Simple proteins
Conjugated proteins
Enzymes

A manual for study of submodule 1 on Biochemistry for second-year students of International Faculty specialty: 7.12010001 “General medicine”
This manual is recommended as additional one for independent work of students at home and in class for submodule 1 of Biochemistry at 2-d year study at specialty 7.12010001 “General Medicine”.

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Наведений навчально-методичний посібник пропонується викладачами кафедри біохімії та лабораторної діагностики для використання у самостійній роботі англомовних іноземних студентів медичного факультету ЗДМУ з метою підготовки до змістового модулю 1 з біохімії. Цей навчальний посібник містить все необхідне для вивчення базових питань зі змістового модулю 1 «Прості та складні білки. Ферменти» відповідно робочої програми з дисципліни «Біохімія» для студентів 2-го курсу медичного факультету вищих навчальних медичних закладів III-IV рівнів акредитації в Україні.

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INTRODUCTION

This methodological manual is recommended for students to use like additional materials in preparation for practical classes on Biochemistry for submodule 1. The plan to work with it: 1) to prepare theoretical questions for topic using literature sources, summaries of lectures and this textbook; 2) to make testing control yourself; 3) to be ready to answer teacher about principles of methods used for the determination of proposed biochemical indexes and about their clinical significance.

Authors
CONTENT

• Chapter 1. Classification, physicochemical properties and functions of simple proteins in humans. The methods for identification, separation and release of proteins from biological fluids…………………………………P. 5

• Chapter 2. Conjugated proteins. The methods of allocation and quantitative determination of proteins in biological fluids………………………………P.16

• Chapter 3. Enzymes: structure and physicochemical properties. A classification and nomenclature of enzymes…………………………………P.24

• Chapter 4. The mechanism of action and kinetic properties of enzymes. The regulation of enzymatic activity……………………………………P.35

• Chapter 5. Principles of enzyme activity determination. Genetic deficiency of enzymes. Medical enzymology…………………………………P.45

• Chapter 6. The investigation of Simple and Conjugated proteins, Enzyme properties and their activity in biological fluids……………………………P.53

• Control testing for chapters 1-5………………………………………………P.65

• Literature……………………………………………………………………P.77
CHAPTER 1
THEME:
CLASSIFICATION, PHYSICOCHEMICAL PROPERTIES AND FUNCTIONS OF SIMPLE PROTEINS IN HUMANS. THE METHODS FOR INDICATION, SEPARATION AND RELEASE OF PROTEINS FROM BIOLOGICAL FLUIDS

Simple proteins are complex nitrogen containing organic compounds (polymers) that are consisted of α-amino acid residues, connected by peptide bonds.

Amino acids are biologically important organic compounds composed of amine (-NH₂) and carboxylic (-COOH) functional groups, along with a side-chain specific to each amino acid. In biochemistry, amino acids having both the amine and the carboxylic acid groups attached to the first (alpha-) carbon atom have particular importance. They are known as 2-, alpha-, or α-amino acids with the general formula represented on the fig. 1. Amino acids can be related to a specific stereochemical lines (D- or L-) using D-Glyceraldehyde as a reference compound.

Figure 1. General structure of α-amino acids, where R is an organic substituent known as a "side-chain"

This group of amino acids includes the 20 proteinogenic ("protein-building") amino acids, which combine into peptide chains ("polypeptides") to form the building-blocks of a vast array of proteins.
There are many ways to classify amino acids. These molecules can be assorted into 7 groups on the basis of their structure and the general chemical characteristics of their side-chain radicals:

### Amino Acids with Hydrophobic Side Chain – Aliphatic

- Alanine, Ala
- Isoleucine, Ile
- Leucine, Leu
- Valine, Val

### Amino Acids with Hydrophobic Side Chain - Aromatic

- Phenylalanine, Phe
- Tryptophan, Trp
- Tyrosine, Tyr

### Sulfur-containing Amino Acid with Hydrophobic Side Chain

- Methionine, Met

### Amino Acids with Polar Neutral Side Chains

- Asparagine, Asn
- Cysteine, Cys
- Glutamine, Gln
Amino Acids with Negatively Charged Side Chains - Acidic

- Aspartic acid, Asp
- Glutamic acid, Glu

Amino Acids with Positively Charged Side Chains - Basic

- Arginine, Arg
- Histidine, His
- Lysine, Lys

Unique Amino Acids

- Glycine, Gly
- Proline, Pro

The other type of amino acids classification based on the ability of organism to synthesized them de novo. By this classification, amino acid can be divided on essential, non-essential and conditional amino acid. Essential amino acids cannot be synthesized by the human organism. As a result, they must come from food (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine). Nonessential amino acids are produced in human organism even if they don't come from food (alanine, asparagine, aspartic acid,
glutamic acid and serine). Conditional amino acids are usually not essential, except in times of illness and stress (arginine, cysteine, glutamine, tyrosine, glycine, proline and serine).

QUALITATIVE REACTIONS FOR PROTEINS AND AMINO ACIDS

1. Piotrovsky’s test or biuretic test. This reaction proves the peptide bond in proteins and peptides (starting from tripeptides). The protein solution during the interaction with copper ions gets blue-violet colour in the alkaline environment.

2. Ninhydrin reaction. There is the formation of blue-violet product after the additional of ninhydrin to protein solution. This reaction is used to prove the presence of α-aminoacids residues.

3. Sakaguchi’s test. Arginine is oxidized with sodium hypobromite and reaction with α-naphthol gives red colouring.

4. Fole’s test. This test is used to prove the presence one amino acid residue, only, in the composition of proteins – cysteine.

5. Millon’s test. Tyrosine, reacting with Milon’s reagent, forms mercurial salt coloured red.

6. Adamkiewicz’s test. Tryptophan can react with glyoxylic acid in acid environment. Red-violet coloured condensation products are formed.

7. Reaction with formaldehyde. Tryptophan, condensing with formaldehyde, forms with mineral acids blue-violet coloured salts.

8. Pauli’s test. The test is used to prove the presence of histidine and tyrosine which react with diazobenzene-sulfonic acid, forming cherry-red coloured complex.

CLASSIFICATION OF PROTEINS

All proteins can be classified:

I. According to their function

1. Catalytic (enzymes) – more than 3000 proteins are enzymes.
2. **Nutritive (reserve)** – casein, ovalbumin etc.
3. **Transport** – blood serum proteins, which are capable to transport different compounds and substances to corresponding target organs (hemoglobin, blood plasma albumins, lipoproteins etc).
4. **Protective** – specific protective antibody proteins in response to the invasion of the organism by bacteria, toxins, or viruses. The coagulation of the blood by plasma protein fibrinogen prevents the organism from blood loss.
5. **Contractile** – a large number of proteinic substances are involved in the act of muscular contraction and relaxation (actin, myosin)
6. **Structural** – collagen in connective tissue, keratin in hair, skin, and nails, elastin in vascular wall etc.
7. **Hormonal** – a number of hormones are proteins or polypeptides (the hypophyseal and pancreatic hormones)
8. **Receptor** – rhodopsin, chemoreceptors etc.
9. **Regulatory** – histones, which stabilize structure of DNA, heat shock proteins etc.
10. Other vitally important functions "performed by proteins may be quoted-for example, the maintenance of oncotic pressure.

II. **According to their three-dimensional structure**
1. **Globular proteins**, or **spheroproteins** are spherical ("globe-like") water-soluble proteins (they form colloids in saline solutions) that perform dynamic functions (enzymes, immunoglobulins, transport proteins). During the formation of globular proteins hydrophobic radicals of the polypeptide chain are located inside the structure, and hydrophilic one – on the surface of globular structure.
2. **Scleroproteins**, or **fibrous proteins** have an elongated form, insoluble in water, because they consist mostly from hydrophobic amino acids (proline, hydroxyproline, etc.), physically lasting, perform both structural and protective function: collagen, elastin, keratin.

III. **According to their composition**
1. **Simple proteins** consist from amino acid residues, only

2. **Conjugated or complex proteins** consist from polypeptide chains and non-protein part – *prostetic group*.

**CLASSIFICATION OF SIMPLE PROTEINS**

1. **Protamines.** A group of the simplest water-soluble basic proteins, which consist mostly from arginine (60 %-85 %) Well known protamines: Salmin – a protein of salmon sperm; Clupein – a protein of herring sperm. They take place in the structure of DNA-containing proteins.

2. **Histones.** A group of simple basic proteins that has high solubility in the water and saline solutions, and consist 20-30 % Arg and Lys. They are part of the DNP structure and has regulative role in the control of genome activity.

3. **Prolamines** and **Glutelines.** Simple proteins located in plants (vegetables). Prolamines contain 20-25% Glu and 10-15% Pro and are soluble in 60-80% aqueous ethanol without denaturation.

4. **Albumins** and **Globulins.** Simple proteins, that are abundant very widely (blood plasma, milk, egg white, muscles) and belong to globular proteins. They have different solubility in saline solutions. Solubility of albumins much higher, but they have the lesser mass in comparison with globulins.

**SIMPLE PROTEINS STRUCTURE**

Each protein in its native state has an unique tree-dimensional structure, which is referred to its conformation. This conformation determined by primary structure of Protein.

**Primary structure** of proteins (fig. 2) is an unique determined sequence of α-amino acid residues connected by peptide bond. This sequence is coded by gene of DNA and determines the native state of protein molecule. Mechanism of peptide bond formation is represented on the fig. 3 and 4.
Secondary structure (fig. 2) is dictated by the primary structure. Secondary structure is a configuration of a polypeptide chain in space. It is formed due to Hydrogen bonds between peptide fragments of polypeptide chain. Each normal peptide fragment binds to each another one by two Hydrogen bonds.

Figure 2. Simple proteins structure

\[
\begin{align*}
\text{Primary Structure} & \quad \text{Secondary Structure} & \quad \text{Tertiary Structure} & \quad \text{Quaternary Structure} \\
& \quad \text{Examples of amino acid subunits} \\
& \quad \text{Examples of amino acid subunits} \\
& \quad \text{Examples of amino acid subunits}
\end{align*}
\]

Figure 3. Mechanism of peptide bond formation

\[
\text{R}^1\text{C} = \text{C} - \text{OH} + \text{H} - \text{N} = \text{C} = \text{OH} \rightarrow \text{R}^1\text{C} = \text{C} = \text{N} - \text{C} = \text{C} - \text{OH}
\]

Figure 4. Mechanism of peptide bond formation of Proline is used
1) If Hydrogen bonds are formed between peptide fragments in the same chain the \( \alpha \)-helix turns. Characteristic of \( \alpha \)-helix:

   a) The polypeptide chain turns to the right.
   b) There are 3.6 amino acid residues per turn of the helix.
   c) The total length of the \( \alpha \)-helix in a globular protein can vary from almost 0 to more then 75% of the total chain length.
   d) \( \alpha \)-Helix chains are much shorter in globular proteins then in fibrous ones.

2) If hydrogen bonds are formed between peptide fragments in different chains, extended structures are formed, such as \( \beta \)-pleated sheet.

The chains lie side by side with the Hydrogen bonds forming between -CO group of one peptide fragment and the –NH group of another peptide fragment in the neighboring chain. The chains may run in the same direction, forming parallel \( \beta \)-sheet or they may run in opposite directions forming anti-parallel \( \beta \)-structure.

The most known protein with \( \beta \)-pleated sheet structure is silk fibroin.

It is impossible to form \( \alpha \)-helix or \( \beta \)-pleated sheet structures if Pro residues are represented mostly in polypeptide chain because peptide fragments are without hydrogen.

**Tertiary structure of Proteins** (fig 2). The secondarily ordered polypeptide chain tends to fold into globular structure (like a ball) because this conformation represents a state of lowest energy and of greatest stability for this structure. The conformation results from various interactions between side chain radicals of amino acid residues in polypeptide chain: Hydrogen bonds, disulfide bonds, ester bonds, non-covalent bonds (electro-static interactions, Van-der-Waals forces – magnetic attraction forces). Disulfide bonds are the strongest among all these because they are covalent non-polar.

**Quaternary Structure** (fig. 2) refers to the spatial relationships between individual polypeptide chains in a multichain protein. Each chain is in tertiary conformation and known as protomer. Disulfide bonds are the most important for formation of Quaternary structure. But if one subunit has an overall charge negative and another subunit is positively charged, they can attract and result in
multichain protein. Example of protein with quaternary level of structural organization is hemoglobin.

It should be noted that fibrous proteins organization is considered with missed tertiary level of organization. All fibrous proteins are with quaternary structure in native state.

