identity, charge, and spatial orientation of the functional groups located there. It regulates cell chemistry so that the proper reactions occur in the proper place at the proper time. Clearly, it is crucial to the proper functioning of the living cell.

## Summary

A substrate binds to a specific region on an enzyme known as the active site, where the substrate can be converted to product. The substrate binds to the enzyme primarily through hydrogen bonding and other electrostatic interactions. The induced-fit model says that an enzyme can undergo a conformational change when binding a substrate. Enzymes exhibit varying degrees of substrate specificity.

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# 19.5: Factors Affecting Enzyme Activity

**Learning Objectives**

- To describe how pH, temperature, and the concentration of an enzyme and its substrate influence enzyme activity.

The single most important property of enzymes is the ability to increase the rates of reactions occurring in living organisms, a property known as *catalytic activity*. Because most enzymes are proteins, their activity is affected by factors that disrupt protein structure, as well as by factors that affect catalysts in general. Factors that disrupt protein structure include temperature and pH; factors that affect catalysts in general include reactant or substrate concentration and catalyst or enzyme concentration. The activity of an enzyme can be measured by monitoring either the rate at which a substrate disappears or the rate at which a product forms.

## Substrate Concentration

In the presence of a given amount of enzyme, the rate of an enzymatic reaction increases as the substrate concentration increases until a limiting rate is reached, after which further increase in the substrate concentration produces no significant change in the reaction rate (part (a) of Figure 19.5.1). At this point, so much substrate is present that essentially all of the enzyme active sites have substrate bound to them. In other words, the enzyme molecules are saturated with substrate. The excess substrate molecules cannot react until the substrate already bound to the enzymes has reacted and been released (or been released without reacting).

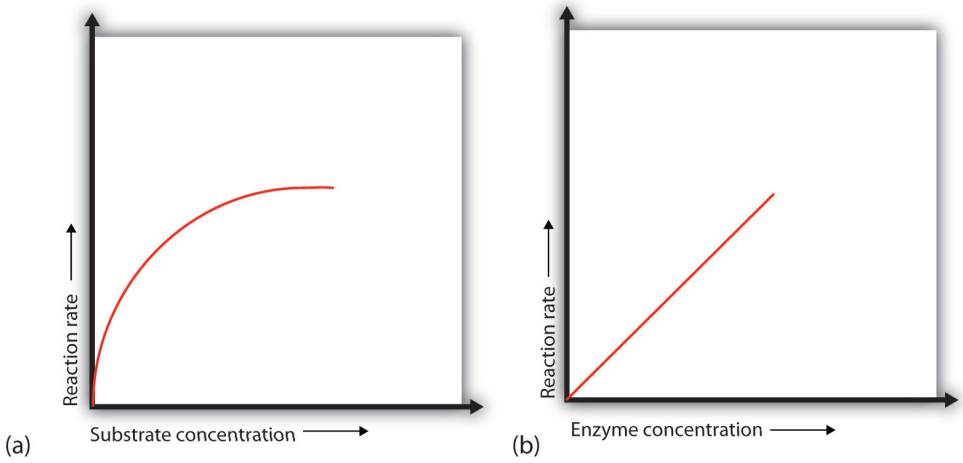


Figure 19.5.1: Concentration versus Reaction Rate. (a) This graph shows the effect of substrate concentration on the rate of a reaction that is catalyzed by a fixed amount of enzyme. (b) This graph shows the effect of enzyme concentration on the reaction rate at a constant level of substrate.

Let's consider an analogy. Ten taxis (enzyme molecules) are waiting at a taxi stand to take people (substrate) on a 10-minute trip to a concert hall, one passenger at a time. If only 5 people are present at the stand, the rate of their arrival at the concert hall is 5 people in 10 minutes. If the number of people at the stand is increased to 10, the rate increases to 10 arrivals in 10 minutes. With 20 people at the stand, the rate would still be 10 arrivals in 10 minutes. The taxis have been "saturated." If the taxis could carry 2 or 3 passengers each, the same principle would apply. The rate would simply be higher (20 or 30 people in 10 minutes) before it leveled off.

