Chapter Objectives:
• Learn about the general characteristics of enzyme catalysts.
• Learn about catalytic efficiency, specificity and enzyme regulation.
• Learn how to name and classify enzymes.
• Learn about enzyme cofactors.
• Learn about the mechanisms of enzyme action (lock-and-key and induced fit theories).
• Learn about enzyme inhibition, and how enzyme activity is regulated.

General Characteristics of Enzymes
• The catalytic behavior of proteins acting as enzymes is one of the most important functions that they perform in living cells.
  – Without catalysts, most cellular reactions would take place too slowly to support life.
  – With the exception of some RNA molecules, all enzymes are globular proteins.
  – Enzymes are extremely efficient catalysts, and some can increase reaction rates by $10^{20}$ times that of the uncatalyzed reactions.
• Enzymes are well suited to their roles in three major ways: they have enormous catalytic power, they are highly specific in the reactions they catalyze, and their activity as catalysts can be regulated.
**Catalytic Efficiency**

- **Catalysts** increase the rate of chemical reactions without being used up in the process.
  
  – Although catalysts participate in the reaction, they are not permanently changed, and may be used over and over.
  
  – Enzymes act like many other catalysts by lowering the activation energy of a reaction, allowing it to achieve equilibrium more rapidly.

  ![Energy Diagram](image)

**Catalytic Efficiency**

- Enzyme-catalyzed reaction accomplish many important organic reactions, such as ester hydrolysis, alcohol oxidation, amide formation, etc.

  – Enzymes cause these reactions to proceed under mild pH and temperature conditions, unlike the way they are done in a test tube.

  – Enzymes can accomplish in seconds what might take hours or weeks under laboratory conditions.

- The removal of carbon dioxide out of the body is sped up by the enzyme *carbonic anhydrase*, which combines \( \text{CO}_2 \) with water to form carbonic acid much more quickly than would be possible without the enzyme (36 million molecules per minute).

\[
\text{CO}_2 + \text{H}_2\text{O} \xrightleftharpoons[\text{anhydrase}]{\text{carbonic}} \text{H}_2\text{CO}_3
\]
Specificity

- Enzymes are often very specific in the type of reaction they catalyze, and even the particular substance that will be involved in the reaction.

  - Strong acids catalyze the hydrolysis of any amide or ester, and the dehydration of any alcohol. The enzyme urease catalyzes the hydrolysis of a single amide, urea.

\[
\begin{align*}
\text{NH}_2\text{C} & \text{NH}_2 + \text{H}_2\text{O} \quad \xrightarrow{\text{urease}} \\
\text{CO}_2 & \quad + 2\text{NH}_3
\end{align*}
\]

Specificity

- An enzyme with **absolute specificity** catalyzes the reaction of one and only one substance.

- An enzyme with **relative specificity** catalyzes the reaction of structurally related substances (*lipases* hydrolyze lipids, *proteases* split up proteins, and *phosphatases* hydrolyze phosphate esters).

- An enzyme with **stereochemical specificity** catalyzes the reaction of only one of two possible enantiomers (*D-amino acid oxidase* catalyzes the reaction of D-amino acids, but not L-amino acids).
**Regulation**

• The catalytic behavior of enzymes can be regulated.

• A relatively small number of all of the possible reactions which could occur in a cell actually take place, because of the enzymes which are present.

• The cell controls the rates of these reactions and the amount of any given product formed by regulating the action of the enzymes.
Enzyme Nomenclature — EC System

- Some of the earliest enzymes to be discovered were given names ending in –in to indicate that they were proteins (e.g. the digestive enzymes pepsin, trypsin, chymotrypsin).

