Practical class №5

Enzymes — Biological Catalysts

In living organisms, countless chemical reactions constantly occur. As a result, all cellular structures are renewed. These transformations in living nature happen millions of times faster than in non-living nature.

These transformations in organisms occur under normal temperature and pressure conditions, and at limited concentrations of hydrogen and hydroxide ions.

For example, the proteins in food are broken down into amino acids during digestion in the human body at 37°C over 2–3 hours, whereas in laboratory conditions, the same process with acids or bases at 100°C can take several hours.

The extremely fast pace of chemical reactions in living organisms is due to the activity of biological catalysts — enzymes or ferments.

While enzymes increase the rate of chemical reactions, they themselves return to their original state after the reaction.

Enzymes are considered organic compounds with a complex chemical composition. They are colloidal solutions, meaning their molecular mass ranges from 10,000,000 and more, and their solutions do not pass through semi-permeable membranes. In solution, enzymes act as amphoteric electrolytes and alter the electric current and pH of molecules. Enzymes can crystallize out of solution while retaining their activity. These crystals, when dissolved again, still retain their catalytic activity. However, if the crystals are dried, their activity is lost.

Enzymes are divided into two types: intracellular and extracellular enzymes.

The intracellular enzymes carry out their activity in the places where they are produced. By entering the structure of complex cell systems, they can form complexes with other enzymes, which not only accelerates the speed of reactions but also regulates many biochemical processes.

Extracellular enzymes are secreted from the cell and help digest food substances in biological fluids, breaking down various complex compounds into simple ones.

7.2. Structure of Enzymes

Like other organic compounds, enzymes are divided into two groups based on their structure: one-component and two-component enzymes, meaning enzymes made of only simple or complex compounds.

Two-component enzymes are formed from a protein part and a non-protein prosthetic group. This prosthetic group may contain ions, vitamins, nucleotides, or other compounds. Enzymes without a prosthetic group are called **apoenzymes**, while those with a prosthetic group are called **holoenzymes**.

Cofactors and apoenzymes alone are not active; when combined, they form a complete enzyme complex and become active, known as a **holoenzyme**.

One of the main features of two-component enzymes is that the prosthetic group gives the enzyme its specific catalytic activity. The prosthetic group is partly responsible for the enzyme's activity, but the enzyme only becomes fully active and performs its function when it is in the holoenzyme form that matches the needs of the organism. The prosthetic group alone cannot ensure full catalytic activity.

Enzyme molecules consist of chains of 100 or more amino acid residues. Each such polypeptide chain has a specific primary, secondary, and tertiary structure. For example, ribonuclease, lysozyme, and similar enzymes are composed of only one polypeptide chain.

Lactate dehydrogenase has 4, catalase has 8, and others have many polypeptide chains — called **protomer units**. Such enzymes consist of multiple protomers.

Such enzymes are called **multimeric enzymes**. Each of them has its own structure. In most multimeric enzymes, each protomer has the same or different amino acid sequence, tertiary structure, and molecular mass. They can function together in one enzyme structure. As a result, an enzyme composed of several different physical-chemical types of protomers is formed. Such enzymes are called **isoenzyme forms** or simply **isoenzymes**. They can be separated from each other using electrophoretic methods.

For example, the molecule of **lactate dehydrogenase (LDG)** is composed of two types of polypeptide chains, forming five different isoenzyme types. LDG obtained from heart muscle consists of 4 identical H-type (heart) chains and is

designated as H_4. LDG from skeletal muscles consists of 4 M-type (muscle) chains and is designated as M_4. Other isoenzymes are mixtures of H and M chains and are denoted as H_3M_1, H_2M_2, H_1M_3. Figure 9 shows their electrophoretic patterns.

A distinct feature of multimeric enzymes is that their structure can change depending on external conditions, meaning they can undergo **protomer dissociation**. This process can be reversible or irreversible. When necessary, multimeric enzymes can switch between active and inactive states. In dissociated form, they often lose catalytic activity. Therefore, the functional state of the multimer complex depends on its full structure. In some cases, multimeric enzymes can work together with each other to perform complex reactions. These reactions take place with the help of intermediate compounds that form between enzyme molecules and later transform into final products.