PHYSICOCHEMICAL PROPERTIES OF GLOBULAR AND FIBROUS PROTEINS

The most characteristic physico-chemical properties inherent in proteins are: 1) high viscosity in solution; 2) low diffusion; 3) pronounced swelling ability; 4) optical activity; 5) mobility in electric field; 6) low osmotic and high oncotic pressures; 7) ability to absorb UV light at 280 nm wavelength (this property which is attributable to the occurrence of aromatic amino acids in proteins, is made use of for protein quantitation).

Proteins, similar to amino acids, are amphoteric owing to the occurrence of free NH$_2$ and COOH groups in their structure and exhibit, accordingly, all properties characteristic of acids and bases. Depending on the pH medium and the percentage of constituent acidic and basic amino acids, proteins in solution develop either a positive, or a negative charge and tend to migrate, respectively, to the anode, or cathode. This property is profitably made use of in the electrophoretic purification of proteins.

Globular proteins solubility in aqueous solutions is due to the presence on their surface of polar amino acid residues

Globular and fibrous proteins have dissimilar physicochemical properties due to their structure formation differences (Table 1).

DENATURATION OF PROTEINS

The process of native protein molecule structure disruption to the primary level is named denaturation. Denaturation is not usually considered to include the
breaking of peptide bonds. Depending on the degree of denaturation, the protein molecule may partially or completely lose its biological activity.

**Denaturing conditions include the following:**

1. **Strong acids or bases.** Changes in pH result in protonation of some protein side groups, which alters hydrogen bonds and salt bridge patterns. As a protein approaches its isoelectric point, it becomes insoluble and precipitates from solution. The structure degradation occurs too.

2. **Organic solvents.** Water-soluble organic solvents such as ethanol interfere with hydrophobic interactions because they interact with non-polar radicals and form hydrogen bonds with water and polar amino acid groups. Non-polar solvents also disrupt hydrophobic interactions.

3. **Detergents.** These amphipathic molecules disrupt hydrophobic interactions, causing proteins to unfold into extended polypeptide chains. (Amphipathic molecules contain both hydrophobic and hydrophilic fragments in structure.)

4. **Reducing agents.** In the presence of reagents such as urea, reducing agents such as β-mercaptoethanol convert disulfide bridges to sulfhydryl groups. Urea disrupts hydrogen bonds and hydrophobic interactions.

5. **Heavy metal ions.** Heavy metals such as mercury (Hg\(^{2+}\)) and lead (Pb\(^{2+}\)) affect protein structure in several ways. They may disrupt salt bridges by forming ionic bonds with negatively charged groups. Heavy metals also bond with sulfhydryl groups, a process that may result in significant changes in protein structure and function. For example, Pb\(^{2+}\) binds to sulfhydryl groups in two enzymes in the haem synthetic pathway. The resultant decrease in hemoglobin synthesis causes severe anemia. (In anemia the number of red blood cells or the hemoglobin concentration is lower than normal.) Anemia is one of the most easily measured symptoms of lead poisoning. This type of denaturation used to reduce the intoxication of the organism after poisoning by lead salts. In this case protein solution is used as a lead scavenger.
6. **Temperature changes.** As the temperature increases, the rate of molecular vibration increases. Eventually, weak interactions such as hydrogen bonds are disrupted and the protein unfolds. Some proteins are more resistant to heat denaturation and this fact can be used in purification procedures.

7. **Mechanical stress.** Stirring and grinding actions disrupt the delicate balance of forces that maintain protein structure. For example, the foam formed when egg white is beaten vigorously contains denatured protein.
Table 1. Differences of physicochemical properties of globular and fibrous proteins

<table>
<thead>
<tr>
<th>Physicochemical properties</th>
<th>Globular protein</th>
<th>Fibrous protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>6000-1000000 Da</td>
<td>6000-1000000 Da</td>
</tr>
<tr>
<td>Shape</td>
<td>Spherical, ellipsoidal</td>
<td>Elongated</td>
</tr>
<tr>
<td>The temperature of the existence of the native molecule</td>
<td>0-40 ºC</td>
<td>0-40 ºC</td>
</tr>
<tr>
<td>The temperature of complete denaturation</td>
<td>More than 70 ºC</td>
<td>More than 70 ºC</td>
</tr>
<tr>
<td>Time of thermal denaturation</td>
<td>1-2 minutes</td>
<td>More than 60 minutes</td>
</tr>
<tr>
<td>Relation to water</td>
<td>Formation of hydrate shell of the micelles</td>
<td>Swelling, only</td>
</tr>
<tr>
<td>Possibility of the formation of saline solution</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Type of solution</td>
<td>Colloidal</td>
<td>No solution</td>
</tr>
<tr>
<td>Relation to:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- mineral and organic acids</td>
<td>- complete denaturation</td>
<td>- complete or partial denaturation</td>
</tr>
<tr>
<td>- salts of heavy metals</td>
<td>- complete denaturation</td>
<td>- complete or partial denaturation</td>
</tr>
<tr>
<td>Presence of isoelectric point</td>
<td>Determined</td>
<td>Absent</td>
</tr>
<tr>
<td>Conduct in electric field</td>
<td>Occurs in protein solution</td>
<td>Not considered</td>
</tr>
<tr>
<td>pH value, that does not cause denaturation</td>
<td>$5 \leq \text{pH} \leq 10$ (depend on structure, location and function of protein in the organism)</td>
<td>Neutral pH</td>
</tr>
<tr>
<td>Passive diffusion</td>
<td>Occurs</td>
<td>Not considered</td>
</tr>
<tr>
<td>Functions:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Structural</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>- Nutrition</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>- Transport</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>- Regulatory</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>- Contractile</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>- Protective (antibody)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>- Protective (mechanical)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>- Enzymatic</td>
<td>Yes</td>
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</tr>
</tbody>
</table>
CHAPTER 2
THEME:

CONJUGATED PROTEINS. THE METHODS OF ALLOCATION AND QUANTITATIVE DETERMINATION OF PROTEINS IN BIOLOGICAL FLUIDS

Many proteins yield, on hydrolysis, some other chemical component in addition to amino acids and they are called conjugated proteins. The non-protein part of a conjugated protein is usually named prosthetic group. Protein part of conjugated protein has a name – apoprotein. Prosthetic groups may be combined with the protein part by the different kinds of bond.

Conjugated proteins are classified on the basis of chemical nature of their prosthetic groups:

1. Chromoproteins
A non-protein component of this class of holoproteins has a special colour. They can be divided on several subgroups:

a) Hemoproteins keep a heme (a special prosthetic group), containing the iron ion (Fe$^{2+}$ / Fe$^{3+}$) or copper ions (Cu$^{+}$ / Cu$^{2+}$). Examples: Hemoglobin. Its native conformation is a quaternary globular structure, composed from four subunits $\alpha_1$, $\alpha_2$, $\beta_1$, $\beta_2$. Each subunit keeps one heme and one polypeptide chain. Hemoglobin is a transfer of oxygen from lungs to any tissue and the transfer of carbon dioxide from tissue to lungs. Molecules of oxygen are connected with iron ions of four hemes, that are contained in hemoglobin.

Cytochromes. Their native conformation is tertiary structure (one polypeptide chain), except Cytochrome oxidase (CChO) (6 subunits in one molecule). The heme of cytochromes contains the iron- ion which can be in two forms: Fe$^{2+}$ / Fe$^{3+}$. CChO keeps two subunits with Copper Cu$^{+}$/Cu$^{2+}$. Cytochromes b, c, c and CChO are used for electrons transfer to molecular oxygen in tissue respiration chain.

The presence of iron ion Fe$^{2+}$explains the colour of hemoproteins – some reddish or reddish – brown shade is found for their molecules.
b) **Flavoproteins** contain in the non-protein part an isoalloxazine fragment from vitamin B$_2$ (riboflavin). A majority of these proteins are enzymes, taking part in oxidation-reduction of some substrates. The prosthetic group of these proteins may be FMN (flavin adenine mononucleotide) or FAD (flavin adenine dinucleotide).

2. **Metalloproteins**

The prosthetic group of these conjugated proteins is represented by metal ions. Depending on nature of ions metalloproteins can be divided to:

a) **Non-heme iron-ions containing.** *Ferritin* is located in spleen, liver, bone marrow and serves for storage of iron in the organism. *Transferrin* is a transfer of iron ions Fe$^{3+}$ from the intestine wall to each tissue. It is indicated in blood plasma in β-globulin fraction. *Hemosiderin* is located in reticuloendotheliocytes of liver and spleen. Its function has been yet little studied.

b) **Copper ions containing proteins.** *Ceruloplasmin* (it is also glycoprotein) is an enzyme with weakly pronounced catalytic activity in oxidation of ascorbic acid, adrenalin, dihydroxyphenylalanine, and a number of compounds.

A lot of enzymes contain other metall-ions: alchohol dehydrogenase (Zn$^{2+}$), phosphotransferases or kinases (Mg$^{2+}$), catalase (Fe$^{2+}$ / Fe$^{3+}$), ATPases (Ca$^{2+}$, Mg$^{2+}$) etc.

3. **Glycoproteins**

This group of conjugated proteins contains carbohydrates and their derivatives as a non-protein part (glucose, mannose, galactose, xylose, arabinose, glucuronic acid derivatives, neuraminic acids, sialic acids, hyaluronic acid, chondroitin sulphuric acid and other glucose aminoglucans).

The last three types of prosthetic group are represented abundantly in proteins of connective tissue. Their function may be structural, protective. All the receptors for hormones and some hormones (gonadotropins, FSH) are glycoproteins. Some glycoproteins may be also enzymes. This type of proteins is also engaged in immune reactions, ion exchange, cellular adhesion.
4. Lipoproteins

They are synthesized in many human tissues or organs: liver, an intestine wall, kidneys, blood. The main function of them in the blood is to transfer lipids from one organ to another one. They are divided in four groups: high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons (ChM). Lipoproteins found in nervous tissue are discussed as structural components of neurons compartments and as transporter of electric impulses.

5. Phosphoproteins

This group of very spread proteins is synthesized in cells in post-translational modification, using special enzymes – protein kinases. Phosphoproteins contain residues of phosphoric acid, which usually are connected to protein part due to serine or threonine side radicals. Phosphoproteins are widely represented in central nervous system (CNS), in the liver, kidneys, bone marrow. A majority of these phosphoproteins are key enzymes of many processes. Caseinogen is also phosphoprotein.

6. Nucleoproteins

This group of conjugated proteins contains nucleic acid as prosthetic group. Depending on the type of nucleic acid nucleoproteins are divided on: DNP (deoxyribonucleoproteins) and RNP (ribonucleoproteins). DNP are found in the nucleus and mitochondrions and RNP — in cytoplasm, endoplasmatic reticulum, in some cases: in nuclei and nucleoli (for high-molecular RNP). Function of these proteins is stipulated by the non-protein part. DNA is a keeper of hereditary information (or genetic information) in a cell. RNAs have such functions:are divided in three groups (types) according their functions:

Ribosomal RNA may be discussed as prosthetic group constantly linked with protein part, because these proteins are in need to create small and big subunits of ribosome.

tRNA may be in linkage with proteins short time during its function: to transfer amino acid residue to the place where the translation occurs.
mRNA messages the information about sequence of amino acid residues in the polypeptide chain that is produced due to translation.

Sharp RNA found in nucleus are enzymes which catalyze splicing (cutting of non-information parts in primary transcription).

METHODS OF CLEANING AND SEPARATION OF PROTEINS

**Dialysis**

This method works on the principles of the diffusion of solutes and ultrafiltration of fluid across a semi-permeable membrane. So that, low molecular weight impurities pass through the pores of the membrane, and macromolecular compounds (proteins) are retained. Thus, proteins are cleaned from impurities. This method is used in the department of "artificial kidney" to purify blood of its low molecular weight compounds.

**Salting-out**

There is no denaturation of protein molecule due to salting-out. When large amount of neutral salt is added to a protein micella in solution, a precipitate forms. The large number of salt ions can effectively compete with the protein for water molecules, that is, the solvation spheres surrounding the protein ionized groups are removed. The net charge of protein molecule becomes zero and it aggregate and then precipitate. This process is referred to as salting out. Because salting out is usually reversible, it is often used as an early step in protein purification without denaturation.

This method is also used for separation of proteins. For example, globulins sediment is formed from 50 % solution of ammonium sulfate and albumins precipitate under the addition crystal form of ammonium sulfate. Difference in solubility give an opportunity to separate these proteins from each other, using salting-out.
**Ultracentrifugation**

Centrifuges of many sizes and speeds are used in the laboratory to remove debris as well as to collect precipitated proteins and other materials at various steps in a purification scheme. They may be used both for separation of molecules and for determination of molecular mass ($M_r$) (Fig. 1). When macromolecules in the solution are subjected to an ultracentrifugal field they are accelerated rapidly to a constant velocity of sedimentation. This is expressed as a sedimentation constant $S$, which is the rate (cm/s) per unit of centrifugal force. The unit of $S$ is the second but it is customary to give it in Svedberg units, $S$ (1 $S = 10^{-13}$ s). Sizes of particles are often cited by their $S$ values. At a constant velocity the equilibrium will eventually be attained in which sedimentation is just balanced by diffusion and a smooth concentration gradient forms from the top to the bottom of the centrifuge cell or tube. After centrifugation, which is usually done in a plastic tube, a hypodermic needle is inserted through the bottom of the tube and the contents are pumped or allowed to flow by gravity into a fraction collector.