- Because of the large number of enzymes that are now known, a systematic nomenclature called the Enzyme Commission (EC) system is used to name them. [International Union of Biochemistry and Molecular Biology]

- Enzymes are grouped into six major classes on the basis of the reaction which they catalyze. Each enzyme has an unambiguous (and often long) systematic name that specifies the substrate of the enzyme (the substance acted on), the functional group acted on, and the type of reaction catalyzed. All EC names end in –ase.
### EC Classification of Enzymes

<table>
<thead>
<tr>
<th>EC Code</th>
<th>Group Name</th>
<th>Type of Reaction Catalyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC 1</td>
<td>Oxidoreductases</td>
<td>Oxidation-reduction reactions</td>
</tr>
<tr>
<td>EC 2</td>
<td>Transferases</td>
<td>Transfer of functional groups</td>
</tr>
<tr>
<td>EC 3</td>
<td>Hydrolases</td>
<td>Hydrolysis reactions</td>
</tr>
<tr>
<td>EC 4</td>
<td>Lyases</td>
<td>Addition to double bonds or the reverse of that reaction</td>
</tr>
<tr>
<td>EC 5</td>
<td>Isomerases</td>
<td>Isomerization reactions</td>
</tr>
<tr>
<td>EC 6</td>
<td>Ligases</td>
<td>Formation of bonds with ATP cleavage</td>
</tr>
</tbody>
</table>

---

### The EC System

- **Enzymic Actions**: An Enzyme, EC Code, and a Group Name are linked as follows:
  - EC 1.1.1.1, oxaloacetate, oxaloacetate
  - EC 1.1.1.2, oxalacetate, oxaloacetate

- **Type of Reaction Catalyzed**: An EC Code is linked to a Group Name and a Type of Reaction as follows:
  - EC 1.1.1.1, oxaloacetate, oxaloacetate, oxaloacetate, oxaloacetate

- **EC Numbers**: An EC Code is linked to another EC Code as follows:
  - EC 1.1.1.1, oxaloacetate, oxaloacetate

---

**Chapter 10 Enzymes**

- **Chapter Title**: EC Classification of Enzymes

---

**Table**: A table is shown with EC Codes, Group Names, and Type of Reaction Catalyzed.

---

**Diagram**: A diagram is shown with links between different EC Codes and Group Names.
Examples

• Enzymes are also assigned common names derived by adding \textit{-ase} to the name of the substrate or to a combination of substrate name and type of reaction:

\[
\begin{align*}
\text{H}_2\text{N}-\text{C}=\text{NH}_2 + \text{H}_2\text{O} \xrightarrow{\text{enzyme}} \text{CO}_2 + 2\text{NH}_3
\end{align*}
\]

IEC name: urea amidohydrolase (EC 3.5.1.5)
Substrate: urea
Functional group: amide
Type of reaction: hydrolysis
Common name: urea + ase = urease

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{OH} + \text{NAD}^+ \xrightarrow{\text{enzyme}} \text{CH}_3\text{CHO} + \text{NADH} + \text{H}^+
\end{align*}
\]

IEC name: alcohol dehydrogenase (EC 1.1.1.1)
Substrate: alcohol (ethyl alcohol)
Type of reaction: dehydrogenation (removal of hydrogen)
Common name: alcohol dehydrogenation + ase = alcohol dehydrogenase

Examples: Enzyme Nomenclature

• Predict the substrates for the following enzymes:

a) maltase
b) peptidase
c) glucose 6-phosphate isomerase
### Examples: Enzyme Substrates

- Match the following general enzyme names and the reactions that they catalyze:

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Reaction Catalyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>decarboxylase</td>
<td>formation of ester linkages</td>
</tr>
<tr>
<td>phosphatase</td>
<td>removal of carboxyl groups from compounds</td>
</tr>
<tr>
<td>peptidase</td>
<td>hydrolysis of peptide linkages</td>
</tr>
<tr>
<td>esterase</td>
<td>hydrolysis of phosphate ester linkages</td>
</tr>
</tbody>
</table>
Enzyme Cofactors

- Conjugated proteins function only in the presence of specific nonprotein molecules or metal ions called prosthetic groups.
  - If the nonprotein component is tightly bound, and forms an integral part of the enzyme structure, it is a true prosthetic group.
  - If the nonprotein component is weakly bound, and easily separated from the rest of the protein, it is called a cofactor.
- When the cofactor is an organic substance, it is a coenzyme. The cofactor may also be an inorganic ion (usually a metal cation, such as Mg$^{2+}$, Zn$^{2+}$, or Fe$^{2+}$).
- The protein portion is called an apoenzyme:

  apoenzyme + cofactor (coenzyme or inorganic ion) $\rightarrow$ active enzyme
**Enzyme Cofactors**

