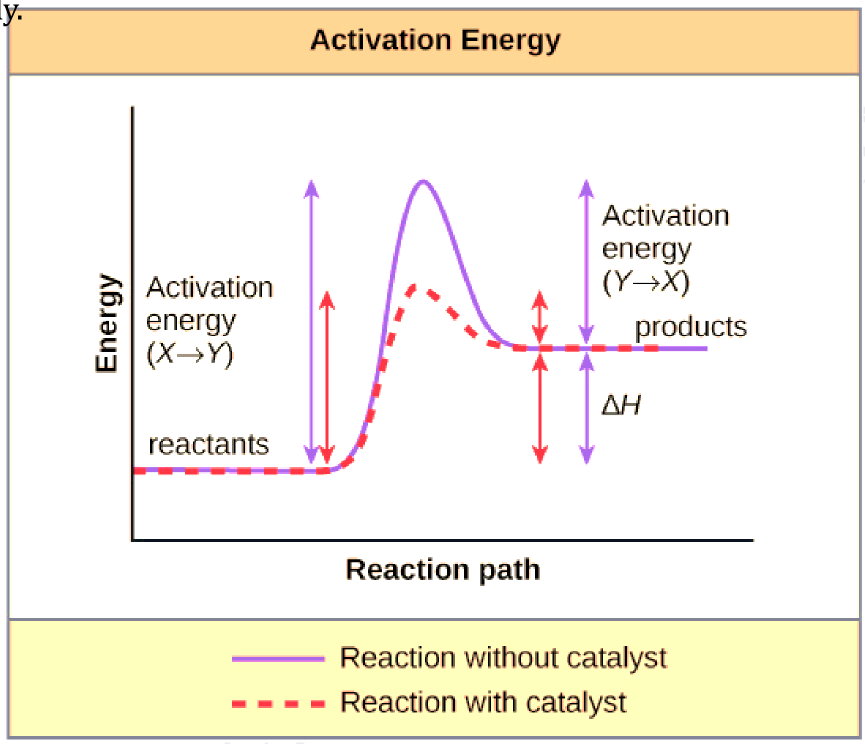
MODULE 6 GENERAL ENZYMOLOGY

Enzymes are biological catalysts that accelerate chemical reactions in living organisms without being consumed in the process. They play crucial roles in almost all biochemical processes, such as digestion, metabolism, DNA replication, and energy production. Understanding enzyme structure and catalysis is fundamental to comprehending how biological reactions are regulated in the body.



1. Structure of Enzymes

Enzymes are **proteins**, and their structure is crucial to their catalytic function. Their structure can be described in several levels of complexity:

A. Primary Structure

The primary structure of an enzyme refers to the **linear sequence of amino acids** in its polypeptide chain. The order of these amino acids is determined by the gene encoding the enzyme and dictates how the enzyme will fold and function.

B. Secondary Structure

The polypeptide chain of the enzyme folds into localized structures such as:

- **Alpha-helices**: Spiral structures stabilized by hydrogen bonds.
- **Beta-sheets**: Pleated sheet structures also held together by hydrogen bonds.

These structures are critical for maintaining the overall shape and stability of the enzyme.

C. Tertiary Structure

The tertiary structure refers to the **three-dimensional folding** of the entire polypeptide chain, forming a compact, globular structure. This level of structure is essential because it creates the **active site**, the region where substrate molecules bind and undergo a chemical reaction.

- **Hydrophobic interactions**, **hydrogen bonds**, **disulfide bridges**, and **ionic bonds** stabilize the tertiary structure.
- The **active site** is typically a small pocket or groove on the enzyme surface, formed by the folding of the protein.

D. Quaternary Structure

Some enzymes are made of multiple polypeptide chains (subunits) that come together to form a **quaternary structure.** Each subunit may have its own active site, or the subunits may work together to form a functional enzyme complex.

- Example: **Haemoglobin** (though not an enzyme) is a classic example of a protein with quaternary structure. For enzymes, the quaternary structure can allow cooperativity, where the binding of a substrate to one subunit affects the function of others.

