

## Chapter 1: General information of enzymes

### 1. Definition of enzymes

Enzymes are **polypeptides** that catalyze biological reactions. They accelerate the metabolic reactions of a living organism, possessing a catalytic power  **$10^6$  to  $10^{12}$  times greater** than that of spontaneous reactions. These **biocatalysts** are generally associated with **small-sized cofactors**; they are involved in processes such as **biosynthesis, degradation, regulation, and reproduction**. Enzymes are **not consumed** during the reaction and are **recovered unchanged at the end** of the catalyzed reaction.

### 2. Classification by location of action

Enzymes are divided into **two main groups**:

- **Exoenzymes** or **extracellular enzymes**: These are synthesized inside the cell and then secreted into the extracellular space. They typically function to break down large macromolecules that cannot pass through the cell membrane.
- **Endoenzymes** or **intracellular enzymes**: These are synthesized and used entirely within the cell. They are responsible for the internal metabolic pathways, such as energy production and DNA replication.

### 3. Nomenclature and classification

#### 3.1. Functional nomenclature

To name an enzyme, **the name of the substrate** is indicated first, followed by the **type of reaction catalyzed**, and finally the suffix **''ase''**.

- **Examples**: Glucose-6-phosphate isomerase and Pyruvate carboxylase.

When an enzyme uses **two substrates**, both indicated by specifying:

1. the **group donor substrate**,
2. the **group acceptor substrate**,
3. the **transferred group**,
4. the **type of reaction**, followed by the suffix **''ase''**.

- **Examples**: Glutamate-pyruvate aminotransferase and Glutamic-oxaloacetic aminotransferase

#### 3.2. Biochemical nomenclature and classification of enzymes

Because the number of enzymes is very large, the International Union of Biochemistry and Molecular Biology (IUBMB) has proposed an official nomenclature and classification system. Each enzyme is assigned a **four-number code**, separated by dots and preceded by the letters **EC** (Enzyme Commission), written as **EC X1. X2. X3. X4**.

- **First number (X1):** Ranges from 1 to 6 and indicates the **type of reaction catalyzed**. Enzymes are classified into six main groups:
  - Oxidoreductases
  - Transferases
  - Hydrolases
  - Lyases
  - Isomerases
  - Ligases
- **Second number (X2):** Designates the **subclass**, specifying the type of chemical group or bond involved.
- **Third number (X3):** Designates the **sub-subclass**, defining the precise nature of the chemical groups involved or the reaction mechanisms when known.
- **Fourth number (X4):** is the **serial number** of the enzyme within its sub-subclass. It allows for further classification based on the specific substrate on which the enzyme acts.
- **Example:** Carboxypeptidase carries the code **EC 3.4.17.1**:
  - **3:** Class of Hydrolase
  - **4:** Subclass of Peptide bond
  - **17:** Sub-subclass of Metalloprotease
  - **1:** Enzyme serial number in this series.

#### 4. Enzymatic reaction

An enzymatic reaction is a **chemical process occurring within the cell** in the presence of a **biocatalyst (an enzyme)**. It is represented by the following equation:



- **S (Substrate):** the molecule that is transformed during the enzymatic reaction.
- **[ES] (Enzyme-Substrate Complex):** The intermediate state where the substrate is bound to the enzyme's active site.
- **P (Product):** The substance resulting from the transformation of the substrate.

There are two main types of biochemical reactions:

- **Catabolism:** Degradation reactions of organic matter (breaking down).
- **Anabolism:** Synthesis reactions of organic matter (building up).

#### 5. Characteristics of Enzymes

- **Low concentration:** Enzymes are effective at very low concentrations.
- **Specificity:** Each enzyme has a specific substrate, though some may accept structurally similar molecules.
- **Speed:** They accelerate reaction rates by a factor of at least  $10^6$ .
- **Not consumed:** The enzyme is not used up; it is released intact.
- **Turnover:** Each enzyme molecule can catalyze an unlimited number of reactions (until it eventually denatures).
- **Activation energy:** Enzymes lower the **free energy of activation** of the substrate

## 6. Determination of enzymatic Activity

The **reaction mixture** is the medium where the reaction occurs, consisting of substrates, cofactors/coenzymes, a buffer solution and the enzyme being studied. Measuring enzymatic activity involves evaluating **the quantity of substrate (S) transformed** or **product (P) formed** per unit of time under specific conditions.

### 6.1. Units of enzymatic activity

**International Unit (IU or U):** The amount of enzyme that converts **1 micromole ( $\mu\text{mol}$ ) of substrate per minute** under specific conditions (temperature, pH, etc.). **Katal (kat):** The amount of enzyme catalyzing the transformation of **1 mole of substrate per second**. The katal is very large:  $1 \text{ U} = 16.67 \text{ nkat}$  So, the **microkatal ( $\mu\text{kat} = 10^{-6} \text{ kat}$ )** is more commonly used.

### 6.2. Enzymatic activity formula

The activity is often measured via spectrophotometry using the Beer-Lambert law:

$$\text{Activit  enzymatique} = \frac{\Delta \text{Abs. min} - 1. V_t}{\epsilon. L. V_e}$$

- **V<sub>t</sub>:** Total volume of the reaction mixture.
- **V<sub>e</sub>:** Volume of the enzyme sample.
- **ε:** Molar extinction coefficient.
- **L:** Optical path length (usually 1 cm).