- Many organic coenzymes are derived from vitamins.
  - For example, nicotinamide adenine dinucleotide (NAD⁺) is a necessary part of some enzyme-catalyzed redox reactions. It is formed from the vitamin precursor nicotinamide.
  - In this reaction, NAD⁺ is the oxidizing agent, and accepts hydrogen from lactate. It can then transfer the H⁺ to other compounds in subsequent reactions.

\[
\text{Lactate} + \text{NAD}^+ \xrightarrow{\text{dehydrogenase}} \text{Pyruvate} + \text{NADH} + \text{H}^+
\]

**Vitamins and their Coenzyme Forms**

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Coenzyme Form</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>biotin</td>
<td>biocytin</td>
<td>Carboxyl group removal or transfer</td>
</tr>
<tr>
<td>folacin</td>
<td>tetrahydrofolic acid</td>
<td>One-carbon group transfer</td>
</tr>
<tr>
<td>lipoic acid</td>
<td>lipoamide</td>
<td>Acyl group transfer</td>
</tr>
<tr>
<td>niacin</td>
<td>nicotinamide adenine dinucleotide (NAD⁺)</td>
<td>Hydrogen transfer</td>
</tr>
<tr>
<td>niacin</td>
<td>nicotinamide adenine dinucleotide phosphate (NADP⁺)</td>
<td>Hydrogen transfer</td>
</tr>
<tr>
<td>pantothenic acid</td>
<td>coenzyme A (CoA)</td>
<td>Acyl group carrier</td>
</tr>
<tr>
<td>pyridoxal, pyridoxamine, pyridoxine (B₆ group)</td>
<td>pyridoxal phosphate</td>
<td>Amino group transfer</td>
</tr>
<tr>
<td>riboflavin</td>
<td>flavin mononucleotide (FMN)</td>
<td>Hydrogen transfer</td>
</tr>
<tr>
<td>riboflavin</td>
<td>flavin adenine dinucleotide (FAD)</td>
<td>Hydrogen transfer</td>
</tr>
<tr>
<td>thiamin (B₁)</td>
<td>thiamin pyrophosphate (TPP)</td>
<td>Aldehyde group transfer</td>
</tr>
<tr>
<td>vitamin B₁₂ (cyanocobalamin)</td>
<td>coenzyme B₁₂</td>
<td>Shift of hydrogen atoms between adjacent carbon atoms; methyl group transfer</td>
</tr>
</tbody>
</table>
The Mechanism of Enzyme Action

Enzyme Action

- Enzymes differ widely in structure and specificity, but a general theory that accounts for their catalytic behavior is widely accepted.

- The enzyme and its substrates interact only over a small region of the surface of the enzyme, called the active site.
  - When the substrate binds to the active site via some combination of intermolecular forces, an enzyme-substrate (ES) complex is formed.
  - Once the complex forms, the conversion of the substrate (S) to product (P) takes place:
Enzyme Action

- The chemical transformation of the substrate occurs at the active site, aided by functional groups on the enzyme that participate in the making and breaking of chemical bonds.
- After the conversion is complete, the product is released from the active site, leaving the enzyme free to react with another substrate molecule.

Lock-and-Key Theory

- The **lock-and-key theory** explains the high specificity of enzyme activity. Enzyme surfaces accommodate substrates having specific shapes and sizes, so only specific substances “fit” in an active site to form an ES complex.
- A limitation of this theory is that it requires enzymes conformations to be rigid. Research suggests that instead enzymes are at least somewhat flexible.
**Induced-Fit Theory**

- A modification of the lock-and-key theory called the **induced-fit theory** proposes that enzymes have flexible conformations that may adapt to incoming substrates.

- The active site adopts a shape that is complementary to the substrate only after the substrate is bound.
Enzyme Activity

**Enzyme Activity**

- **Enzyme activity** refers to the catalytic ability of an enzyme to increase the rate of a reaction.

- The **turnover number** is the number of molecules of substrate acted on by one molecule of the enzyme per minute.
  
  - Carbonic anhydrase is one of the highest at 36 million molecules per minute.
  