![Figure 1. Ultracentrifugation](image)

**Electrophoresis**

Electrophoresis, the process of separating molecules, which based on the difference of their net charges, by migration in an electrical field, is conducted in many ways. In zone electrophoresis, a tiny sample of protein solution is placed in a thin line on a piece of paper or cellulose acetate. The sheet is moistened with a buffer and electrical current is passed through it. An applied voltage of a few hundred volts across a 20-cm strip suffices to separate serum proteins in an hour.
To hasten the process and to prevent diffusion of low-molecular-weight materials, a higher voltage may be used. Two to three thousand volts may be applied to a sample cooled by water-chilled plates. Large-scale electrophoretic separations may be conducted in beds of starch or of other gels.

One of the most popular and sensitive methods for separation of proteins is **electrophoresis in a column filled with polyacrylamide or agarose gel or on a thin layer of gel on a plate**. The method depends upon both electrical charge and molecular size and has been referred to as electrophoretic molecular sieving. This method, which is often referred to as SDS–PAGE, has the advantage of breaking up complex proteins composed of more than one subunit and sorting the resultant monomeric polypeptide chains according to molecular mass (fig. 2, 3).

![Illustration of SDS-PAGE](image)

**Figure 2. Electrophoresis in a column filled on a thin layer of gel on a plate**

**Capillary electrophoresis** is increasingly popular and can be used to separate attomole amounts ($10^{-18}$ mole). It may be used not only for separation of proteins but also for rapid estimation of the net charge of a protein molecule.

Whereas in conventional **zone electrophoresis** most of the electrical current is carried by the buffer, in **isotachophoresis** the ions being separated carry most of the current. In **isoelectric focusing**, a pH gradient is developed electrochemically
in a vertical column or on a thin horizontal plate between an anode and a cathode. Proteins within the column migrate in one direction or the other until they reach the pH of the isoelectric point where they carry no net charge and are “focused” into a narrow band. As little as 0.01 pH unit may separate two adjacent protein bands which are located at positions corresponding to their isoelectric points. The isoelectric point (pI), is the pH at which a particular molecule carries no net electrical charge and their electrophoretic mobility are absent. If pH value more than pI protein particle will move to anode, if less – to cathode.

Such two-dimensional method in which proteins are separated by isoelectric focusing in the first dimension and by SDS-gel electrophoresis in the second has become a popular and spectacularly successful method for studying complex mixtures of proteins.

Figure 3. Polyacrylamide-gel electrophoresis (SDS-PAGE)

**Affinity chromatography**

Affinity chromatography is a method of separating biological mixtures based on a highly specific interaction such as that between antigen and antibody, enzyme and substrate, or receptor and ligand. The stationary phase is typically a gel matrix, often of agarose. Usually the starting point is an undefined heterogeneous group of
molecules in solution, such as a cell lysate, growth medium or blood serum. The molecule of interest will have a well known and defined property, and can be exploited during the affinity purification process. The process itself can be thought of as an entrapment, with the target molecule becoming trapped on a solid or stationary phase or medium. The other molecules in the mobile phase will not become trapped as they do not possess this property. The stationary phase can then be removed from the mixture, washed, and the target molecule is released from the entrapment in a process known as elution. Possibly the most common use of affinity chromatography is for the purification of recombinant proteins.

Affinity chromatography may be used to:
1. purify and concentrate a substance from a mixture into a buffering solution;
2. reduce the amount of a substance in a mixture;
3. discern what biological compounds bind to a particular substance;
4. purify and concentrate an enzyme solution.
CHAPTER 3

THEME:

ENZYMES: STRUCTURE AND PHYSICOCHEMICAL PROPERTIES.
CLASSIFICATION AND NOMENCLATURE OF ENZYMES

Enzymes are catalysts of protein nature. Enzymes share some properties with chemical catalysts.

**Shared properties:**

1. Enzymes are neither consumed nor produced during the course of a reaction.
2. Enzymes do not cause reactions to take place; they expedite reactions that would ordinarily proceed, but at a much slower rate, in their absence. They don’t alter the equilibrium constants of reactions that they catalyze.

**Differences between enzymes and chemical catalysts:**

1. Enzymes are invariably proteins
2. Enzymes are highly specific for the reactions they catalyze and produce only the expected products from the given reactants (or substrates)
3. Enzymes often show a high specificity toward one substrate, although some enzymes have a broader specificity, using more than one substrate.
4. Enzymes function within a moderate pH and temperature range.

A majority of enzymes are globular proteins. So, all specified properties of globular proteins are introduced in enzymes.

**Composition and structure of enzymes**

Enzymes may be simple or conjugated, it depends upon the presence of the non-protein part (see below):
Cofactors may be non-organic or organic compounds.

**Non-organic cofactors:**
1. Metal ions: Ca$^{2+}$, Mg$^{2+}$, Zn$^{2+}$, Co$^{2+}$, K$^+$, Na$^+$, Cu$^{2+}$, selenium for glutathione peroxidase, etc.
2. Phosphoric acid residues: $\text{H}_2\text{PO}_4^-$, $\text{HPO}_4^{2-}$, $\text{PO}_4^{3-}$.

**Organic compounds-cofactors:**
1. Nucleotides: ATP, AMP, ADP, etc.
2. Carbohydrates: glucose, galactose, mannose, etc.
3. Vitamins and their derivatives (look figure N1)
4. Heme and its derivatives: 1) Cytochromes $b$, $c$, $aa_3$, $P_{450}$; 2) Catalase, Peroxidase
5. Short peptides: Glutathione (GSH/GS-GL), etc.

Figure N1. The use of some vitamins in the structure of enzymes catalyzed special type of the reaction

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Coenzyme or prosthetic group</th>
<th>Type of the reaction catalyzed by the enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamine</td>
<td>TPP (thiamine pyrophosphate)</td>
<td>Oxidative decarboxylation of keto acids;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Transketolase reactions</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td><strong>Riboflavin</strong></td>
<td>FMN (Flavin MonoNucleotide) FAD, Flavin Adenine Dinucleotide</td>
<td>Oxidation-Reduction</td>
</tr>
<tr>
<td><strong>Pantothenic acid</strong></td>
<td>CoASH (Coenzyme A) ACP (Acyl carrier protein)</td>
<td>Activation of free acids Palmitate synthetase complex</td>
</tr>
<tr>
<td><strong>Nicotinic acid or nicotinamide</strong></td>
<td>NAD⁺, NADP⁺ (Nicotinamide Adenine Dinucleotide, Nicotinamide Adenine Dinucleotide Phosphate) and their reduced forms</td>
<td>Oxidation-Reduction Hydroxylation (NADPH mainly)</td>
</tr>
<tr>
<td><strong>Pyridoxin</strong></td>
<td>Pyridoxal phosphate, Pyridoxamine</td>
<td>Alpha-decarboxylation, Transamination of amino acids</td>
</tr>
<tr>
<td><strong>Lipoic acid</strong></td>
<td>Lipoamide</td>
<td>Oxidative decarboxylation of keto acids</td>
</tr>
<tr>
<td><strong>Biotin</strong></td>
<td>Carboxybiotin</td>
<td>Carboxylation of some acids</td>
</tr>
</tbody>
</table>

It should be noted that some medicines may be found in the formation of so named pseudo-coenzymes thus they can block activity of enzymes, for example: isoniazide is precursor for pseudo-coenzyme similar in structure to NAD⁺.

**Specific sites of enzyme**

The most important part of any enzyme is ***the active centre. It is a structural fragment of enzyme which attaches a substrate (one or more), and there is a conversion of substrates to the products of enzymatic reaction*** in this centre. There are two parts in each active centre of enzymes: a **catalytic site** and **binding site** for substrates.

Active centre of simple enzymes is composed from amino acid residues, only. The most frequently used amino acid residues in active centre of many enzymes are: Serine, Aspartic acid, Histidine, Lysine, Glutamic acid, Cysteine.
Active centre of conjugated enzymes usually keeps the non-protein part, for example:

a) Alcohol Dehydrogenase has NAD$^+$;

b) Cytochrome oxidase has heme-containing Fe$^{2+}$/Fe$^{3+}$ and Cu$^+$/Cu$^{2+}$.

As a rule vitamin derivatives are in the active centre of conjugated enzymes. There are some amino acid residues in the active centre of conjugated enzymes, too. A conformation of active centre is formed only when a three-dimensional structure of enzymes is formed.

A majority of enzymes are synthesized as precursors of enzymes (inactive form, proenzyme). There are some ways of activation of proenzymes to form active enzymes:

1. **Non-complete proteolysis of precursor**: a part of polypeptide chain of precursor is eliminated by some another enzyme (protease). For example: 1) Enteropeptidase action on trypsinogen: N-terminal hexopeptide is eliminated from precursor to form active enzyme trypsin; 2) Trypsin produces chymotrypsin from its precursor chymotrypsinogen. The subtype of limited proteolysis is Autocatalysis: ability of active form of enzyme to produce itself from proenzyme. This way is discussed for pepsin, trypsin and chymotrypsin formation.

2. **Allosteric activation** of proenzyme. As a rule the key enzymes of process have allosteric centers. Allosteric centre is a site in the enzyme molecule structure which is able to adopt some organic or non-organic compounds. They are named effectors. The effector changes the conformation of enzyme (or proenzyme) after its linkage:

   1) to form the active centre in the structure of proenzyme. In this case it is named allosteric activator;

   2) to destroy the active centre of enzyme. In this case it is named allosteric inhibitor.

**Phosphorylation–Dephosphorylation** is this type of enzyme activation (or inhibition). As example, look in your textbook at two key enzymes regulation in glycogen metabolism: glycogen phosphorylase and glycogen synthetase.
Properties of enzymes

1. Specificity of enzymes

   **Absolute specificity.** This is specificity of enzyme action that is determined by its ability to act with only one substrate. For example: enzyme *urease* can destroy the urea, only, and can’t react with any other substrate.

   **Relative group specificity.** Many enzymes in nature have more then one substrate. This type of specificity may be named as *relative group* one. Term “relative” is used for the enzyme catalyzing the conversion of the same fragment in the structure of its substrate molecules. For example: A salivary amylase has the relative group specificity. It can destroy the α–1,4–glycosidic bond in the structure of polysaccharides such as starch, glycogen and their non-complete digestion products. But this enzyme can’t react with disaccharides such as sucrose or maltose and monosaccharides as substrates. Second enzyme from saliva named lysozyme (or muramidase) can destroy proteoglycans in bacterial wall and has the same type of specificity.

   **Stereochemical specificity.** For example: There are two types of alanine oxidase in the liver: L–oxidase and D–oxidase. L–oxidase can react with L–alanine, only. D–oxidase can react with D–alanine, only.

   Any type of specificity of enzyme is determined by:

   1) The functional groups of the substrate (or product);
   2) The functional groups in the active centre of enzyme and its cofactors (coenzymes)
   3) The physical proximity of these various functional groups during the duration of the reaction.

2. Thermolability of enzymes

   High temperature of environment (more then 60°C) should be considered as a factor for denaturation of human enzymes. Optimal temperature for enzymatic action in human organism is about 38–40°C. The low enzymatic activity is keeping
at low temperature in region -8°C – 0°C. So the curve of enzymatic activity (A) dependence on temperature is like this one:

![Figure 2. A temperature of environment influences the enzyme activity.](image)

### 3. Effect of pH medium on enzymatic activity

Each enzyme-catalyzed reaction has its pH optimum. For majority tissue enzymes in humans pH optimum is about 7.2–7.4. Pepsin of gastric juice has very low pH optimum 1.5–2.5 at healthy adults. Enzymes of small intestine have the pH optimum about 8.0–8.4. So, the pH optimum of enzymes is very individual characteristic for them. The curve of the enzymatic activity (A) dependence on pH environment may be shown for tissue enzymes like this graph curve:

![Figure 3. The influence of pH medium on enzyme activity.](image)

There is the denaturation of tissue enzymes at points K and M because of strong acidic (point K) or strong alkalic (point M) medium around enzyme is found as denaturation factor. There is the lower enzyme activity in point X and Y in comparison with the point L because the charge of amino acid residues in active
centre of enzyme is changed at pH values related to points X and Y. This change influences the rate of enzymatic reaction, and it is decreased.