E. Cofactors and Coenzymes

Some enzymes require additional molecules called **cofactors** or **coenzymes** to function. These can be:

- **Metal ions** (e.g., zinc, magnesium, or iron) that assist in stabilizing enzyme structure or participate in the catalytic process.
- **Coenzymes** are organic molecules (often derived from vitamins, such as NAD^+ or FAD) that transfer electrons, atoms, or functional groups during the reaction.

F. Prosthetic Groups

Some enzymes have non-protein molecules called **prosthetic groups** tightly bound to them. These groups are often permanently attached to the enzyme and play a critical role in its function.

2. Mechanism of Enzyme Catalysis

Enzymes accelerate reactions by lowering the **activation energy** required to convert reactants (substrates) into products. Here's a breakdown of how enzymes achieve this through different mechanisms:

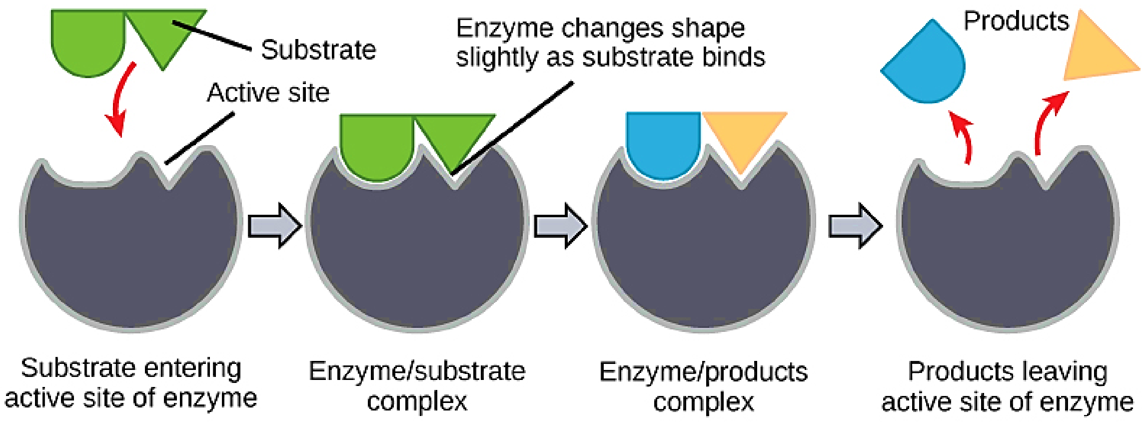
A. The Active Site and Substrate Binding

The active site of an enzyme is the region where the substrate binds and where the reaction takes place. The active site is highly specific for the substrate due to:

- **Geometric complementarity**: The shape of the substrate fits the active site, like a key fits a lock.
- **Chemical complementarity**: The active site contains amino acid residues that form temporary bonds with the substrate, stabilizing the transition state.

There are two models that describe substrate binding:

1. **Lock-and-Key Model**: The enzyme's active site is perfectly complementary to the substrate's shape.
2. **Induced Fit Model**: The enzyme undergoes a conformational change upon substrate binding, which allows for a tighter fit and optimizes the orientation of the catalytic residues.



B. Lowering the Activation Energy

For a reaction to occur, reactants must overcome an energy barrier called the **activation energy**. Enzymes lower this barrier by stabilizing the **transition state**, the high-energy intermediate between the reactants and the products.

Enzymes can reduce activation energy through several mechanisms:

1. **Proximity and Orientation**: By binding substrates in the correct orientation and bringing them into close proximity, enzymes increase the likelihood of a reaction occurring.
2. **Transition State Stabilization**: Enzymes stabilize the unstable transition state, reducing the energy required to reach it.

3. **Microenvironment**: The active site may provide a unique microenvironment, such as a hydrophobic region or a site with charged amino acids, that facilitates the reaction.
4. **Covalent Catalysis**: In some reactions, the enzyme forms a temporary covalent bond with the substrate, helping to break bonds in the substrate and stabilize the transition state.
5. **Acid-Base Catalysis**: Some enzymes can donate or accept protons, aiding in the breaking and forming of bonds during the reaction.