  - More common numbers are closer to 1000 molecules per minute.

**Table 10.2: Examples of Enzyme Turnover Numbers**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Turnover Number (per minute)</th>
<th>Reaction Catalyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>carbonic anhydrase</td>
<td>36,000,000</td>
<td>( \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 )</td>
</tr>
<tr>
<td>cholinesterase</td>
<td>5,000,000</td>
<td>( 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 )</td>
</tr>
<tr>
<td>chainotrispine</td>
<td>1,500,000</td>
<td>Hydrolysis of ester bonds</td>
</tr>
<tr>
<td>DNA polymerase I</td>
<td>6,000</td>
<td>Addition of nucleotide monomers to DNA chains</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>900</td>
<td>( \text{pyruvate} + \text{NAD}^+ \rightarrow \text{lactate} + \text{NAD}^+ )</td>
</tr>
<tr>
<td>peroxidase</td>
<td>80,000</td>
<td>Hydrolysis of peroxides</td>
</tr>
<tr>
<td></td>
<td>120,000</td>
<td>Hydrolysis of proteins</td>
</tr>
</tbody>
</table>
**Enzyme Activity**

- Enzyme assays are experiments that are performed to measure enzyme activity.
- Assays for blood enzymes are routinely performed in clinical labs.
- Assays are often done by monitoring the rate at which a characteristic color of a product forms or the color of a substrate decreases. For reactions involving H⁺ ions, the rate of change in pH over time can be used.

**Enzyme International Units**

- Enzyme activity levels are reported in terms of **enzyme international units (IU)**, which defines enzyme activity as the amount of enzyme that will convert a specified amount of substrate to a product within a certain time.
  - One standard IU is the quantity of enzyme that catalyzes the conversion of 1 micromole (1 μmol) of substrate per minute under specified conditions.
  - Unlike the turnover number, IUs measure how much enzyme is present. (An enzyme preparation having an IU of 40 is forty times more concentrated than the standard solution.)
Factors Affecting Enzyme Activity

Enzyme Concentration

• The concentration of an enzyme, [E], is typically low compared to that of the substrate. Increasing [E] also increases the rate of the reaction:

\[
E + S \rightarrow ES
\]

increased [E] gives more [ES]

• The rate of the reaction is directly proportional to the concentration of the enzyme (doubling [E] doubles the rate of the reaction), thus, a graph of reaction rate vs. enzyme concentration is a straight line:

Factors Affecting Enzyme Activity

Substrate Concentration

• The concentration of substrate, [S], also affects the rate of the reaction.

• Increasing [S] increases the rate of the reaction, but eventually, the rate reaches a maximum (\(v_{\text{max}}\)), and remains constant after that.

• The maximum rate is reach when the enzyme is saturated with substrate, and cannot react any faster under those conditions.
Factors Affecting Enzyme Activity

Temperature

- Like all reactions, the rate of enzyme-catalyzed reactions increases with temperature.
- Because enzymes are proteins, beyond a certain temperature, the enzyme denatures.
- Every enzyme-catalyzed reaction has an **optimum temperature** at which the enzyme activity is highest, usually from 25º-40ºC; above or below that value, the rate is lower.

Factors Affecting Enzyme Activity

The Effect of pH

- Raising or lowering the pH influences the acidic and basic side chains in enzymes. Many enzymes are also denatured by pH extremes. (E.g., pickling in acetic acid [vinegar] preserves food by deactivating bacterial enzymes.)
- Many enzymes have an **optimum pH**, where activity is highest, near a pH of 7, but some operate better at low pH (e.g., pepsin in the stomach).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Optimum pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>pepsin</td>
<td>Gastric mucosa</td>
<td>1.5</td>
</tr>
<tr>
<td>β-glucosidase</td>
<td>Almond</td>
<td>4.5</td>
</tr>
<tr>
<td>sucrase</td>
<td>Intestine</td>
<td>6.2</td>
</tr>
<tr>
<td>trypsin</td>
<td>Soybean</td>
<td>6.8</td>
</tr>
<tr>
<td>catalase</td>
<td>Liver</td>
<td>7.3</td>
</tr>
<tr>
<td>succinic dehydrogenase</td>
<td>Beef heart</td>
<td>7.6</td>
</tr>
<tr>
<td>aspartase</td>
<td>Beef heart</td>
<td>9.0</td>
</tr>
<tr>
<td>alkaline phosphatase</td>
<td>Bone</td>
<td>9.5</td>
</tr>
</tbody>
</table>
**Enzyme Inhibition — Irreversible Inhibition**