**Classification and nomenclature of enzymes**

The International Union of Biochemistry recommended to introduce a decimal system of enzymes based on the nature of the catalyzed reaction. In 1972, the Commission for Biochemical Nomenclature of the International Union of Pure and Applied Chemistry (IUPAC) published a new addition of enzyme nomenclature. Before this time the substrate name was usually taken and the suffix “ase” was attached. In other cases, the suffix was attached to the name of the catalyzed reaction. Some of the yearly described enzymes have special names, such as trypsin, pepsin, catalase. Today according to the classification there are six classes of enzymes. Each enzyme receives a four-part-number code and is also given a systematic name and recommended trivial name. For example: membrane carrier proteins that facilitate diffusion are named permeases, because it is difficult to estimate type of the reaction catalyzed by them.

**Example of enzyme class code:**

Tyrosine amino transferase:

E.C.2.6.1.5. the ordinal number of this enzyme

**Sub-sub-class:**

The nature of substrate (donor)

**Class:**

Subclass:

Transferase the type of the group that is transferred

**Classes of enzymes**

1. **Oxido reductases** are involved in oxidation and reduction. The trivial names: dehydrogenases, oxidases, oxygenases, cytochromes. All the enzymes of this class
are conjugated proteins. The cofactors of this class: FAD/FADH₂, FMN/FMNH₂, NAD⁺/NADH, NADP⁺/NADPH, heme (Fe²⁺/Fe³⁺), Cu⁺/Cu²⁺

**Scheme of reactions, related to the oxidation / reduction:**

1) \( \text{H}_2\text{C} = \text{C} = \text{CH}_2 \rightarrow \text{H} \text{C} = \text{C} = \text{H} \quad 2\text{H}^+, 2 \overline{e} \)

2) \( A + 2 \overline{e} \rightarrow B \)

3) \( A + \text{O}_2 \rightarrow \text{A} \text{O}_2 \)

4) \( \text{S} - \text{OH} + \text{O}_2 \quad \text{S} - \text{OH} + \text{H}_2\text{O} \quad 2\text{H}^+, 2 \overline{e} \)

**2. Transferases** transfer structural fragment from one substrate (donor) to another one (acceptor)

**Scheme of this type of reaction:**

<table>
<thead>
<tr>
<th>Group transported by the enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-X + B ← B-X + A</td>
</tr>
<tr>
<td>Donor</td>
</tr>
</tbody>
</table>

Transferases catalyze usually reversible reactions. Fragments that may be transported:
Amino – NH₂; Methyl – CH₃; Acetyl – CH₃CO⁻; Phosphate – OPO₃H₂ and many others.

**3. Hydrolases** catalyze the hydrolysis of a substrate. The structural fragment (or bond) of a substrate is digested, water molecule is used in the formation of products. A scheme of this type of reaction:

\[ A-X + \text{H-OH} \rightarrow \text{H-X} + \text{A-OH} \]

<table>
<thead>
<tr>
<th>Substrate hydrolyzed by the enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two products</td>
</tr>
</tbody>
</table>
The digestion of proteins, polysaccharides, some lipids is carried out by this class of enzymes. Invasive properties of phytopathogenic microorganisms are due to this enzyme class.

4. **Lyases** add (or remove) the elements of water, ammonia, or carbon dioxide (CO₂) to (or from) double bonds. They can destroy the bond without water molecule utilization.

1) alpha-decarboxylation of amino acid

\[ \text{A-X} \xrightarrow{\text{enzyme}} X + A \]

Substrate destroyed by the enzyme

Two products

2) dehydration of beta-hydroxyacyl-CoA fragment:

\[ \text{CHCH₂OH} \xrightarrow{\text{H₂O}} \text{HCH₂} \]

3. **Isomerases** catalyze changes within one molecule; they include **racemases** and **mutases**, as well as **epimerases**. Isomers are different in structures, but quantitative composition is the same for both substances.

For example, reaction catalyzed by glucose-6-phosphate isomerase:

\[ \text{Glucose-6-P} \xleftrightarrow{\text{ISOMERS}} \text{Fructose-6-P} \]

A sign for this type of reaction: the reaction is obligatory reversible!

6. **Ligases** (trivial: synthetases) join two or more molecules (substrates) together at the expense of energy released after degradation of high-energy bond of nucleoside triphosphate (ATP, GTP, UTP and others).

The schemes of this type of reaction are:
X + A + ATP → A-X + ADP + H₃PO₄

Three substrates

Energy source

Three products

(1)

or:

A + B + ATP → A \ − \ B + AMP + H₄P₂O₇

(2)

ATP may be used as the agent for phosphorylation (as a donor of phosphate group) catalyzed by phosphotransferase:

S + ATP → S-OPO₃H₂ + ADP

(3)

Compare equation (1) and (3) and care for the transformation of ATP molecule in both reactions to differ them.

**Isozymes: the definition and properties**

The genetic information about the same enzyme may be represented in different tissues of human organism by variation of genes. In this case genetic forms of this enzyme may differ partially in variation of subunits which are in creation of the native molecule of the enzyme. As example, let us consider those genetic forms for lactate dehydrogenase (LDH):

<table>
<thead>
<tr>
<th>Subunit</th>
<th>Myocardium</th>
<th>Liver</th>
<th>Skeletal muscle</th>
<th>Kidneys</th>
<th>Erythrocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH1</td>
<td>LDH2</td>
<td>LDH3</td>
<td>LDH4</td>
<td>LDH5</td>
<td>LDH1</td>
</tr>
<tr>
<td>H₄</td>
<td>H₃M</td>
<td>H₂M₂</td>
<td>HM3</td>
<td>M4</td>
<td></td>
</tr>
</tbody>
</table>

H and M – subunits of the quaternary structure of enzyme

These genetic forms are named **isozymes**. The active site in isozymes structure is the same, and any isozyme of LDH catalyzes the same reaction. But they are
different in quaternary structures, physicochemical properties and location in tissues, that is because the determination of activity and concentration of each isozyme in the blood serum may be used in clinical diagnostics of diseases.

**Multienzyme complexes**

This is a complex of enzymes that are located together and carry out the same reaction or process.  

*For example:* Pyruvate dehydrogenase complex is composed from three enzymes:

1) Pyruvate Dehydrogenase: \( E_1 \)-TPP  
2) Dihydrolipoyl transacetylase: \( E_2 \) (Lipoic Acid in two forms, CoA~SH)  
3) Dihydrolipoyl dehydrogenase: \( E_3 \) (FAD, NAD\(^+\))

The inhibition of any one enzyme from this complex causes the inactivation of the whole system. There are many Multienzyme complexes (MC) in cells: MC for High Fatty Acids Synthesis; MC for Oxidative decarboxylation of alpha–ketoglutarate; MC for \( \beta \)–oxidation of HFA; a respiratory chain in the inner membrane of mitochondria, etc.
A mechanism of Enzymes action

Enzymes decrease the energy of activation. A chemical reaction occurs when a certain proportion of the substrate molecules are sufficiently energized to reach a transition state in which there is high probability that a chemical bond will be made or to form the product. The effect of enzymes is to decrease the energy of activation (fig. 1).

\[ E_{\text{activation}} = E_{\text{transition state}} - E_{\text{initial state}} \]

Figure 1. Free energy of chemical reaction for uncatalyzed reaction and catalyzed by enzyme. Energy activation for enzymatic reaction is lower!

In 1913, L. Michaelis and M. Menten noted that an enzyme – substrate complex ES is formed which undergoes a chemical reaction and is broken down to free enzyme E and the product P.

So, the common equation of reversible enzymatic reaction must be:

\[ E + S \rightarrowright ES \rightarrowright EP \rightarrowright E + P \quad (1) \]
\[ E + S \rightarrowright ES \rightarrowright EP \rightarrowright E + P \quad (2), \]

Where case (1) – equation for reversible reaction; case (2) - equation for irreversible reaction.
The rate of both reactions is depended on the substrate, enzyme concentration, and the rate to reach transition state is promoted by ES complex concentration. Product concentration influences the rate of reaction (2), only.

**The types of bonds for ES complex formation:** Hydrogen bonds; Electro-static interactions; Covalent bonds; Magnetic attractions.

**Two theories have been proposed to explain specificity of enzyme action:**

*a) The lock and the key theory (Fisher E., 1940th)*

The active centre of the enzyme (the lock) is complementary in conformation to the substrate (the key), so that enzyme and substrate “recognize” one other.

*b) The induced-fit theory (D.E. Koshland, 1950th)*

The enzyme changes shape upon binding the substrate, so that the conformations of a substrate and enzyme protein are only complementary after binding reaction. The “induced-fit” hypothesis presumes the existence between the enzyme and the substrate of not only spatial ore geometrical complanarity, but also electrostatic charge complementary: it means interactions of oppositely charged groups of the substrate and the active centre of the enzyme.

Today a majority of scientists agree with the second theory, because it can explain any type of specificity of enzymes, and the least level of energy activation for enzymatic reaction. Step by step whole mechanism of enzymatic reaction may be explained so:

- there is a moment of orientation and approach of enzyme and substrate relatively (may be at the expense of high-energy bond digestion) one to another in space;
- then it is a moment of an enzyme contact with the substrate – as the result ES complex is formed, and there is the induced fit of enzyme to substrate at this moment too. The attachment of a substrate provokes the spatial changes in the enzyme conformation. There is some strain in the conformation of active centre, and there is some deformation in substrate structure attached to the active centre. All these changes promote quickly the reaching of the transition state of the reaction.
Enzymes catalyze reactions by utilizing the same general reactions as studied in organic chemistry:

- **Covalent catalysis**
- **Metal ion catalysis**
- **Catalysis by alignment (approximation)**
- **Acid-base catalysis**

  - Additional free energy is obtained through the “Binding Energy” (binding of the substrate to the enzyme);
  - Binding energy often helps stabilize the transition state, lowering energy for activation of enzyme.

**Acid-base catalysis.** There are some specific amino acid residues in active centre of enzymes that can be donors or acceptors of protons during the catalysis. Such as:

<table>
<thead>
<tr>
<th>Donors</th>
<th>Acceptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>- COOH</td>
<td>- COO⁻</td>
</tr>
<tr>
<td>- NH₃⁺</td>
<td>- NH₂</td>
</tr>
<tr>
<td>- SH</td>
<td>- S⁻</td>
</tr>
</tbody>
</table>

These groups take part in catalysis of many organic reactions in water phase.

**Covalent catalysis (fig.2).** In some cases enzyme (E-OH) can replace the functional group in a substrate RCO-X to form the covalent complex E-OCOR and first product HX (step A: acylation). This complex is not stable and is quickly hydrolyzed due to water use (step B: deacylation):

![Figure 2. Covalent catalysis mechanism in steps (A, B) for chymotrypsin.](image-url)
The hydroxylic group –OH in the enzyme active site may be from amino acid residues such as Serine or Threonine. This mechanism of enzymatic action is discussed for chymotrypsin and is named as covalent catalysis.

**Kinetics of enzymatic reactions**

Kinetic is the trend of enzymology that is concerned with study of all the factors which can influence the rate of enzymatic reaction. The determination of special indexes for each enzyme (Km and V) at normal condition (or in a case of some factors influence the rate of enzymatic reaction) is made. These indexes can help us to estimate the behavior of enzyme in living system.

**Substrate concentration influences the rate of enzymatic reaction**

Common equation of reversible enzymatic reaction is:

\[
E + S \xrightleftharpoons[K_1]{K_{+1}} ES \xrightleftharpoons[K_2]{K_{+2}} EP \xrightarrow{K_{-2}} E + P
\]  

(1)

K_{+1} – the rate constant for the formation of ES
K_{-1} – the rate constant for dissociation of ES
K_{+2} – the rate constant for dissociation of ES to E plus P.
K_{-2} – the rate constant of ES formation from E and P.

If the substrate concentration [S] equals zero, the rate of enzymatic reaction equals zero, too. The rate of enzymatic reaction depends upon the rate of saturation of active centers of enzyme by substrate molecules. The curve of reaction velocity (V) dependence on the substrate concentration [S] is this one (fig.3):

Figure 3. The curve of reaction velocity (V) dependence on the substrate concentration [S]
1) when the [S] is low, the reaction is first-order with respect to substrate: 
\[ V \sim [S] \rightarrow \text{intercept } 0A \]
2) in the middle of the curve (part AB) the reaction is mixed-order.
3) the part BC is discussed as a complete saturation of active centers of enzyme by substrate mole-cules. The velocity is maximal \( V = V_{\text{max}} \). The [S] corresponding to the point B is named as the substrate concentration for saturation of active centers.