## Enzyme Concentration

When the concentration of the enzyme is significantly lower than the concentration of the substrate (as when the number of taxis is far lower than the number of waiting passengers), the rate of an enzyme-catalyzed reaction is directly dependent on the enzyme concentration (part (b) of Figure 19.5.1). This is true for any catalyst; the reaction rate increases as the concentration of the catalyst is increased.

## Effect of Temperature on Activity

A general rule of thumb for most chemical reactions is that a temperature rise of 10°C approximately doubles the reaction rate. To some extent, this rule holds for all enzymatic reactions. After a certain point, however, an increase in temperature causes a decrease

in the enzyme reaction rate, due to *denaturation* of the protein structure and disruption of the active site (part (a) of Figure 19.5.2). For many proteins, denaturation occurs between 45°C and 55°C. Furthermore, even though an enzyme may appear to have a maximum reaction rate between 40°C and 50°C, most biochemical reactions are carried out at lower temperatures because enzymes are not stable at these higher temperatures and will denature after a few minutes.

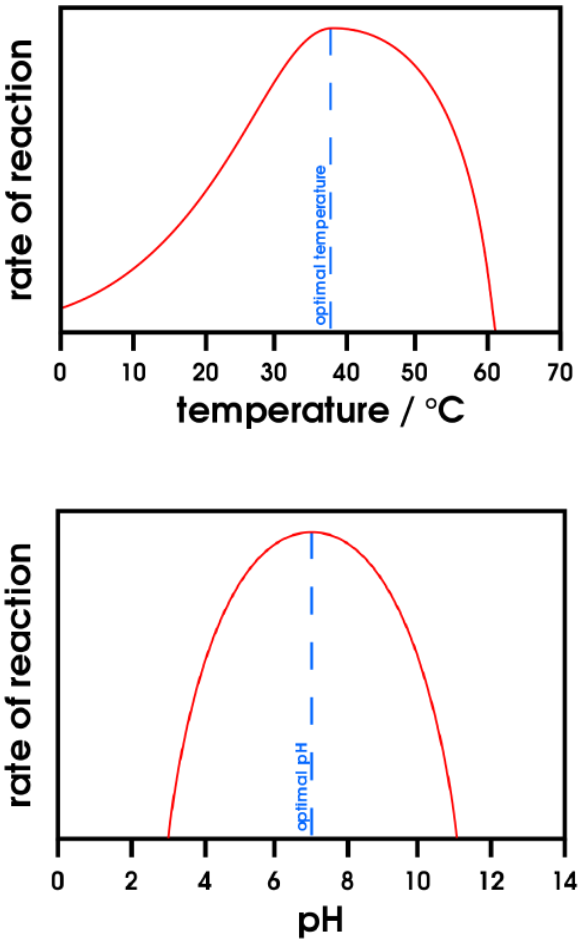


Figure 19.5.2: Temperature and pH versus Concentration. (a) This graph depicts the effect of temperature on the rate of a reaction that is catalyzed by a fixed amount of enzyme. (b) This graph depicts the effect of pH on the rate of a reaction that is catalyzed by a fixed amount of enzyme.

At 0°C and 100°C, the rate of enzyme-catalyzed reactions is nearly zero. This fact has several practical applications. We sterilize objects by placing them in boiling water, which denatures the enzymes of any bacteria that may be in or on them. We preserve our food by refrigerating or freezing it, which slows enzyme activity. When animals go into hibernation in winter, their body temperature drops, decreasing the rates of their metabolic processes to levels that can be maintained by the amount of energy stored in the fat reserves in the animals' tissues.