- An enzyme inhibitor is a substance that decreases the rate of an enzyme-catalyzed reaction.
  - Many poisons and medicines inhibit one or more enzymes and thereby decrease the rate of the reactions they carry out.
  - Some substances normally found in cells inhibit specific enzymes, providing a means for internal regulation of cell metabolism.

- Irreversible inhibition occurs when an inhibitor forms a covalent bond with a specific functional group of an enzyme, thereby inactivating it.
  - Cyanide ion, $\text{CN}^-$, is a rapidly-acting, highly toxic inhibitor, which interferes with the iron-containing enzyme cytochrome oxidase:

$$\text{CN}^- + \text{Fe}^{3+} \rightarrow \text{CN}^- \text{Fe}^+$$

- Cell respiration stops, and death occurs within minutes.
- An antidote for cyanide poisoning is sodium thiosulfate, which converts cyanide into thiocyanate, which does not bind to cytochrome:

$$\text{CN}^- + \text{S}_2\text{O}_3^{2-} \rightarrow \text{SCN}^- + \text{SO}_3^{2-}$$
Enzyme Inhibition — Irreversible Inhibition

- **Heavy metal poisoning** results when mercury or lead ions bind to —SH groups on enzymes. Heavy metals can also cause protein denaturation. Pb and Hg can cause permanent neurological damage.

- **Heavy-metal poisoning** is treated by administering chelating agents, which bind tightly to metal ions, allowing them to be excreted in the urine.

\[
\text{CaEDTA}^{2-} + \text{Pb}^{2+} \rightarrow \text{PbEDTA}^{2-} + \text{Ca}^{2+}
\]

Antibiotics

- **Antibiotics** are enzyme inhibitors that act on life processes that are essential to certain strains of bacteria.

- **Sulfa drugs** (Gerhard Domagk, 1935; Nobel Prize 1939)

- **Penicillins** (Alexander Fleming, 1928; Nobel Prize, 1945) — interfere with transpeptidase, which bacteria use in the construction of cell walls.

<table>
<thead>
<tr>
<th>Name</th>
<th>Year Marketed</th>
<th>Side Chain (R—)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G</td>
<td>1943</td>
<td>—CH₃</td>
<td>Usually given by injection</td>
</tr>
<tr>
<td>Penicillin V</td>
<td>1953</td>
<td>O—CH₃</td>
<td>An oral penicillin; resistant to stomach hydrolysis</td>
</tr>
<tr>
<td>Methicillin</td>
<td>1960</td>
<td>—CO₂H</td>
<td>Given by injection</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1965</td>
<td>—CH—NH₂</td>
<td>Given by injection or taken orally; effective against a broad spectrum of organisms</td>
</tr>
</tbody>
</table>
Enzyme Inhibition — Reversible Inhibition

- A reversible inhibitor binds reversibly to an enzyme, establishing an equilibrium between the bound and unbound inhibitor:

  \[ E + I \rightleftharpoons EI \]

  - Once the inhibitor combines with the enzyme, the active site is blocked, and no further catalysis takes place.
  - The inhibitor can be removed from the enzyme by shifting the equilibrium.
  - There are two types of reversible inhibitors: competitive and noncompetitive.

Enzyme Inhibition — Competitive Inhibitors

- Competitive inhibitors bind to the enzyme’s active site and compete with the normal substrate molecules. They often have structures that are similar to those of the normal substrate.
**Enzyme Inhibition — Competitive Inhibitors**

- **Sulfa drugs** such as sulfanilamide are similar in structure to \( p \)-aminobenzoic acid (PABA), which bacteria need to build folic acid in order to grow. Sulfanilamide blocks PABA from fitting into the active site of the enzyme which builds folic acid, causing the bacteria to eventually die. Since humans obtain folic acid from their diet rather than by manufacturing it, it is not harmful to the patient.