C. Enzyme-Substrate Complex (ES Complex)

The enzyme catalyzes the reaction by forming a transient **enzyme-substrate complex** (ES complex). This complex undergoes several transformations before converting into the **enzyme-product complex** (EP complex) and then releasing the products. The enzyme remains unchanged at the end of the reaction, ready to catalyze another reaction cycle.

The overall process can be represented as:

$E+S \rightleftharpoons ES \rightarrow EP \rightleftharpoons E+P$

Where:

- **E** = Enzyme
- **S** = Substrate
- **ES** = Enzyme-substrate complex
- **EP** = Enzyme-product complex
- **P** = Product

3. Factors Affecting Enzyme Activity

Enzyme activity can be influenced by various factors:

A. Temperature

- Enzymes have an optimal temperature at which they function most efficiently. For most human enzymes, this is around 37°C (body temperature).
- Higher temperatures increase molecular motion and reaction rates up to a point. Beyond the optimal temperature, enzymes can become **denatured**, losing their functional shape.

B. pH

- Enzymes also have an optimal pH range. Extremes in pH can affect the ionization of amino acids at the active site, leading to reduced activity or denaturation.

  - Example: **Pepsin** (found in the stomach) works best in highly acidic conditions (pH 2), whereas **trypsin** (in the small intestine) operates optimally at pH 8.

C. Substrate Concentration

- At low substrate concentrations, enzyme activity increases as the substrate concentration increases. However, once all the active sites of the enzyme molecules are occupied, the reaction reaches a **maximum rate** (Vmax). At this point, increasing the substrate concentration further will not increase the rate of reaction (enzyme saturation).

D. Enzyme Concentration

- Increasing enzyme concentration (provided there is enough substrate) will proportionally increase the rate of reaction, as more active sites become available for catalysis.

E. Inhibitors

Enzyme activity can be inhibited by molecules that reduce or block their function. Inhibitors can be:

- **Competitive inhibitors**: Molecules that resemble the substrate and compete for the active site, blocking substrate binding.
- **Non-competitive inhibitors**: Molecules that bind to a site other than the active site (an allosteric site), causing a change in the enzyme's shape and reducing its catalytic activity.

F. Cofactors and Coenzymes

As mentioned earlier, some enzymes require cofactors or coenzymes to function. If these molecules are not available, enzyme activity will be reduced or completely halted.

4. Enzyme Kinetics

The study of enzyme kinetics involves measuring the rate of enzymatic reactions and how this rate changes in response to factors such as substrate concentration, enzyme concentration, and the presence of inhibitors.

The **Michaelis-Menten equation** describes the relationship between the reaction rate (v) and substrate concentration

$$[S]: v = \frac{V_{max}[S]}{Km+[S]}$$

Where:

- **Vmax** is the maximum rate of the reaction.
- **Km** is the Michaelis constant, a measure of the enzyme's affinity for its substrate. A low Km indicates high affinity, while a high Km indicates low affinity.

This equation helps understand how enzymes function under different conditions and can be used to compare different enzymes.

5. Allosteric Regulation

Some enzymes are regulated through **allosteric regulation**, where the binding of a molecule at a site other than the active site (an **allosteric site**) causes a conformational change that alters enzyme activity. This can either:

- **Activate** the enzyme, enhancing its activity.
- **Inhibit** the enzyme, reducing its activity.

Allosteric enzymes often play key roles in regulating metabolic pathways and can exhibit **cooperativity**, where the binding of a substrate to one active site affects the activity of other active sites on the enzyme.

Enzymes are remarkable biological catalysts that accelerate reactions by lowering the activation energy. Their structure, especially the shape and characteristics of their active sites, determines their specificity and efficiency. Understanding enzyme catalysis and the factors that affect enzyme activity is crucial for many fields, including medicine, biotechnology, and agriculture, as enzymes are involved in virtually all biological processes.

ENZYMATIC REACTIONS

Enzymatic reactions can be classified based on the type of biochemical transformation they catalyze. These reactions fall into several categories, and each enzyme type typically performs a specific kind of reaction. Below are the **six major types of enzymatic reactions**, along with the class of enzymes responsible for catalyzing them:

1. Oxidoreductases

Oxidoreductases are enzymes that catalyze oxidation-reduction reactions, where electrons are transferred between molecules. These reactions are essential in cellular respiration and metabolic processes.