## Effect of Hydrogen Ion Concentration (pH) on Activity

Because most enzymes are proteins, they are sensitive to changes in the hydrogen ion concentration or pH. Enzymes may be denatured by extreme levels of hydrogen ions (whether high or low); *any* change in pH, even a small one, alters the degree of ionization of an enzyme's acidic and basic side groups and the substrate components as well. Ionizable side groups located in the active site must have a certain charge for the enzyme to bind its substrate. Neutralization of even one of these charges alters an enzyme's catalytic activity.

An enzyme exhibits maximum activity over the narrow pH range in which a molecule exists in its properly charged form. The median value of this pH range is called the optimum pH of the enzyme (part (b) of Figure 19.5.2). With the notable exception of

gastric juice (the fluids secreted in the stomach), most body fluids have pH values between 6 and 8. Not surprisingly, most enzymes exhibit optimal activity in this pH range. However, a few enzymes have optimum pH values outside this range. For example, the optimum pH for pepsin, an enzyme that is active in the stomach, is 2.0.

## Summary

Initially, an increase in substrate concentration leads to an increase in the rate of an enzyme-catalyzed reaction. As the enzyme molecules become saturated with substrate, this increase in reaction rate levels off. The rate of an enzyme-catalyzed reaction increases with an increase in the concentration of an enzyme. At low temperatures, an increase in temperature increases the rate of an enzyme-catalyzed reaction. At higher temperatures, the protein is denatured, and the rate of the reaction dramatically decreases. An enzyme has an optimum pH range in which it exhibits maximum activity.

## Concept Review Exercises

1. The concentration of substrate X is low. What happens to the rate of the enzyme-catalyzed reaction if the concentration of X is doubled?
2. What effect does an increase in the enzyme concentration have on the rate of an enzyme-catalyzed reaction?

## Answers

1. If the concentration of the substrate is low, increasing its concentration will increase the rate of the reaction.
2. An increase in the amount of enzyme will increase the rate of the reaction (provided sufficient substrate is present).

## Exercises

1. In non-enzyme-catalyzed reactions, the reaction rate increases as the concentration of reactant is increased. In an enzyme-catalyzed reaction, the reaction rate initially increases as the substrate concentration is increased but then begins to level off, so that the increase in reaction rate becomes less and less as the substrate concentration increases. Explain this difference.
2. Why do enzymes become inactive at very high temperatures?
3. An enzyme has an optimum pH of 7.4. What is most likely to happen to the activity of the enzyme if the pH drops to 6.3? Explain.
4. An enzyme has an optimum pH of 7.2. What is most likely to happen to the activity of the enzyme if the pH increases to 8.5? Explain.

## Answers

1. In an enzyme-catalyzed reaction, the substrate binds to the enzyme to form an enzyme-substrate complex. If more substrate is present than enzyme, all of the enzyme binding sites will have substrate bound, and further increases in substrate concentration cannot increase the rate.
3. The activity will decrease; a pH of 6.3 is more acidic than 7.4, and one or more key groups in the active site may bind a hydrogen ion, changing the charge on that group.

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# 19.6: Enzyme Regulation - Inhibition

**Learning Objectives**

- Explain what an enzyme inhibitor is.
- Distinguish between reversible and irreversible inhibitors.
- Distinguish between competitive, noncompetitive, and uncompetitive inhibitors.

Previously, we noted that enzymes can be inactivated at high temperatures and by changes in pH. These are *nonspecific* factors that would inactivate any enzyme. The activity of enzymes can also be regulated by more *specific* **inhibitors** that slow or stop catalysis. Enzyme inhibition can either be *reversible* or *irreversible*. In reversible inhibition, the inhibitor can bind (usually non-covalently) and dissociate, allowing enzyme activity to return back to its original, uninhibited level. Irreversible inhibitors bind to the enzyme permanently and thus permanently inhibit enzyme activity.