![Chemical structures of PABA, folic acid, and sulfanilamide]

- In competitive inhibition, there are two equilibria taking place:

  \[
  \begin{align*}
  \text{equilibrium 1:} & \quad E + S \underset{\text{decreasing [E]}}{\overset{\text{increasing [S]}}{\rightleftharpoons}} ES \\
  \text{equilibrium 2:} & \quad E + I \underset{\text{decreasing [E]}}{\overset{\text{increasing [I]}}{\rightleftharpoons}} EI
  \end{align*}
  \]

  - Competitive inhibition can be reversed by either increasing the concentration of the substrate, or decreasing the concentration of the enzyme, in accordance with Le Châtelier’s principle.
**Enzyme Inhibition — Noncompetitive Inhibitors**

- **Noncompetitive inhibitors** bind reversibly to the enzyme at a site *other than the active site*, changing the 3D shape of the enzyme and the active site, so that the normal substrate no longer fits correctly.
  - Noncompetitive inhibitors do not look like the enzyme substrates.
  - Increasing the substrate concentration does not affect noncompetitive inhibition because it can’t bind to the site occupied by the inhibitor.

**Summary of Enzyme Inhibitors**

- **Irreversible inhibitor** (cyanides, heavy metals)
  - Binds tightly to enzyme
  - Binds to active site

- **Competitive inhibitor** (malonate, ethanol)
  - Binds to active site

- **Noncompetitive inhibitor** (isoleucine — feedback inhibitor)
  - Binds at other than active site
The Regulation of Enzyme Activity

**Enzyme Regulation**

- Enzymes work together to facilitate all the biochemical reactions needed for a living organism. To respond to changing conditions and cellular needs, enzyme activity requires very sensitive controls:
  - activation of zymogens
  - allosteric regulation
  - genetic control
Activation of Zymogens

- **Zymogens** or **proenzymes** are inactive precursors of an enzyme.
  - Some enzymes in their active form would degrade the internal structures of the cell.
  - These enzymes are synthesized and stored as inactive precursors, and when the enzyme is needed, the zymogen is released and activated where it is needed.
  - Activation usually requires the cleavage of one or more peptide bonds.
  - The digestive enzymes pepsin, trypsin, and chymotrypsin, as well as enzymes involved in blood clotting, are activated this way.

### Examples of Zymogens

<table>
<thead>
<tr>
<th>Zymogen</th>
<th>Active enzyme</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>chymotrypsinogen</td>
<td>chymotrypsin</td>
<td>digestion of proteins</td>
</tr>
<tr>
<td>pepsinogen</td>
<td>pepsin</td>
<td>digestion of proteins</td>
</tr>
<tr>
<td>procarboxypeptidase</td>
<td>carboxypeptidase</td>
<td>digestion of proteins</td>
</tr>
<tr>
<td>proelastase</td>
<td>elastase</td>
<td>digestion of proteins</td>
</tr>
<tr>
<td>prothrombin</td>
<td>thrombin</td>
<td>blood clotting</td>
</tr>
<tr>
<td>trypsinogen</td>
<td>trypsin</td>
<td>digestion of proteins</td>
</tr>
</tbody>
</table>
Allosteric Regulation

- Compounds that alter enzymes by changing the 3D conformation of the enzyme are called modulators.
- They may increase the activity (activators) or decrease the activity (inhibitors). (Noncompetitive inhibitors are examples of this activity.)
- Enzymes with quaternary structures with binding sites for modulators are called allosteric enzymes.
- These variable-rate enzymes are often located at key control points in cell processes.
- Feedback inhibition occurs when the end product of a sequence of enzyme-catalyzed reactions inhibits an earlier step in the process. This allows the concentration of the product to be maintained within very narrow limits.

Allosteric Regulation

- The synthesis of isoleucine from threonine is an example of allosteric regulation.
  - Threonine deaminase, which acts in the first step of the conversion pathway, is inhibited by the isoleucine product.
  - When isoleucine builds up, it binds to the allosteric site on threonine deaminase, changing its conformation so that threonine binds poorly. This slows the reaction down so that the isoleucine concentration starts to fall.
  - When the isoleucine concentration gets too low, the enzyme becomes more active again, and more isoleucine is synthesized.
Genetic Control

- The synthesis of all proteins and enzymes is under **genetic control** by nucleic acids. Increasing the number of enzymes molecules present through genetic mechanisms is one way to increase production of needed products.