This curve may be described by mathematic equation (Michaelis-Menten equation):
\[ V = V_{\text{max}} [S] / (K_s + [S]) \]  
where \( V_{\text{max}} \) - maximal reaction velocity; \( K_s \) - dissociation constant of enzyme-substrate complex ES.

Briggs and Haldeine later decided to replace the constant \( K_s \) by a new one \( K_m \) (Michaelis constant), that may be calculated as:
\[ K_m = K_s + \frac{K_{+2}}{K_{-1}} \]  
and the new equation is
\[ V = \frac{V_{\text{max}} [S]}{K_m + [S]} \]  

**Physical sense of** \( K_m \):

**\( K_m \) equals to the substrate concentration at which the velocity is half-maximal**, that is because it may found using the curve (fig.4)

![Figure 4. An example of Km determination for enzyme using the graph.](image-url)

**The affinity of an enzyme for its substrate is estimated by** \( K_m \):

**The lower the value of** \( K_m \) **the greater the affinity of the enzyme for its substrate**
$V_{\text{max}}$ and $K_m$ are very important characteristics which are placed in special reference books for each enzyme.

Because it is difficult to estimate $V_{\text{max}}$ from the position of an asymptote, as in the plot of a rectangular hyperbola (Michaelis-Menthen curve), linear transforms of the Michaelis-Menten equation are often used. The equation (3) is transformed into (4) and (5).

The reverse value to $V$ are produced from equation (3):

$$\frac{1}{V} = \frac{K_m + [S]}{V_{\text{max}} \cdot [S]} \quad (4)$$
$$\frac{1}{V} = \frac{K_m}{V_{\text{max}}} \cdot \frac{1}{S} + \frac{1}{V_{\text{max}}} \quad (5)$$

This method is named as **Lineweaver-Burk method**. It shows the straight-line graph obtained by plotting $1/V$ opposite $1/[S]$ (fig.5), where the y-intercept equals $1/V_{\text{max}}$, and the x–intercept equals $-1/K_m$, and the slope equals $K_m/V_{\text{max}}$. This method is often used at the research of inhibitors’ influence on the rate of enzymatic reaction.

![Lineweaver-Burk method graph](image)

**Enzyme concentration**

Enzyme activity is regulated by Enzyme concentration (fig.5). This dependence is considered only if:

$$\text{pH, } t^\circ \text{C – optimal, }$$
$$[S] \gg [S]_{\text{saturation}}$$

![First-order dependence of V from [E]](image)
**Reversible inhibition of enzyme activity**

Different types of reversible inhibition are possible, and they may be easily distinguished by analysis of Lineweaver-Burk plots.

**Competitive inhibition features**

a) Inhibitor (I) is similar in a structure to S.

b) I makes linkage only with active centre of E. The inhibition is observed if \([I] > [S]\).

c) If \([I] << [S]\), I is displaced by substrate molecule from active centre of E.

![Succinate (Amber acid) to Fumarate](image)

Figure 7. Lineweaver-Burk plots for competitive inhibition investigation and succinate dehydrogenase reaction.

**Example 1:** Malonic acid HOOC – CH₂ – COOH is the competitive I for succinate dehydrogenase reaction in Krebs cycle. Thanks to two carboxylic groups in structure Malonic acid blocks active centre of E. It increases the \(K_m\), but \(V_{max}\) is not changed in value. Lineweaver-Burk plots are as shown in fig.7. **Example 2:** Proserin preparation influences the acetylcholine esterase activity: proserin competes with acetylcholine to attach active site of this enzyme, thus it decreases activity of acetylcholine esterase in treatment of myasthenia.
**Example 3:** Antimicrobial effect of Sulfonamide preparations is associated with the damage of folic acid (vitamin B₉) synthesis from para-amino benzoic acid, and sulfonamide competes with para-amino benzoic acid to be linked to active site of the enzyme involved in production of this very important vitamin.

**Example 4:** Ethanol is used for treatment of patients with methanol poisoning (per os or intravenously) in a quantity that can cause separately toxicity for healthy person. The effect of ethanol use as drug in this case is explained so: affinity of ethanol to active site of alcohol dehydrogenase is much higher then for methanol, and it can replace methanol by itself under condition of ethanol excess intake.

**Non-competitive inhibition features**

a) \( I \) has another structure in a comparison with \( S \)

b) \( I \) may be attached not only with active centre of \( E \).

c) The complex \( \text{EIS} \) is formed due to weak or covalent bonds.

d) \( I \) changes the \( V_{\text{max}} \) value, but \( K_m \) is not changed.

Lineweaver-Burk plots for this type of inhibition are shown in fig.8.

![Lineweaver-Burk plots](image)

Figure 8. Lineweaver-Burk plots for non-competitive inhibition investigation. For example: \( E \) – cytochrome C oxidase (heme-containing); \( I \) – cyanide ions \( \text{CN}^- \)

Heavy metal ions (lead, mercury), arsenic ions toxicity is explained from their influence on enzymes as non-competitive inhibitors to block SH-groups in active site of enzymes. Reversibility for this type of inhibition may be due to decrease of their concentration in the reaction medium due to dilution of solution where reaction occurs.
**Allosteric inhibition features**

It is usually the reversible inhibition. That is because the I makes linkage with allosteric centre by non-covalent bonds to change conformation of enzyme molecule (fig.9). Allosterically regulated enzymes are key enzymes for metabolic processes. So, allosteric activation and inhibition are the most important regulative processes in promotion of homeostasis in a cell. Feed-back inhibition is discussed as the case of allosteric inhibition. Sometimes a product of enzymatic reaction (or terminal product of a process) may be as allosteric inhibitor at condition of its accumulation in a cell.

*Allosteric inhibitor connects with allosteric centre*

\[ \downarrow \]

*An enzyme conformation is changed at this moment*

\[ \downarrow \]

*The conformation of active centre is changed, too (or there is the degradation of active centre)*

\[ \downarrow \]

*Result: it is impossible to create the ES*

Figure 9. All the steps for the influence of allosteric inhibitor on enzyme.

**Example 1:** NADH is produced due to isocitrate dehydrogenase reaction, under condition of its accumulation the enzyme activity is blocked. The terminal product of a process may be the feed-back inhibitor, too.

**Example 2:** Cholesterol synthesis from acetyl-SCoA is controlled so: the key enzyme – β hydroxy-β—methyl-glutaryl~SCoA-reductase is inactivated by cholesterol if its concentration is increased in a cell.

**Example 3:** Acetyl-CoA-carboxylase (the key enzyme in fatty acid synthesis) is regulated by feed-back influence of end-product – Palmityl-CoA.
**Irreversible inhibition of enzyme activity**

This type of inhibitors binds covalently or so tightly to the active centre of enzymes that they are inactivated irreversibly. There are those subtypes:

**Affinity labels.** There are substrate analogs that possess a highly reactive group that is not present on the natural substrate. The reactive group of \( I \) permanently blocks the active centre of the \( E \) from the \( S \) because the group reacts covalently with amino acid residue. The residue that is modified is not necessarily involved in catalysis.

**Mechanism-based or suicide inhibitors.** These are substrate analogs that are transformed by the catalytic action of the enzyme. Their structures are such that the product of this reaction is highly reactive and subsequently combines covalently with an amino acid residue in the active centre, thus inactivating the enzyme.

**Transition – state analogs** There are substrate-analogs which do not covalently modify the enzyme but bind the active centre so tightly that they irreversible inactivate the \( E \).

Many highly toxic, naturally occurring and man-made compounds are irreversible enzyme inhibitors. Some organic compounds are poisons for humans (diisopropyl fluorophosphate, organophosphorus insecticides are among them). Phosphor-containing organic compounds inhibit acetyl choline transferase across blockage of OH-groups of serine residues in active sites of enzyme to cause CNS paralysis

Natural compounds used as drugs can also inhibit enzymes. For example:

1) Penicillin, which is a transition-state analog that inhibits the reaction with transpeptidase that is important in the development of bacterial membranes, thus destroying normal growth of the bacteria.

2) Allopurinol is the suicide inhibitor of xanthine oxidase and is used in the treatment of gout.
CHAPTER 5

THEME:

PRINCIPLES OF ENZYME ACTIVITY DETERMINATION. GENETIC DEFICIENCY OF ENZYMES. MEDICAL ENZYMEOLOGY

A determination of enzyme activity in biological fluids

The determination of enzyme activity is of great importance for scientists. The enzyme activity is determined for tissue enzymes in homogenates of tissues or cellular fractions at research works. It is determined in whole blood, plasma or serum, in saliva, in gastric juice, in the urine, in cerebrospinal fluid for disease diagnostics in patients.

There are some methodic requirements for the enzyme activity determination in biological fluids:

- The substrate concentration must be more than the substrate concentration for saturation of active centers of enzyme molecules found in investigated sample;
- The pH and temperature of the environment must be optimal;
- The activator for the enzyme in some cases must be added.

Total activity (T.A.) units:

- An International Unit (IU) is the amount of enzyme that catalyzes the transformation of 1 μmole of a substrate per minute under optimal conditions of measurement.
- Katal is the amount of enzyme that catalyzes the transformation of 1 mole of a substrate per second under optimal conditions of measurement.

These units are used for Total enzyme Activity (T.A.) determination.

Specific activity (S.A.) is the number of units of total activity per milligram of total protein [C] present in a sample:  

\[ S.A. = \frac{T.A}{[C]} \] . This type of activity is used in researching works in biochemistry.
**Turnover number (N)** is the number of substrate molecules metabolized per one enzyme molecule per unit of time. For example, Carbonic unhydrase has turnover number 36000000/min.

**Clinical significance of some enzymes activity determination in biological fluids**

The determination of enzyme activity in the blood plasma is of great importance for medicine. It helps to make diagnosis for some diseases, to find out the tissue damage, to differentiate the type of infringements for the same organ when other indexes of the blood plasma can’t help.

**Example 1: Aspartate aminotransferase (AsAT) activity** in the blood plasma increases 10-100 times more then normal value at myocardium infarction in the first 3-4 hours of the damage development when cardiogram may be normal for patient.

**Example 2:** The knowledge about tissue distribution of isozymes of Lactate Dehydrogenase (LDH):

<table>
<thead>
<tr>
<th>Tissue</th>
<th>LDH activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardium</td>
<td>LDH₁ and LDH₂</td>
</tr>
<tr>
<td>Liver</td>
<td>LDH₃ &lt; LDH₄ &lt; LDH₅</td>
</tr>
<tr>
<td>Skeletal muscular tissue</td>
<td>LDH₃ &gt; LDH₄ &gt; LDH₅</td>
</tr>
<tr>
<td>Kidneys</td>
<td>LDH₃</td>
</tr>
</tbody>
</table>

It helps to find out the damage of some tissues if special type of isozymes is allocated from damaged tissue cell into the blood plasma. The determination of isozymes activity in the blood plasma is very important for diagnostic of heart, liver disease and many others (Fig. 1).

**Example 3:** Bilirubin indexes (conjugated and unconjugated) may be high in blood serum at various types of jaundice. The hepatic jaundice is accompanied with parenchyma damage of the liver. The liver parenchyma damage may be proved by the determination of alanine aminotransferase (ALAT) activity and choline esterase activity in the blood plasma of patients. Beside this the dynamic of choline
esterase activity plays a valuable prognostic role at the treatment of patient: the decrease of the cholinesterase activity plays a role of a harbinger of the aggravation.

Example 4: Amylase activity in blood plasma and in urine may be increased in 10-60 times or more at sharp pancreatitis in patients. This test is used also to check up the pancreatic gland function after treatment of patient with parotitis (mumps).

Genetic disorders of enzyme synthesis

A lot of genetic disorders are associated with the damage of enzymes synthesis or with the infringements of their regulation in tissues. These disorders are the most difficult in treatment, and the diagnosis is made at newborns or at prenatal state using the determination of some substrates or products concentration for enzymes that are in deficiency. DNA probes are available for prenatal diagnosis using amniotic liquid.

Example 1: Defects in the Phenylalanine 4-monooxygenase (hyperphenylalaninemia type I) or classic phenylketonuria. There is no transformation of phenylalanine into tyrosine in patient. Phenylalanine is accumulated in tissues and in the blood; the transformation of it may be to phenyl pyruvate, only. Phenyl pyruvate levels are also high in tissues and all the liquids. The major consequence of untreated type I hyperphenylalaninemia is mental retardation. Additional clinical signs include seizures, psychoses, eczema and a mould odour of urine. Screening of newborn infants for phenylketonuria now is compulsory (in a few days after born, 1-6 days).