## Reversible Inhibition

Reversible enzyme inhibition can be *competitive*, *noncompetitive*, or *uncompetitive*, depending on where the inhibitor binds to the enzyme, substrate, or enzyme-substrate complex.

**Competitive inhibition** is when an inhibitor reversibly binds to an enzyme at the enzyme active site; competing with the substrate for binding. A competitive inhibitor must be a molecule that is *structurally similar* to the substrate molecule, allowing it to interact with the enzyme active site through similar non-covalent interactions, but it does not, or cannot, undergo the same chemical reaction. When the inhibitor is bound to the active site, it blocks the correct substrate from binding and catalysis from occurring. However, as a reversible inhibitor, it can disassociate from the enzyme eventually allowing for the correct substrate to bind and the catalysis to occur. Because the inhibitor and substrate are in competition for the same active site, inhibition is concentration-dependent. As shown in the below plot of rate of reaction vs. substrate concentration (Figure 19.6.1), the competitive inhibitor slows the rate of reaction, but at higher substrate concentrations, the normal maximum rate can be reached.

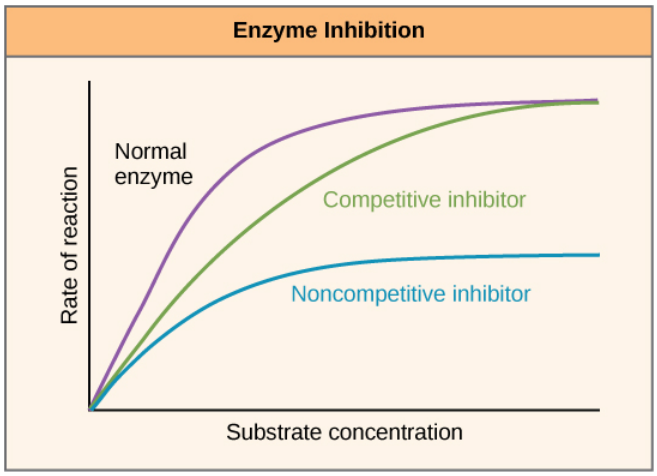



Figure 19.6.1: Plots of reaction rate vs. substrate concentration with and without inhibitors. Competitive inhibitors bind reversibly at the active site and therefore, compete with the substrate for binding. As substrate concentration increases, it can outcompete the inhibitor allowing enzyme activity to reach a normal maximum (green line). A noncompetive inhibitor binds at a site separate from the active site, the enzyme activity can only reach a lower than normal maximum reaction rate even as substrate concentration increases (blue line). Uncompetitive inhibition is not represented on this plot, but would be similar to the noncompetitive inhibitor, reaching a lower maximum rate. (Figure from OpenStax Biology)

Studies of competitive inhibition have provided helpful information about certain enzyme-substrate complexes and the interactions of specific groups at the active sites. As a result, pharmaceutical companies have synthesized drugs that competitively inhibit metabolic processes in bacteria and certain cancer cells. Many drugs are competitive inhibitors of specific enzymes.

A classic example of competitive inhibition is the effect of malonate on the enzyme activity of succinate dehydrogenase (Figure 19.6.2). Malonate and succinate are the anions of dicarboxylic acids and contain three and four carbon atoms, respectively. The

malonate molecule binds to the active site because the spacing of its carboxyl groups is not greatly different from that of succinate. However, no catalytic reaction occurs because malonate does not have a $CH_2CH_2$ group to convert to CH=CH. This reaction will also be discussed in connection with the Krebs cycle and energy production in a later chapter.