Classification of Enzymes

Most enzymes are named by adding the suffix **-ase** to the name of the substrate. For example, the enzyme that accelerates the hydrolysis of starch is called **amylase** (from the Greek *amylum* — starch), the enzyme that catalyzes fat hydrolysis is **lipase** (from *lipos* — fat), and the enzyme that facilitates the hydrolysis of urea is **urease** (from Latin *urea* — urea).

There are nearly 1,000 known enzymes in living organisms, all of which accelerate specific chemical reactions.

At the 5th International Congress of Biochemists, an international classification system for enzymes was proposed and adopted. According to this system, enzymes are divided into **six major classes**. Each class is further divided into subclasses and sub-subclasses. All known enzymes are listed in a register, where each enzyme is given a four-part identification number. The first digit represents the main class, the second — subclass, the third — sub-subclass, and the fourth — the specific enzyme serial number.

Class 1: Oxidoreductases

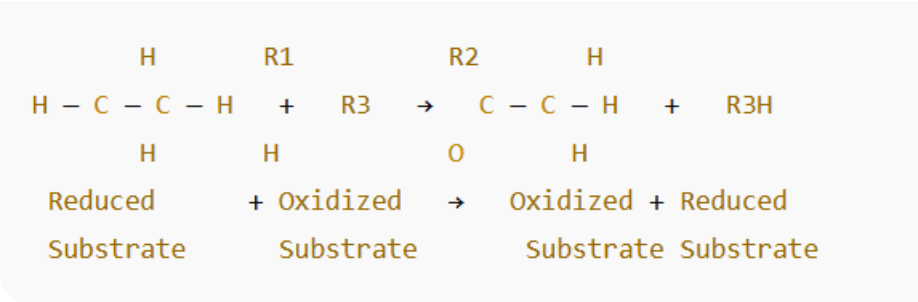
This class of enzymes catalyzes **oxidation-reduction (redox) reactions**, in which electrons are transferred from one molecule (the **donor**) to another (the **acceptor**). Oxidoreductases include the following subclasses:

- **Dehydrogenases**
- **Oxidases**
- **Hydroxylases**

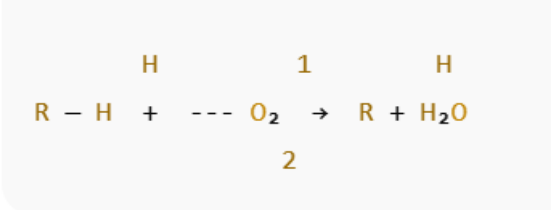
Dehydrogenases

These enzymes participate in redox reactions by removing electrons and protons (hydrogen atoms) from one substrate and transferring them to another.

Reaction Example:



Oxidases catalyze the transfer of hydrogen from the substrate to oxygen:



Hydroxylases and oxygenases — These enzymes speed up certain reactions in biological oxidation by combining oxygen or hydroxyl groups with oxidized substances.

Class 2: Transferases

This class includes enzymes that catalyze the transfer of atoms or groups of atoms within a molecule or between molecules.

- **Methyltransferases** transfer the methyl group (—CH_3)
- **Acyltransferases** transfer an acid residue:
- R — C — (Acid residue)
- ||

- O
- **Glycosyltransferases** transfer monosaccharide residues (glycosyl groups)
- **Aminotransferases** transfer an amino group (—NH_2)
- **Phosphotransferases** transfer phosphate groups:
- OH
- |
- — O — P = O
- |
- OH

(phosphoryl group from phosphoric acid)

Class 3: Hydrolases

This large class includes enzymes that catalyze the cleavage of various bonds in organic molecules using water (hydrolysis):

$R_1 — R_2 + HOH \rightarrow R_1 — H + R_2 — OH$

There are about 500 known individual enzymes in the hydrolase class. These enzymes break various bonds such as esters, peptides, glycosides, amides, and anhydrides using water. Hydrolases include enzymes that break down all food macromolecules. The classification of hydrolases depends on the chemical structure of the substrate and the nature of the bond being hydrolyzed.