<table>
<thead>
<tr>
<th></th>
<th>Normal Plasma / Urine</th>
<th>Phenylketonuric patient Plasma / Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine, mg/DL</td>
<td>1 - 2 / 30</td>
<td>15 - 63 / 300 - 1000</td>
</tr>
<tr>
<td>Phenylpyruvate, mg/DL</td>
<td>- / -</td>
<td>0,3 - 1,8 / 300 - 2000</td>
</tr>
</tbody>
</table>
**Example 2: Alkaptonuria** is caused by a defect in Homogentisate oxidase used for transformation of homogentesic acid to 4-maleylacetoacetate (tyrosine conversions). Homogentisate is accumulated in the blood and is excreted in large amounts in the urine causing the urine to darken after being exposed to air. Later in life, patients may develop pigmentation of connective tissue and suffer from arthritis.

**Figure 1. Enzymes with their respective substrates and inhibitors:**
the use in medicine

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Inhibitor</th>
<th>Significance of inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoamine oxidase</td>
<td>Epinephrine, norepinephrine</td>
<td>Ephidrene, amphetamine</td>
<td>Useful for elevating catecholamine levels</td>
</tr>
<tr>
<td>Dihydrofolate reductase</td>
<td>Dihydrofolate acid</td>
<td>Aminopterin, amethopterin, methotrexate</td>
<td>Employed in the treatment of leukemia and other cancers</td>
</tr>
<tr>
<td>Acetylcholine esterase</td>
<td>Acetylcholine</td>
<td>Succinyl choline</td>
<td>Used in surgery for muscle relaxation, in anaesthetised patients</td>
</tr>
<tr>
<td>Dihydropteroate synthase</td>
<td>Para-Amino Benzoic Acid</td>
<td>Sulfonamide</td>
<td>Prevents bacterial synthesis of folic acid</td>
</tr>
<tr>
<td>Vitamin K epoxide reductase</td>
<td>Vitamin K</td>
<td>Dicumarol</td>
<td>Acts as an anticoagulant</td>
</tr>
<tr>
<td>Betta-Hydroxy-betta-Methyl-Glutaryl-CoA (HMG CoA)-reductase</td>
<td>HMG CoA</td>
<td>Lovastatin, compactin</td>
<td>Inhibits cholesterol biosynthesis</td>
</tr>
</tbody>
</table>
### Figure 2. Enzymes with their respective substrates and inhibitors: the use in medicine

#### Irreversible inhibitor

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Inhibitor</th>
<th>Significance of inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldehyde Dehydrogenase</td>
<td>Acetaldehyde</td>
<td>Disulfiram (antabuse)</td>
<td>Used in the treatment of alcoholism</td>
</tr>
<tr>
<td>Xanthine oxidase</td>
<td>Xanthine hypoxanthine</td>
<td>Allopurinol (suicide inhibitor)</td>
<td>Used in the control of gout to reduce excess production of uric acid from hypoxanthine</td>
</tr>
<tr>
<td>Cyclooxygenase</td>
<td>Arachidonic acid</td>
<td>Acetyl salicylic acid (aspirin)</td>
<td>Anti-inflammatory drug: antipyretic (fever-reducing) and analgesic (pain relieving)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phenyl butazone</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Indomethacin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ibuprofen</td>
<td></td>
</tr>
<tr>
<td>Aldehyde dehydrogenase</td>
<td>Acetyc aldehyde produced</td>
<td>Teturam</td>
<td>Accumulation of acetic aldehyde in the blood of alcoholics will be, and it causes the aversion to alcohol under its use</td>
</tr>
<tr>
<td></td>
<td>from ethanol</td>
<td></td>
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<tr>
<td>Trypsin</td>
<td>Proteins of blood plasma</td>
<td>Trasilol and other protease</td>
<td>To prevent spread proteolysis of proteins in pancreas and in the blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td>inhibitors</td>
<td></td>
</tr>
<tr>
<td>Kallikrein</td>
<td>Proteins-regulators of</td>
<td>Contrical</td>
<td>To control blood pressure in norm</td>
</tr>
<tr>
<td></td>
<td>blood vessels tonicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic sections</td>
<td>Enzyme name</td>
<td>Examples of use</td>
<td></td>
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<td>----------------------</td>
<td>--------------------------------------------------</td>
<td>------------------------------------------------------</td>
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<tr>
<td>Diagnostics</td>
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<tr>
<td>a) serum enzyme</td>
<td>Lactate dehydrogenase</td>
<td>Heart attack</td>
<td></td>
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<td></td>
<td>[isozyme LDH&lt;sub&gt;1&lt;/sub&gt;]</td>
<td></td>
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<td></td>
<td>[isozyme LDH&lt;sub&gt;5&lt;/sub&gt;]</td>
<td>Liver diseases</td>
<td></td>
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<tr>
<td>The increased level</td>
<td>Aspartate aminotransferase [SGOT], AsAT</td>
<td>Heart attack (myocardial infarction)</td>
<td></td>
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<tr>
<td></td>
<td>Alanine aminotransferase [SGPT], A1AT</td>
<td>Viral hepatitis, liver damage</td>
<td></td>
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<tr>
<td></td>
<td>Creatine phosphokinase [CPK]:</td>
<td>Muscle disorders</td>
<td></td>
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<tr>
<td></td>
<td>Isozyme MM (CPK&lt;sub&gt;3&lt;/sub&gt;)</td>
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<tr>
<td></td>
<td>Isozyme MB (CPK&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>Heart attack</td>
<td></td>
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<tr>
<td></td>
<td>Acid phosphatase [ACP]</td>
<td>Prostate cancer</td>
<td></td>
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<tr>
<td></td>
<td>Alcaline phosphatase [ALP]</td>
<td>Liver diseases, bone disorders</td>
<td></td>
</tr>
<tr>
<td>b) serum enzyme</td>
<td>Choline esterase [ChE]</td>
<td>Liver parenchyma damage, hypothyroidism, nephritic syndrome, myocardial infarction</td>
<td></td>
</tr>
<tr>
<td>The lowered level</td>
<td>γ-Glutamyl transpeptidase [GGT]</td>
<td>Alcoholism</td>
<td></td>
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<tr>
<td></td>
<td>α-Amylase</td>
<td>Acute pancreatitis</td>
<td></td>
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<tr>
<td></td>
<td>Lipase</td>
<td>Acute pancreatitis</td>
<td></td>
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<tr>
<td></td>
<td>Aldolase</td>
<td>Muscular dystrophy</td>
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<tr>
<td></td>
<td>5’-Nucleotidase</td>
<td>Hepatitis</td>
<td></td>
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<tr>
<td></td>
<td>Glucose 6-phosphate dehydrogenase [G6PD]</td>
<td>Congenital deficiency with hemolytic anemia</td>
<td></td>
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<tr>
<td></td>
<td>Ceruloplasmin</td>
<td>Wilson’s disease</td>
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<td></td>
<td>Pepsin</td>
<td></td>
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<tr>
<td>A treatment of patients using enzyme preparation</td>
<td>Disordered digestion of proteins in stomach, deranged synthesis or secretion of pepsin</td>
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<td>-----------------------------------------------</td>
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<tr>
<td>Trypsin, chymotrypsin (immobilized forms)</td>
<td>Treatment of purulent wounds</td>
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<tr>
<td>Streptokinase, urocaninase</td>
<td>Prevention of clots formation at transplantation of organs and other operations</td>
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<tr>
<td>Hyaluronidase, Lidase</td>
<td>Resorption of a scar tissue, keloids due to the degradation of substrate - hyaluronic acid</td>
<td></td>
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<tr>
<td>Asparaginase</td>
<td>Treatment of some malignant neoplasms, leucosis to prevent the accumulation of tumor growth factor - asparagine</td>
<td></td>
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</tr>
<tr>
<td>Nucleases (DNAase)</td>
<td>Viral conjunctivitis, rhinitis, purulent bronchitis</td>
<td></td>
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<tr>
<td>Urease</td>
<td>Removal of urea from an organism in artificial kidney apparatus</td>
<td></td>
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<tr>
<td>Streptodekase (immobilized enzyme)</td>
<td>To promote fibrinolysis at patients without normal duration of this pathway</td>
<td></td>
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<tr>
<td>Glucose oxidase</td>
<td>The determination of glucose</td>
<td></td>
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<tr>
<td>Use of enzymes as analytical reagents</td>
<td>content in blood</td>
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<tr>
<td>Cholesterol oxidase</td>
<td>The determination of cholesterol content in blood</td>
<td></td>
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<tr>
<td>Lipase</td>
<td>The determination of triacylglycerols content in blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urease</td>
<td>The determination of urea content in blood</td>
<td></td>
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</tr>
</tbody>
</table>
CHAPTER 6

THEME:

THE INVESTIGATION OF SIMPLE AND CONJUGATED PROTEINS, ENZYME PROPERTIES AND THEIR ACTIVITY
IN BIOLOGICAL FLUIDS

1. Qualitative reactions for proteins

1.1. Biuretic reaction (Piotrovsky's test)

This reaction proves the peptide bond in proteins and peptides (starting from tripeptides). The protein solution during the interaction with copper ions gets blue-violet color complex in the alkaline environment. And incomplete hydrolysis products of it (peptones) give pink coloring.

THE COURSE OF THE WORK:

Add to 5 drops of 1 % egg protein solution 5 drops of 10 % NaOH solution, 2 drops of 1 % copper sulfate solution, and all of them mix. The test tube contents will get violet colour. A copper sulfate shouldn't be added surplussly, as the dark blue residue of the copper hydroxide masks the characteristic violet colouring of the biuretic protein complex.

1.2. Fole's test.

This test is used to prove the presence one amino acid residue, only, in the composition of proteins - Cysteine. The sodium hydroxide under the boiling will cause the denaturation of egg proteins to get free cysteine residues in polypeptide chains which are involved in the reaction with lead acetate to give the product – lead sulfide (black sediment)

THE COURSE OF THE WORK:

Add 10 drops of Fole’s reactive (30% NaOH : Pb(CH₃COO)₂ in correlation 1:1) to the 10 drops of 1 % egg protein solution, then boil intensively for 1 min and wait for 1-2 minutes. The black or brown sediment of lead sulfide (PbS) should be formed.
1.3. The reaction with sulfosalicylic acid.

**THE COURSE OF THE WORK:**

Pour 2-3 ml solution of protein (or researched fluid) into a test tube and add 5-6 drops of 20% sulfosalicylic acid solution. You can see the appearance of white colour precipitate at the presence of protein. This test is the most sensitive reaction for proteins.

**Clinical significance**

This test is used to prove the presence of proteins in the urine of patients at nephritis, some cardiac diseases, during some forms of idiopathic hypertension and during pregnancy pathology.

2. The separation of egg albumins and globulins by salting-out

**THE PRINCIPLE OF THE METHOD:**

Salting-out is a reversible reaction of the protein sedimentation from solutions by means of big neutral salts concentration: sodium, ammonium sulfates, magnesium sulfate. Albumins are besieged in saturated solutions of sulfate ammonium, globulins are besieged in the half-saturated one; because the globulins molecular weight is bigger, than the albumins one. And albumins have the higher solubility in solution.

**THE COURSE OF THE WORK:**

Add 20 drops of the saturated ammonium sulfate solution to 20 drops of egg protein solution and mix. The half saturated solution is got, where the egg globulin sediment falls out. In 5 minutes it must be separated by filtration. There is another protein in solution. It is an egg albumin. For albumins salting-out the crushed powder of ammonium sulfate is added till full saturation, i.e. while a new portion of powder remains not dissolved. The dropped out albumin’s sediment is filtered out. The biuretic reaction is made with the filtrate. The negative reaction proves the absence of a protein in the filtrate.
3. The determination of total protein content in the blood serum (Biuretic method)

**THE PRINCIPLE OF THE METHOD:**

The coloured complex is formed under the protein interaction with biuretic reagent. Its colouring intensity corresponds to the protein concentration in the researching test. The optical density of the skilled test is determined on photocolorimeter (PC). The result correlates with the protein concentration in the test tube.

**THE COURSE OF THE WORK:**

Add 5 ml of the biuretic reagent to 0.1 ml of blood serum and mix, avoiding a foam formation. The optical density is measured in 30 minutes on PC in cuvettes (10 mm of layer) at the 540-560 nm wave length against the control. Control test tube is prepared so: to 5 ml of the worker biuretic reagent add 0.1 ml of 0,9 % sodium chloride solution and it is be processed as an experimental test tube. The calculation is conducted according to the graph.

**Clinical significance of proteins content determination in the blood serum:**

Normal value - 65 - 85 g/l (plasma or serum)

**Hyperproteinemia** is a pathologic condition manifested by an increased content of blood plasma proteins. An increase in the blood plasma protein concentration can be caused by diarrhea in children, by vomiting (due to an obstruction of the upper small intestine), or by extensive burns.