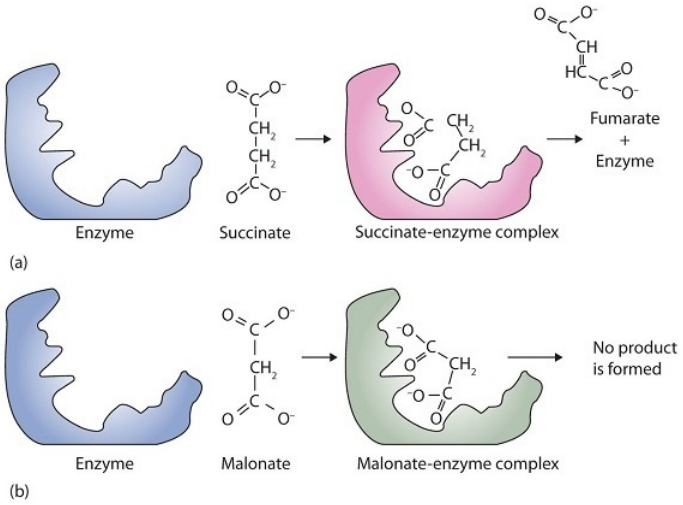



Figure 19.6.2: Competitive Inhibition. (a) Succinate binds to the enzyme succinate dehydrogenase. A dehydrogenation reaction occurs, and the product—fumarate—is released from the enzyme. (b) Malonate, a competitive inhibitor, also binds to the active site of succinate dehydrogenase. In this case, however, no subsequent reaction occurs while malonate remains bound to the enzyme.

In **uncompetitive inhibition**, the inhibitor can only bind the enzyme when the substrate is already bound, in other words it binds the enzyme-substrate complex but not the enzyme alone. The maximum reaction rate in the presence of an uncompetitive inhibitor is lowered, however, unlike with competitive inhibition, the rate cannot be increased by adding more substrate. This type of inhibition is most commonly seen when the enzyme reaction involves two substrates and as long as the concentration of inhibitor remains constant, the maximum reaction rate does not change.

A **noncompetitive inhibitor** can bind to either the free enzyme or the enzyme-substrate complex because its binding site on the enzyme is distinct from the active site. Binding of this kind of inhibitor alters the three-dimensional conformation of the enzyme, changing the configuration of the active site with one of two results. Either the enzyme-substrate complex does not form at its normal rate, or, once formed, it does not yield products at the normal rate (see Figure 19.6.1). Because the inhibitor does not structurally resemble the substrate, nor is it competing with the substrate for the active site, the addition of excess substrate does *not* reverse the inhibitory effect.

*Chemotherapy* is the strategic use of chemicals (that is, drugs) to destroy infectious microorganisms or cancer cells without causing excessive damage to the other, healthy cells of the host. From bacteria to humans, the metabolic pathways of all living organisms are quite similar, so the search for safe and effective chemotherapeutic agents is a formidable task. Many well-established chemotherapeutic drugs function by inhibiting a critical enzyme in the cells of the invading organism.

An *antibiotic* is a compound that kills bacteria; it may come from a natural source such as molds or be synthesized with a structure analogous to a naturally occurring antibacterial compound. Antibiotics constitute no well-defined class of chemically related substances, but many of them work by effectively inhibiting a variety of enzymes essential to bacterial growth.

### To Your Health: Penicillin

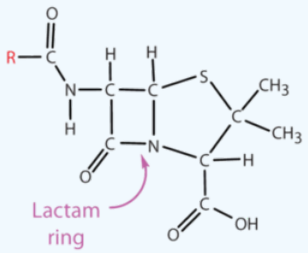
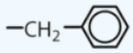
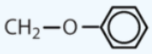
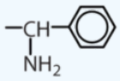
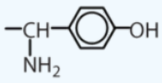
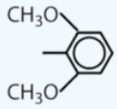
Penicillin, one of the most widely used antibiotics in the world, was fortuitously discovered by Alexander Fleming in 1928, when he noticed antibacterial properties in a mold growing on a bacterial culture plate. In 1938, Ernst Chain and Howard Florey began an intensive effort to isolate penicillin from the mold and study its properties. The large quantities of penicillin needed for this research became available through development of a corn-based nutrient medium that the mold loved and through the discovery of a higher-yielding strain of mold at a United States Department of Agriculture research center near Peoria, Illinois. Even so, it was not until 1944 that large quantities of penicillin were being produced and made available for the treatment of bacterial infections.