- **Oxidation** involves the loss of electrons, while **reduction** involves the gain of electrons.
- Subtypes include **dehydrogenases**, **oxidases**, **peroxidases**, and **reductases**. **Example:**
- **Lactate dehydrogenase** catalyzes the oxidation of lactate to pyruvate, transferring electrons to NAD^+ to form NADH.

General Reaction:

$$A^{reduced} + B^{oxidized} \rightleftharpoons A^{oxidized} + B^{reduced}$$

2. Transferases

Transferases are enzymes that catalyze the transfer of functional groups (such as methyl, phosphate, or amino groups) from one molecule (the donor) to another (the acceptor). These enzymes are critical in metabolic pathways, such as those involved in amino acid and nucleotide synthesis.

Example:

- **Kinases** are a type of transferase that transfers phosphate groups from ATP to other molecules (e.g., **hexokinase** transfers a phosphate group to glucose during glycolysis).

General Reaction:

$$A+B \rightarrow A\text{-}X+B$$

(Where "X" represents the functional group being transferred.)

3. Hydrolases

Hydrolases catalyze the cleavage of bonds (such as C-O, C-N, or C-C bonds) by adding water molecules. These reactions are commonly involved in digestion and the breakdown of large biomolecules.

Examples:

- **Proteases** (like trypsin) break down proteins into peptides and amino acids by hydrolyzing peptide bonds.
- **Lipases** catalyze the hydrolysis of lipids into fatty acids and glycerol.

General Reaction:

$$A\text{-}B + H_2O \rightarrow A\text{-}H + B\text{-}OH$$

4. Lyases

Lyases catalyze the breaking of various chemical bonds (C-C, C-O, C-N) by means other than hydrolysis or oxidation, often forming a new double bond or ring structure. These reactions are commonly seen in metabolic pathways like glycolysis and the Krebs cycle.

Example:

- **Aldolase** catalyzes the cleavage of fructose 1,6-bisphosphate into two three-carbon sugars during glycolysis.
- **Decarboxylases** catalyze the removal of a carboxyl group, releasing carbon dioxide.

General Reaction:

$$A\text{-}B \rightarrow A+B$$

(or the reverse, depending on the reaction conditions.)

5. Isomerases

Isomerases catalyze the rearrangement of atoms within a molecule, converting it from one isomer to another. These reactions are important in metabolic pathways where intermediates are converted into forms that can be used for subsequent steps. **Example:**

- **Phosphoglucose isomerase** converts glucose-6-phosphate into fructose-6-phosphate in glycolysis. **General Reaction:**

$$A \rightarrow B$$

(Where A and B are isomers of each other.)

6. Ligases

Ligases catalyze the joining of two molecules by forming a new bond, typically with the use of energy derived from ATP hydrolysis. Ligases are important for DNA replication and repair, where they join DNA fragments together. **Example:**

- **DNA ligase** is responsible for sealing nicks in the DNA backbone by forming a phosphodiester bond between adjacent nucleotides during DNA replication and repair.

General Reaction:

$$A + B + ATP \rightarrow A\text{-}B + ADP + Pi$$

Summary of Enzyme Classes and Their Reactions:

Enzyme Class	Reaction Type	Examples
Oxidoreductases	Oxidation-reduction	Dehydrogenases (e.g., lactate dehydrogenase)
Transferases	Transfer of functional groups	Kinases (e.g., hexokinase)
Hydrolases	Hydrolysis reactions	Proteases (e.g., trypsin)
Lyases	Breaking of bonds (not by hydrolysis)	Decarboxylases, Aldolases
Isomerases	Rearrangement of molecules	Phosphoglucose isomerase
Ligases	Bond formation using ATP	DNA ligase

Enzymatic reactions are highly specific and essential to life processes. Each class of enzyme specializes in a particular type of chemical reaction, facilitating biochemical pathways that support growth, reproduction, and maintenance of cellular function.