**Hyperproteinemia** may be caused by an elevated level of α-globulins, for example, hyperproteinemia sequent to an infection or a toxic disturbance of the macrophage system. Among such states may also be classified hyperproteinemia in multiple myeloma (myelomatosis). In the blood serum of patients with myelomatosis, specific “myelomatous” proteins are detected. The occurrence in the blood plasma of proteins, normally untypical of the healthy organism, is conventionally referred to as paraproteinemia. Quite often, in such pathology, the concentration of proteins in the blood plasma may be as high as 100-160 g/litre.
**Hypoproteinemia**, or a decrease in the total concentration of blood plasma proteins, is mainly due to a lowered percentage of albumins. The manifest hypoproteinemia is a permanent and pathogenically important symptom of nephrotic syndrome. The total protein content drops to a level of 30-40 g/litre. Hypoproteinemia is also observed in affected liver cells (acute atrophy of the liver, toxic hepatitis, and other states). Moreover, hypoproteinemia may develop as sequent to a drastically increased permeability of the capillary wall, or protein deficiency (affected gastrointestinal tract, carcinoma, etc.)

It is to be inferred therefore that hyperproteinemia is, as a rule, associated with hyperglobulinemia, and hypoproteinemia, with hypoalbuminemia.

4. **Specificity of salivary amylase**

**The principle of the method:**

Amylase splits starch, glycogen to form glucose and does not react with sucrose. The specificity of the amylase action is proved by Trommer's test result.

**The course of the work:**

Pour 5 drops of the saliva dissolved in correlation (1:4) into 2 test tubes. Add 10 drops of 1% starch solution into the 1-st test tube, and 10 drops 1% of the sucrose solution into the 2-nd one. Put the both test tubes into the thermostat at 38°C for 10 minutes. Then carry out the Trommer's test.

**Trommer's test:**

Pour 3 drops of 5% copper sulfate (II) solution and a few drops of 10% sodium hydroxide solution into each test tube until the blue transparent solution appears. Shake up the content of the test tubes. Then cautiously heat up the test tubes and boil for 1 minute. The appearance of red color sediment proves the glucose presence.
5. The thermolability of salivary amylase.

The principle of the method:

The influence of temperature on salivary amylase activity is judged at splitting of starch by this enzyme at various temperature conditions (100°C and 38°C). The degree of starch splitting is determined by iodine test, the product (glucose) formation might be proved by the Trommer's test.

The course of the work:

Collect 3 ml of saliva into a test tube. Take away 2 ml of saliva into another tube for to boil 5 minutes, and then cool it under the water. Into the third test tube add 1 ml of saliva and dissolve the volume in correlation (1:4). Take the new three test tubes, and pour into each test tube 10 drops of 1% starch solution, after that add 10 drops of the dissolved saliva into the 1-st test tube. Add 10 drops of boiled saliva into the 2-nd test tube. Add 10 drops of water into the 3-rd test tube (control sample). All three test tubes put into the thermostat for 10 minutes at 38°C. Then divide the content of each test tube into 2 parts and carry out qualitative reactions to starch and glucose (Trommer's test).

a) Reaction to starch (iodine test):

Pour 1 drop of the 1% solution of iodine in 0.2% potassium iodide into all three test tubes. At the starch presence the blue color complex will appear.

b) Trommer's test (has been discussed above)

6. The influence of the pH environment on the salivary amylase activity

The principle of the method:

The influence of the pH-environment on amylase activity is judged by the starch splitting in various pH values. The degree of starch splitting is determined by iodic test, the optimum of pH corresponds to a negative iodic test.

The course of the work:

The saliva volume is dissolved in correlation (1:100). Take 6 test tubes and pour 2 ml of the phosphate buffer with various value of pH medium: 6.0; 6.4; 6.8; 7.2; 7.6; 8.0 into each test tube. Then add 1 ml of 0.5 % starch solution and 1 ml of the
dissolved saliva into each one. Mix the content of the test tubes and place them into thermostat at 38°C for 10 minutes. Then pour 1 drop of the 1% solution of iodine in 0.2% potassium iodide into each tube, and mix. You can observe the color in each tube and mark the pH optimum (at optimal pH the mixture must be colorless or with some yellow shade).

7. Lipase action on neutral fats

The principle of the method:

Neutral fats are split under the action of lipase by hydrolytic way, reaction of environment is shifted in the acidic medium due to fat acids formation, and pink color disappears at the presence of phenolphthalein. The method helps in understanding of hydrolase function at condition in vitro.

The course of the work:

Pour 10 drops of milk in 2 test tubes. Add 5 drops of pancreatine that contains lipase to the first test tube, and add 5 drops of water to the second test tube. Put into both test tubes 1 drop of 0.5% phenolphthalein solution and drop by drop of 1% sodium carbonate solution before occurrence of slight pink color at pH=8.0 (it is impossible to flow surplus of sodium carbonate solution). Place test tubes into thermostat at 38°C for 30 minutes. Observe disappearance of color in the test tube that contains lipase.

8. The influence of activators and inhibitors on the salivary amylase activity

The principle of the method:

The activator of salivary amylase is sodium chloride (Cl−), and the irreversible inhibitor of salivary amylase is copper sulfate (Cu2+). The influence of these substances on the amylase activity is judged by the degree of starch hydrolysis under the enzyme influence at the presence of sodium chloride and copper sulfate. Iodine test helps to estimate the degree of starch hydrolysis in prepared mixtures.

The course of the work:

The saliva is dissolved in correlation (1: 200). Take 3 test tubes. Pour on 2 drops of 1% sodium chloride solution into the 1-st one, and 2 drops of 1% copper
sulfate solution into the 2-nd one, and 2 drops of water into the 3-rd one (control sample). Add 1 ml of the dissolved saliva and 5 drops of 1% starch solution into each test tube. Mix the content and keep test tubes at a room temperature for 2 minutes. Pour 1 drop of the 1% solution of iodine in 0.2% potassium iodide into each tube, mix and observe the color. As the result the lightest mixture must be in the test tube with sodium chloride, the darkest color is at the presence of copper sulfate.

9. The dependence of enzyme reaction velocity on enzyme concentration

The principle of the method:

Splitting of starch by salivary amylase gives products - erythrodextrins, their formation is proved by red color of iodine test. The rate of enzymatic reaction is fixed as the time for this color appearance, and it depends upon the dilution of saliva (relative amylase concentration).

The course of the work:

Number 4 test tubes and put 1 ml of the saliva dissolved in 10, 20, 40 and 80 times into each test tube accordingly. Add 5 ml of 1 % starch solution into each test tube. Mix the content of test tubes quickly, place the test tubes into thermostat at 38 °C and note the time of the reaction beginning. Using a glass stick take out 1-2 drops of the solution on the watch crystal every 2 minutes and add 1 drop of 0,1 % of iodine solution. First tests give dark blue color, then - violet, then - red-violet and at the end of the reaction - red color.

Fix the time of red color appearance (by iodine test) from the beginning of reaction for each test tube. Show the results on graph, marking relative amylase concentration (dilutions) on X-line and time (min) of erythrodextrin formation on Y-line, and make the curve. It has to be as first-order dependence curve.
10. Determination of amylase activity in the urine (Volgemut`s method)

The principle of the method:

The Volgemut's method is based on the minimal quantity of the enzyme determination, which is capable to split completely 2ml of 1% starch solution. This quantity of enzyme is accepted for a unit of the amylase activity.

The course of the work:

Pour 1 ml of 0.85% sodium chloride solution into each test tube (8 test tubes). Add 1 ml of patient’s urine into the 1-st test tube and mix thoroughly. Then transfer 1 ml of the mixture into the 2-nd test tube and repeat all the operations with the test tubes: from the 2-nd one into the 3rd one, etc. Pour 1 ml of liquid out of the 8-th test tube. Add 2 ml of 0.1% starch solution into each test tube, mix and put them into the thermostat at 38°C for 30 minutes. At the end of the incubation take the test tubes out, cool them and add 2 drops of the iodine solution into each one. Mix the content of the tubes and mark the latest test tube with no colored solution (where there was full starch splitting).

The calculation is made according to the formula:

\[ X (\text{units}) = 1 \cdot 2 \cdot \text{dilution value}; \]

1 - urine volume (1ml);
2 - volume of 0.1% starch solution in ml;
X - salivary amylase activity in standard units.

Dilution is in each test tube (respectively): N1 - 2; N2 - 4; N3 - 8; N4 - 16; N5 - 32; N6 - 64; N7 - 128; N8 - 256.

The clinical significance of the test:

Normal values of the amylase activity in the urine (by Volgemut) are 16-64 units. At sharp pancreatitis the activity of amylase in the urine and the blood serum arises 10-60 times.
11. Cholinesterase activity determination in the blood serum

The principle of the method:

Cholinesterase (CE) hydrolyzes acetylcholine to obtain an acetic acid and choline. The acetic acid decreases the pH value of solution that is because an indicator changes its color from crimson color to yellow one.

The course of the work:

<table>
<thead>
<tr>
<th>Add, ml</th>
<th>N1 (test sample)</th>
<th>N2 (control of turbidity)</th>
<th>N3 (empty sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indicator solution</td>
<td>2.5</td>
<td>-</td>
<td>2.5</td>
</tr>
<tr>
<td>Blood serum</td>
<td>0.05</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>0, 9% NaCl solution</td>
<td>-</td>
<td>2.7</td>
<td>0.05</td>
</tr>
<tr>
<td>Acetylcholine solution</td>
<td>0.1</td>
<td>-</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Put all the test tubes into the thermostat (37°C) and keep them there for 30 minutes. Then add 0.1 ml of stop-reagent to the experimental and empty test tubes, only, and mix. You have to determine an optical density of each sample against distilled water at 540 nm (green color filter) in cuvettes (5 mm layer). Calculate the E according the formula:

\[ E = E(\text{empty}) + E(\text{control}) - E(\text{test}) \]

Use the E value and the graph curve to make the determination of choline esterase activity.

The creation of the graph curve

Prepare, please the dilution from 0,1 mol/L solution of acetic acid, using the information from the table below:
Control samples for each dilution are prepared using 0.1 ml of dilution, 2.5 ml of indicator solution, 0.05 ml 0.9% NaCl solution, and 0.1 ml of stop-reagent.

The empty sample is made in the same way that is described above. Determine the optical density of each dilution sample (5 values) and empty sample opposite the distilled water. The calculation is using the same formula but without E (control of turbidity). Create the graph curve for optical density-activity relation

**The clinical significance of the cholinesterase (CE) determination in the blood serum:**

The normal value of CE activity is 45-95 micromole/sec•L. The distinct decrease of the CE activity in blood serum takes place at the diseases of the liver, hypothyroidism, the bronchial asthma, articulate rheumatism, heart attacks of the myocardium, burns, traumatic shocks, in postoperative conditions. In severe forms of Botkin's disease the CE activity is decreased. In a case of the aggravation of this disease the decrease of the cholinesterase activity outstrips the bilirubin peak, playing a role of a harbinger of the aggravation. The dynamics of CE activity change plays a valuable prognostic role at the patient's treatment [*].
12. The determination of alanine aminotransferase activity in the blood serum (proposed by Reitman S., Frenkel S.)

The principle of the method:
Glutamate and pyruvate are formed under the action of alanine aminotransferase (AlAT) from $\alpha$-ketoglutarate and alanine. The pyruvate can act with 2,4-dinitrophenylhydrazine to produce dinitrophenylhydrazone of a brown color at alkaline medium. The intensity of color is proportional to the quantity of pyruvic acid released during the reaction and the activity of enzyme.

The course of the work:
Prepare reactive solutions according to scheme:

<table>
<thead>
<tr>
<th>Add, in ml</th>
<th>Test sample</th>
<th>Control sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate-buffer solution</td>
<td>0,5</td>
<td>0,5</td>
</tr>
<tr>
<td>Incubation in a dry-air thermostat at 37°C for 3 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stop reagent</td>
<td>-</td>
<td>0,5</td>
</tr>
<tr>
<td>Blood serum</td>
<td>0,1</td>
<td>0,1</td>
</tr>
<tr>
<td>Incubation in a dry-air thermostat at 37°C for 30 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stop reagent</td>
<td>0,5</td>
<td>-</td>
</tr>
<tr>
<td>Let them stay at room temperature for 20 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0,4 N NaOH</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Let them stay at room temperature for 10 min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Measure the optical density of experimental sample opposite control one in cuvettes (10mm) at light-green filter (490-540 nm).

Calculation of enzyme activity in the blood serum is made by the graph. The graph curve is created using the information from assay kit produced by the firm «Філісіт-Діагностика».
The clinical significance of aminotransferases determination in the blood serum:

Alanine aminotransferase activity of the blood serum at healthy people equals 0.1-0.68 micromole/ml•hour at 37°C.