Penicillin functions by interfering with the synthesis of cell walls of reproducing bacteria. It does so by inhibiting an enzyme—transpeptidase—that catalyzes the last step in bacterial cell-wall biosynthesis. The defective walls cause bacterial cells to burst.

Human cells are not affected because they have cell membranes, not cell walls.

Several naturally occurring penicillins have been isolated. They are distinguished by different R groups connected to a common structure: a four-member cyclic amide (called a lactam ring) fused to a five-member ring. The addition of appropriate organic compounds to the culture medium leads to the production of the different kinds of penicillin.

The penicillins are effective against gram-positive bacteria (bacteria capable of being stained by Gram's stain) and a few gram-negative bacteria (including the intestinal bacterium *Escherichia coli*). They are effective in the treatment of diphtheria, gonorrhea, pneumonia, syphilis, many pus infections, and certain types of boils. Penicillin G was the earliest penicillin to be used on a wide scale. However, it cannot be administered orally because it is quite unstable; the acidic pH of the stomach converts it to an inactive derivative. The major oral penicillins—penicillin V, ampicillin, and amoxicillin—on the other hand, are acid stable.

| Penicillin Structure | R Group | Drug Name |
|---|---|---|
|   |  | penicillin G |
| |  | penicillin V |
| |  | ampicillin |
| |  | amoxicillin |
| |  | methicillin |

Some strains of bacteria become resistant to penicillin through a mutation that allows them to synthesize an enzyme—penicillinase—that breaks the antibiotic down (by cleavage of the amide linkage in the lactam ring). To combat these strains, scientists have synthesized penicillin analogs (such as methicillin) that are not inactivated by penicillinase.

Some people (perhaps 5% of the population) are allergic to penicillin and therefore must be treated with other antibiotics. Their allergic reaction can be so severe that a fatal coma may occur if penicillin is inadvertently administered to them. Fortunately, several other antibiotics have been discovered. Most, including aureomycin and streptomycin, are the products of microbial synthesis. Others, such as the semisynthetic penicillins and tetracyclines, are made by chemical modifications of antibiotics; and some, like chloramphenicol, are manufactured entirely by chemical synthesis. They are as effective as penicillin in destroying infectious microorganisms. Many of these antibiotics exert their effects by blocking protein synthesis in microorganisms.

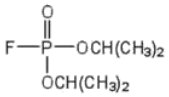
Initially, antibiotics were considered miracle drugs, substantially reducing the number of deaths from blood poisoning, pneumonia, and other infectious diseases. Some seven decades ago, a person with a major infection almost always died. Today, such deaths are rare. Seven decades ago, pneumonia was a dreaded killer of people of all ages. Today, it kills only the very old or those ill from other causes. Antibiotics have indeed worked miracles in our time, but even miracle drugs have limitations. Not long after the drugs were first used, disease organisms began to develop strains resistant to them. In a race to stay ahead of resistant bacterial strains, scientists continue to seek new antibiotics. The penicillins have now been partially displaced by related compounds, such as the cephalosporins and vancomycin. Unfortunately, some strains of bacteria have already shown resistance to these antibiotics.

## Irreversible Inhibition

An **irreversible inhibitor** inactivates an enzyme by bonding covalently to a particular group at the active site. When the inhibitor is bound, the enzyme active site is blocked, the substrate does not bind, and catalysis cannot occur, similar to competitive inhibition. The difference here is that the inhibition is *irreversible*, meaning that the inhibitor remains bound and does not dissociate from the enzyme because the enzyme-inhibitor covalent bonds are not easily broken. In the presence of an irreversible inhibitor, the substrate cannot bind the active site at all, nor can high substrate concentrations outcompete the inhibitor, hence the

enzyme is completely inactivated. Many of the known irreversible inhibitors are *poisons* because they inactivate an enzyme completely. Some examples are provided in Table 19.6.1 below.