- **Enzyme induction** occurs when enzymes are synthesized in response to cell need.

- This kind of genetic control allows an organism to adapt to environmental changes. The coupling of genetic control and allosteric regulation allows for very tight control of cellular processes.

---

Genetic Control

- β-galactosidase is an enzyme in the bacterium *Escherichia coli* that catalyzes the hydrolysis of lactose to D-galactose and D-glucose.

- In the absence of lactose in the growth medium, there are very few β-galactosidase molecules.

- In the presence of a lactose-containing medium, thousands of molecules of enzyme are produced.

- If lactose is removed, the production of the enzyme once again decreases.
Enzymes in Clinical Diagnosis

- Some enzymes are found exclusively in tissue cells. If they are found in the bloodstream, it indicates damage to that tissue; the extent of cell damage can sometimes be estimated from the magnitude of serum concentration increase above normal levels.

- The measurement of enzyme concentrations in blood serum is a major diagnostic tool, especially in diseases of the heart, liver, pancreas, prostate, and bones.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Pathological condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>acid phosphatase</td>
<td>prostate cancer</td>
</tr>
<tr>
<td>alkaline phosphatase (ALP)</td>
<td>liver or bone disease</td>
</tr>
<tr>
<td>amylase</td>
<td>diseases of the pancreas</td>
</tr>
<tr>
<td>creatine kinase (CK)</td>
<td>heart attack</td>
</tr>
<tr>
<td>aspartate transaminase (AST)</td>
<td>heart attack or hepatitis</td>
</tr>
<tr>
<td>alanine transaminase (ALT)</td>
<td>hepatitis</td>
</tr>
<tr>
<td>lactate dehydrogenase (LDH)</td>
<td>heart attack, liver damage</td>
</tr>
<tr>
<td>lipase</td>
<td>acute pancreatitis</td>
</tr>
<tr>
<td>lysozyme</td>
<td>monocytic leukemia</td>
</tr>
</tbody>
</table>
**Isoenzymes**

- Isoenzymes are slightly different forms of the same enzyme produced by different tissues. Although all forms of a particular isoenzyme catalyze the same reaction, their structures are slightly different and their location within body tissues may vary.

- The enzyme lactate dehydrogenase (LDH) has a quaternary structure that consists of four subunits of two different types:
  - H — main subunit found in heart muscle cells.
  - M — main form in other muscle cells.
  - There are five possible ways to combine these subunits to form the enzyme (see next slide); each of these forms has slightly different properties, which allow them to be separated and identified.

![Isoenzymes Diagram](image)

**Table 10.8 Tissue Distribution of LDH Isoenzymes**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>LDH (1) (%)</th>
<th>LDH (2) (%)</th>
<th>LDH (3) (%)</th>
<th>LDH (4) (%)</th>
<th>LDH (5) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>23</td>
<td>34</td>
<td>30</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Heart</td>
<td>50</td>
<td>36</td>
<td>9</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Kidney</td>
<td>28</td>
<td>34</td>
<td>21</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Liver</td>
<td>4</td>
<td>6</td>
<td>17</td>
<td>16</td>
<td>57</td>
</tr>
<tr>
<td>Lung</td>
<td>10</td>
<td>20</td>
<td>30</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>Serum</td>
<td>28</td>
<td>41</td>
<td>19</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>22</td>
<td>58</td>
</tr>
</tbody>
</table>
Isoenzymes

– Each type of tissue has a distinct pattern of isoenzyme percentages.

– Serum levels of LDH can be used in the diagnosis of a wide range of diseases, such as anemias involving the rupture of red blood cells, acute liver diseases, congestive heart failure, and muscular diseases such as muscular dystrophy.

– Elevated levels of LDH$_1$ and LDH$_2$ indicate myocardial infarction

– Elevated levels of LDH$_5$ indicate possible liver damage.

The End