Organic infringements at sharp and chronic lesions are accompanied with cell destruction and as result there is an output of aminotransferases from damaged tissue to the blood. AlAT activity is increased in the blood plasma of patient with viral hepatitis type A at preicteric period of the disease development, and the determination of its activity is very important for person who was in a contact with diseased human. The change of AlAT activity is associated also with other types of liver disease followed by parenchyma damage.

Change activity of aspartate aminotransferase (AsAT; normal value region 0.1-0.45 micromole/ml•hour at 37°C) increases as early as in 3-6 hours after the attack of acute paroxysmal pain (20-30 times as much) in the heart and is high during 3-7 days after myocardium infarction. De Rittis's factor (AsAT/AlAT = 1.33±0.42 at healthy people) grows considerably at severe infarction of myocardium and is decreased up to the value 0.8 at liver parenchyma damage.
CONTROL TESTING FOR CHAPTERS 1-5

1. The reason of the damage of alpha-helical structure of polypeptide chain may be the large concentration (> 30 %) of one amino acid residue. Name it:
   A. Asp
   B. Pro
   C. Tyr
   D. Ser
   E. Gly

2. The tertiary structure of protein is formed mainly due to disulfide bonds between side radicals of one amino acid, only. Point out it:
   A. Cys
   B. Met
   C. Asp
   D. Lys
   E. His

3. Primary structure of proteins is formed due to one type of bonds. Point out it:
   A. Peptide bond
   B. Disulfide bond
   C. Ester bond
   D. Hydrogen bond
   E. Metal bond

4. Point out the minimal quantity of amino acid residues in the polypeptide chain allowing the formation of the tertiary structure:
   A. 10
   B. 12
   C. 5
   D. 40
   E. 3

5. Polypeptide chains of collagen include specific amino acids. Name one of them:
A. Hydroxyproline
B. Formyl-methyonine
C. Cysteine
D. L-alanine
E. Ornithine

6. The beta-pleated sheet structure is very seldom in nature. Name the protein whose structure is based on it:
   A. Albumin of eggs
   B. Alpha-Keratin of hair
   C. Fibroin of a silk
   D. Elastin of cartilages
   E. Protamine of plants

7. There are many important protein functions in the human organism. Point out that of them, which isn't peculiar for proteins:
   A. Catalyst
   B. Transfer of substances
   C. Antibody
   D. Structural component of a cell
   E. Solvent

8. The solubility of proteins in saline solutions is determined by their native structure. Point out the protein, which will swell only in saline solution:
   A. Elastin
   B. Albumin
   C. Myoglobin
   D. Immunoglobulin
   E. Pepsin

9. The proteins are able to carry out the regulatory function. Find out those protein:
   A. Aminopeptidase
   B. Insulin
C. Collagen
D. Hemoglobin
E. Immunoglobulin G

10. All proteins are divided into simple and conjugated ones. Find out the simple protein among these ones:
   A. Albumin of egg
   B. Histone
   C. Globulin of egg
   D. Protamine
   E. All the proteins above

11. Choose the proteins which are included into the deoxyribonucleoprotein composition in eukaryotic cells:
   A. Albumins
   B. Globulins
   C. Glutelins
   D. Histones
   E. Collagens

12. Find out the conjugated protein among following ones:
   A. Albumin
   B. Protamine
   C. Prolamine
   D. Hemoglobin
   E. Histone

13. The conjugated protein necessarily contains special component as a non-protein part. Choose the substance that can't carry out this function:
   A. Glucose
   B. HNO$_3$
   C. Fe$^{2+}$
   D. Haem
   E. Phosphate
14. Which method is better suited to separate a mixture of compounds into its individual components and detects small amounts (microgram or even picogram) of material:
   A. Dialysis
   B. Paper chromatography
   C. Ultracentrifugation
   D. Salting out
   E. Spectrophotometry

15. Point out a possible cause of hypoproteinemia:
   A. Affected liver cells
   B. Multiple myeloma
   C. Decreased permeability of the capillary wall
   D. Overeating
   E. Paraproteinemia

16. Point out a possible cause of hyperproteinemia
   A. Increased permeability of the capillary wall
   B. Infection (disturbed the macrophage system)
   C. Affected gastrointestinal tract
   D. Nephritic syndrome
   E. Diabetes mellitus

17. Which method is appropriate for the determination of total protein content in the blood serum:
   A. Salting out
   B. Fole’s test
   C. Dialysis
   D. Electrophoresis
   E. Biuretic method

18. Choose the conjugated protein in possession of following characteristics: quaternary structure - 4 polypeptide chains; non-protein part – 4 haem; function – oxygen transport in the blood:
19. What compound serves as non-protein part of glyco-proteins:

A. Cu$^{2+}$
B. Fe$^{2+}$
C. Galactose
D. Haem
E. Phospholipid

20. Which group of proteins being phosphoproteins posses an activity but being dephosphorylated have lost the activity:

A. Hormones
B. Transfer of lipids
C. Transfer of vitamins
D. Enzymes
E. Carriers through membrane

21. Enzymes are the catalysts of protein nature. Name the property of enzymes which is not presented at the inorganic catalysts:

A. Ability to be denaturated
B. Wide specificity
C. To be Inert to chemical substrates
D. Big half-life
E. Ability to lower the energy activation for the reaction

22. One of the important properties of enzymes is their specificity of action. Check up a type of specificity for salivary amylase:

A. Absolute
B. Absolute group
C. Absolute relative
D. Relative group
E. Stereochemical

23. Some terms are used for the description of non-protein part of an enzyme. Point out the term of non-protein part that easily dissociates from polypeptide chain:
   A. Apoenzyme
   B. Coenzyme
   C. Prosthetic group
   D. Cofactor
   E. Metall ions

24. Oxidoreductase can contain prosthetic group with vitamin B$_2$. Name it:
   A. Retinal
   B. Flavin adenine dinucleotide (FAD)
   C. Nicotinamide adenine dinucleotide (NAD)
   D. Pyridoxal phosphate
   E. Ascorbic acid

25. The change of the temperature of environment from 0$^\circ$C to 38$^\circ$C can cause this effect:
   A. The probability of ES complex formation is increased
   B. A denaturation of enzymes occurs
   C. The enzyme molecular charge changes
   D. The substrate molecular charge changes
   E. Enzyme action specificity varies

26. The optimum pH for cytoplasmic enzymes activity varies from 7.2 to 7.6. Point out all possible changes in active centre structure of such enzyme at pH=7.1:
   A. Changes are not presented
   B. Radicals of amino acids get negative charge
   C. Neutralization of negatively charged radicals
   D. Formation of ester bonds between radicals
27. A substrate molecule is destructed upon enzyme action, and the water is used for the products structure formation. Name the enzyme class:
   A. Oxidoreductase
   B. Hydrolase
   C. Lyase
   D. Ligase
   E. Isomerase

28. A qualitative composition of product molecule is completely identical to substrate one, but the structure is different. Name the enzyme class:
   A. Oxidoreductase
   B. Hydrolase
   C. Lyase
   D. Ligase
   E. Isomerase

29. ATP molecules may be used for Transferases and Ligases function. Point out the signs of ATP use for Ligases class:
   A. ATP is used for a substrate dephosphorylation
   B. ATP is used for a substrate phosphorylation
   C. ATP is used for hydrolysis of a substrate bond
   D. ATP is used for the new bond formation during the interaction of two substrates
   E. ATP is used for a substrate decarboxylation.

30. Choose the factor which can cause the block of enzyme activity in human tissue:
   A. The pH value about 2
   B. The temperature about 60°C
   C. The presence of heavy metal ion as Hg^{2+}
   D. The presence of the substrate
   E. Positions A, B, C are right, only
31. E. Fisher`s theory explains the mechanism of enzyme action with the fixed type of specificity, only. Name it:
   A. Absolute
   B. Absolute group
   C. Absolute relative
   D. Relative group
   E. Stereochemical

32. There are some factors influencing enzyme activity. Point out one of them resulting in complete loss of enzymatic activity:
   A. Vitamin H
   B. Oxygen
   C. \( t^0 \text{ C} = 100^0 \text{ C} \)
   D. \( P = 101325 \text{ Pa} \)
   E. Sodium chloride solution

33. There are some characteristic sites in the enzyme structure. Choose the most important site for enzyme function:
   A. Allosteric centre
   B. Active centre
   C. Cofactor
   D. Apoenzyme
   E. Catalytic site, only

34. Choose the factor that changes the cytoplasmic enzyme conformation mainly:
   A. Suicide inhibitor
   B. Environmental pH value about 7.4
   C. Environmental temperature value about \( 25^0 \text{ C} \)
   D. Allosteric inhibitor
   E. Water

35. Point out the way of proenzyme transformation to the active enzyme:
   A. Limited proteolysis
   B. Dehydration
C. Decarboxylation
D. Inhibitor action
E. Vitamin non-protein part dissociation from enzyme

36. Competitive inhibitor always interacts with enzyme active centre. Find out the explanation of this phenomenon:
   A. Inhibitor causes the denaturation of active centre
   B. Inhibitor is similar to a substrate structure
   C. Inhibitor is an exact copy of a substrate structure
   D. Inhibitor is similar to the product's structure
   E. Inhibitor forms a covalent type of bonds with amino acid residues of active centre

37. Covalent modification of inactive form of enzyme may be catalyzed by special enzyme in a cell. Name it:
   A. Esterase
   B. Ligase
   C. Protein kinase
   D. Hydroxylase
   E. Oxygenase

38. Find out the irreversible type of enzyme inhibition:
   A. Competitive
   B. Noncompetitive
   C. Uncompetitive
   D. Allosteric
   E. Suicide

39. Find out the mathematic sense of Michaelis constant (Km):
   A. It is a time for complete degradation of a substrate
   B. It is a 1/2 of a substrate concentration for obtaining of Vmax
   C. It is a substrate concentration for obtaining of 1/2 Vmax
   D. It is a constant for ES-complex dissociation
   E. It is a product concentration formed after enzymatic reaction
40. The active centre of the enzyme contains amino acid residues of Aspartic acid. The substrate for this enzyme is cyclic organic alcohol. Point out the type of bond that may be formed between this substrate molecule and active centre of this enzyme:
   A. Glycosidic bond only
   B. Hydrogen bond mainly
   C. Peptide bond
   D. Ester bond mainly
   E. Disulfide bond

41. Lactate dehydrogenase (LDH) isozymes catalyze the transformation of pyruvate to lactic acid in different types of tissues. Point out the structural distinctive peculiarity of each LDH isozyme:
   A. Different active centre structure
   B. Different level of structural organization in native molecule
   C. Different by the type of coenzyme in native molecule
   D. Different by the quantity of subunits
   E. Different by the combination of subunits, forming a native molecule

42. Point out the activator, used for the determination of urine amylase activity under Volgemut's method:
   A. CuSO₄
   B. NaCl
   C. H₃PO₄
   D. ATP
   E. Ca²⁺

43. Patient's amylase activity in the urine excesses the normal values in ten times as much. Point out the possible diagnosis:
   A. Viral hepatitis
   B. Diabetes mellitus
   C. Sharp pancreatitis
   D. Influenza
E. Angina

44. Point out the signs of multiple enzyme systems (MS):
   A. The MS enzymes are united by their intracellular localization
   B. The MS enzymes form a single structural-functional complex
   C. The MS enzymes form several different metabolic products at once
   D. The MS enzymes use one and the same cofactor
   E. Positions A,B,C are right

45. Find out the term for unit of enzyme activity that is estimated as the number of molecules of a substrate catalyzed upon in a period 1 second by a single enzyme molecule:
   A. Total activity
   B. Specific activity
   C. Turnover number
   D. Katal
   E. The Unit of an enzyme activity

46. Find out the substrate used for amylase activity determination in the urine of patient:
   A. Glucose
   B. Pyruvate
   C. Maltose
   D. Glycogen
   E. Starch

47. Find out the method for separation of isozymes to determine their content in the blood serum of patient:
   A. Dialysis
   B. Electrophoresis
   C. Spectrophotometry
   D. Gel chromatography
   E. Salting-out
48. Pyruvate dehydrogenase complex is multiple enzyme system because it contains:
   A. Two enzymes and one coenzyme
   B. Two enzymes and five coenzymes
   C. Three enzymes and three coenzymes
   D. Three enzymes and five coenzymes
   E. Five enzymes and five coenzymes

49. There is the treatment of patients with achlorhydria (the absence of free hydrochloric acid in the gastric juice of patient) by enzyme as a drug. Name it:
   A. Rennin
   B. Pyruvate
   C. Pepsin
   D. Trypsin
   E. Chymotrypsin

50. Choose the enzyme used as diagnostic reagent for glucose content determination in the blood:
   A. Glucose-6-phosphatase
   B. Pyruvate kinase
   C. Maltase
   D. Glucose oxidase
   E. Amylase
LITERATURE