Table 19.6.1: Poisons as Enzyme Inhibitors

| Poison | Formula | Example of Enzyme Inhibited | Action |
|---|---|---|---|
| arsenate | $AsO_4^{3-}$ | glyceraldehyde 3-phosphate dehydrogenase | substitutes for phosphate |
| iodoacetate | $ICH_2COO^-$ | triose phosphate dehydrogenase | binds to cysteine SH group |
| diisopropylfluoro-phosphate (DIFP; a nerve poison) |  | acetylcholinesterase | binds to serine OH group |

## Summary

An irreversible inhibitor inactivates an enzyme by bonding covalently to a particular group at the active site. A reversible inhibitor inactivates an enzyme through noncovalent, reversible interactions. A competitive inhibitor competes with the substrate for binding at the active site of the enzyme. A noncompetitive inhibitor binds at a site distinct from the active site.

## Concept Review Exercises

1. What are the characteristics of an irreversible inhibitor?
2. In what ways does a competitive inhibitor differ from a noncompetitive inhibitor?

## Answers

1. It inactivates an enzyme by bonding covalently to a particular group at the active site.
2. A competitive inhibitor structurally resembles the substrate for a given enzyme and competes with the substrate for binding at the active site of the enzyme. A noncompetitive inhibitor binds at a site distinct from the active site and can bind to either the free enzyme or the enzyme-substrate complex.

## Exercises

1. What amino acid is present in the active site of all enzymes that are irreversibly inhibited by nerve gases such as DIFP?
2. Oxaloacetate (OOCCH2COCOO) inhibits succinate dehydrogenase. Would you expect oxaloacetate to be a competitive or noncompetitive inhibitor? Explain.

## Answer

1. serine

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# 19.7: Enzyme Regulation- Allosteric Control and Feedback Inhibition

**Learning Objectives**

- Objective 1
- Objective 2

In the previous section you learned about the different types of enzyme inhibitors and how they can be used to *slow* or *stop* enzyme activity by binding to an enzyme or enzyme-substrate complex. All of these inhibitor types, except noncompetitive inhibitors, work by binding to enzyme active sites. Noncompetitive inhibitors, however, work by binding to an enzyme at a location other than the active site, an *allosteric site*. Inhibitors and other molecules, called activators, that bind to enzymes at allosteric sites are considered an important part of enzyme regulation called **allosteric control**. In this section, we will take a look at allosteric control and feedback control, two ways in which enzyme activity is regulated differently.

## Allosteric Control

**Allosteric enzymes** have both a binding site, for substrate binding and catalysis, and an allosteric site, for regulation of enzyme activity. When a regulator molecule binds to the allosteric site of an enzyme, usually by noncovalent interactions, a conformational change occurs in the enzyme active site, which affects substrate binding and reaction rates. Allosteric regulation of enzyme activity can be either positive, increasing reaction rates, or negative, decreasing reaction rates.

When an enzyme binds a negative regulator (or inhibitor), it will undergo a change in the active site in a way that *prevents* substrate binding, thereby lowering the reaction rate. As illustrated in the left-hand panel in Figure 19.7.1, the active site changes (becomes smaller in this case) and the substrate can no longer bind. Positive regulators (activators) bind to allosteric sites and cause conformational changes that open up an active site to *promote* substrate binding, allowing catalysis or increasing the reaction rate. The right panel of Figure 19.7.1 shows an enzyme that will only bind substrate when the active site is formed after the allosteric activator binds.

Some enzymes will have more than one allosteric site that can interact with one another, which allows for highly-controlled or finely-tuned reaction rates.