(Hydrolases that catalyze hydrolysis reactions) are further divided into sub-classes:

- **Esterases**,
- **Phosphatases**,
- **Glycosidases**,
- **Peptidases**.

Esterases

Esterases accelerate the hydrolysis of esters (both lipid and other types), breaking them into acids and alcohols:

$$R—\overset{\overset{O}{\|}}{C}—O—R_1 + H_2O \rightarrow R—\overset{\overset{O}{\|}}{C} + R_1—OH$$

OH

Complex ester → acid + alcohol

Phosphatases

Phosphatases catalyze the hydrolytic cleavage of phosphate groups from nucleotides and other phosphate esters:

OH O

R—O—P=O + H_2O → R—OH + H_3PO_4

OH

Phosphate ester → alcohol + phosphoric acid

Glycosidases

Glycosidases catalyze the hydrolysis of complex carbohydrates (polysaccharides), for example:

$(C_6H_{10}O_5)$□ + H_2O → $(C_6H_{10}O_5)$□$_{-1}$ + $C_6H_{12}O_6$

Polysaccharide + water → shortened polysaccharide + monosaccharide

Peptidases

Peptidases accelerate the hydrolysis of peptide bonds in proteins and peptides:

O O OH

R—C—NH—R_1 + H_2O → R—C + NH_2—R_1

OH

Protein → amino acid + peptide fragment

Class 4: Lyases

Lyases catalyze reactions that break C–C, C–N, C–O bonds *without* hydrolysis. For example:

O O

R—C—C—OH → R—C + CO_2

OH

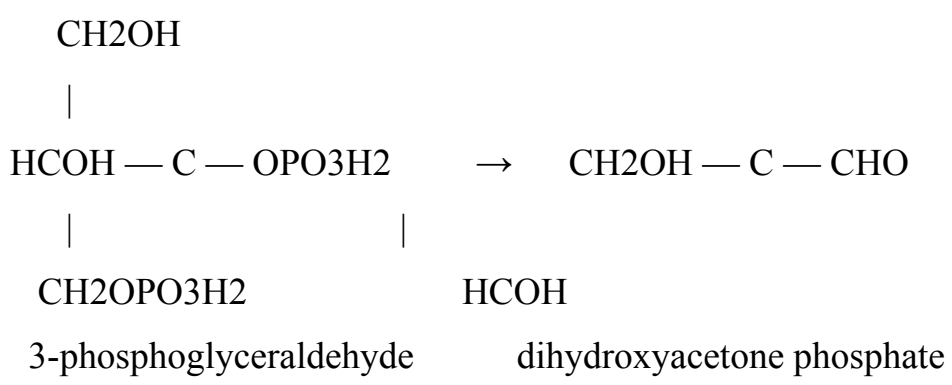
Ketocarboxylic acid → aldehyde + carbon dioxide

Class 5: Isomerases

This is the smallest enzyme class compared to others, including about 90 individual enzymes. These enzymes catalyze internal rearrangement reactions within molecules — including oxidation-reduction, geometric, and structural rearrangements — resulting in the transformation of molecules into different isomeric forms.

Example:

Triosephosphate isomerase



Class 6: Ligases or Synthetases

This class includes enzymes that catalyze the formation of various chemical bonds between molecules found in living organisms. Ligases facilitate synthetic reactions by forming bonds that typically require energy input from ATP or similar molecules.

The main feature of ligase-catalyzed reactions is that they involve **bond formation** during **synthetic processes**, unlike reactions where bonds are broken down to release energy. These enzymes usually catalyze the formation of the following types of bonds:

- C–O
- C–S
- C–N
- C–C

Review Questions

1. What types of substances are enzymes similar to in terms of chemical composition?

2. What is the difference between coenzyme and apoenzyme?
3. What are activators and inhibitors?
4. Describe the mechanism of enzyme action.
5. Explain competitive and non-competitive inhibition.