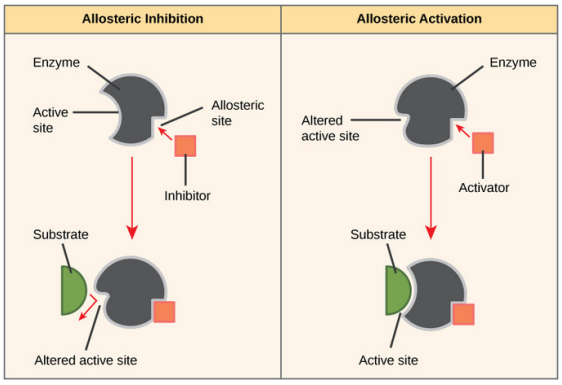


Figure 19.7.1: **Allosteric inhibitors and activators**: Allosteric inhibitors modify the active site of the enzyme so that substrate binding is reduced or prevented. In contrast, allosteric activators modify the active site of the enzyme so that the affinity for the substrate increases.

## Feedback Control

Many biological processes involve the sequential action of multiple enzymes, a reaction pathway, in which the product of one reaction is the substrate for the next enzyme and so on until the final product is formed. Positive or negative regulation of these pathways often occurs by **feedback control**, where a product from one of the steps in the path *feedback* to an earlier step in the process to increase or decrease production. It may help to visualize a factory assembly line with each person responsible for one step (catalytic reaction) in making a perfect box of 12 donuts. If the last person in the line, who is responsible for putting 12 donuts in the box, falls behind, donuts will start to pile up. In order to not waste donuts or have less than full boxes at the end, it would be beneficial to signal to the other people to slow down or take a break. The process is similar in biochemical pathways: if too much product is being formed, the pathway needs to be turned off so energy and resources are not wasted.

Consider the pathway shown below in which substrate A is converted to product D through three enzymes and two intermediate products (B and C):

$$A \xrightarrow{Enzyme\ 1} B \xrightarrow{Enzyme\ 2} C \xrightarrow{Enzyme\ 3} D$$

If there is a lot of product D formed, there would be enough to bind to Enzyme 1, which would inhibit formation of products B, and subsequently product C, and D. This type of *feedback control* is useful to prevent waste of substrate A and any energy that is needed for the activity of Enzymes 1-3. As you will see in later chapters, there are many different types of feedback control that can both negatively and positively regulate pathways. Typically, feedback control occurs at points in pathways where it would be energetically unfavorable to proceede if the final product is not needed.

Feedback inhibition is used to regulate the synthesis of many amino acids. For example, bacteria synthesize isoleucine from threonine in a series of five enzyme-catalyzed steps. As the concentration of isoleucine increases, some of it binds as a noncompetitive inhibitor to the first enzyme of the series (threonine deaminase), thus bringing about a decrease in the amount of isoleucine being formed (Figure 19.7.2).

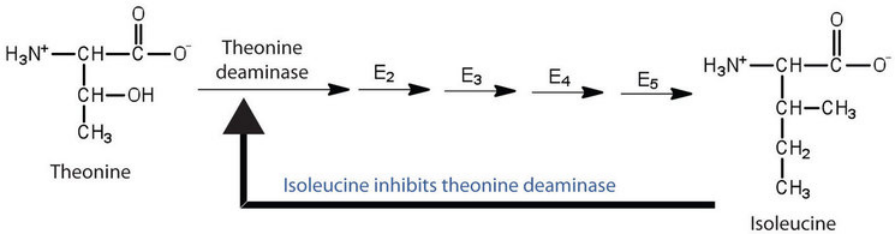


Figure 19.7.2: Feedback Inhibition of Threonine Deaminase by Isoleucine. Threonine deaminase is the first enzyme in the conversion of threonine to isoleucine. Isoleucine inhibits threonine deaminase through feedback inhibition.

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# 19.8: Enzyme Regulation - Covalent Modification and Genetic Control

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# 19.9: Vitamins, Antioxidants, and Minerals

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