**MINISTRY OF SCIENCE AND HIGHER EDUCATION
OF THE REPUBLIC OF KAZAKHSTAN M.UTEMISOV
WEST KAZAKHSTAN UNIVERSITY**

MOLECULAR BIOLOGY
(workshop)

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The workshop includes laboratory works, the purpose of which is to familiarize students with the classical methods of biochemical analysis, to consolidate the skills of working with laboratory equipment. Descriptions of laboratory works according to a standard scheme, are presented, as well as illustrative material, tables, control questions and tasks for self-testing, test tasks, a bibliographic list, a dictionary of terms.

For students studying Biology, Biology and Chemistry, Ecology.

Introduction

Molecular biology is one of the fundamental branches of modern biology, which studies the creation of a clear system of scientific knowledge in the field of molecular biology and in mastering its basics, namely, the features of the structure and properties of nucleic acid and protein molecules, the structural and functional organization of the genetic apparatus of cells.

Course objectives: formation of ideas about the organization of living systems at the molecular level and the unity of their origin; familiarization with the processes of transformation of substances and energy occurring in living organisms and their regulation; study of the role and prospects of molecular biology in solving practical problems of physiology, biotechnology, agriculture and medicine; familiarization with fundamental knowledge in all sections of the course: biological polymers, their structure and functions, genomes of prokaryotes and eukaryotes, protein synthesis, DNA replication and its molecular mechanisms, molecular fundamentals of biotechnology with the basic principles and methods of biochemical research; to form an idea of the system of concepts, terms, research methods in molecular biology

The educational and methodical manual is aimed at improving the efficiency and effectiveness of independent work of bachelor students in the study of molecular biology course and ensuring control over the progress of independent work.

The manual presents laboratory works on molecular biology. After the description of each work, a list of self-testing questions is provided. There are also control tasks for independent work of students on the main topics of the course, including test tasks. At the end of the manual, a list of the most common terms in modern molecular biology including their definitions is given. The list of recommended bibliographic sources, the main and additional literature, is presented.

The laboratory practice on molecular Biology includes 6 works based on quantitative or qualitative analysis of biological material. The purpose of the educational and methodical manual is to familiarize students with the basic methods of biochemical analysis, as well as to consolidate the skills of working with laboratory equipment.

1. Safety requirements for laboratory works on molecular biology.

1.1 General safety requirements.

- The use of molecular biology laboratory for other purposes is prohibited.
- Equipment coming for verification or after verification is stored in laboratories in specially designated places.
- The relative humidity in the laboratory premises should not exceed 80%, except in cases when other humidity standards are established by the relevant instructions for certain types of work.
- Workplaces and walkways should not be cluttered with instruments, equipment and furniture.
- The use of the chemical laboratory premises for cooking, warming up and eating is prohibited.
- Smoking is prohibited in laboratories.
- The laboratory, as well as workplaces and equipment must be kept clean and tidy.
- Cleaning of laboratory and household premises is carried out daily.
- Floors, walls, ceilings of the laboratory premises must be kept in good condition, repaired in a timely manner.
- Installations the operation of which is related with the release of harmful vapors and gases must be under constant supervision.
- All premises must be provided with fire-fighting equipment in accordance with the current instructions and rules.
- The premises must be provided with fire extinguishers suitable for extinguishing burning electrical wiring and electrical equipment. Fire-fighting equipment should be located in a visible and accessible place.

1.2 Ventilation and heating.

- All laboratory and auxiliary premises, regardless of the degree of air pollution, must be provided with natural and forced ventilation, performed in accordance with sanitary requirements and taking into account the types of work carried out.

- Heating and ventilation equipment must be kept in a state of complete serviceability and suitability for normal operation, systematically inspected and cleaned, and in case of damage must be immediately repaired.

- Without the permission of the person responsible for the condition of the heating and ventilation system, it is not allowed to make changes to it that could disrupt the proper operation of the system (connect additional equipment, remove and replace heating elements of installations).

1.3 Lighting.

- In the laboratory and in office premises, measures should be taken to maximize the use of natural light.

- It is not allowed to obstruct the light area with equipment and devices both inside and outside the building.

- The glass surface of the light area must be periodically cleaned.

1.4. Workplace organization.

- The workplace in the laboratory is a laboratory table with a set of dishes and reagents necessary for the analysis.

- When starting analytical work, you should calculate the number of necessary dishes.

- In addition to items of individual use, the laboratory has equipment for general use: a reactive shelf with reagents for various purposes, a titration unit with titrated solutions, muffle furnaces, drying cabinets, water and sand baths, electric stoves, flask heaters, devices for stirring extracts from soils (rotators), water jet pumps, etc.

- The equipment and dishes received for individual use should be placed on the surface and inside the table in a convenient order for work.

You should take care not only of cleanliness, but also of the safety of the table: glass or ceramic tiles should be placed under flasks with caustic substances (concentrated acids and alkalis). Special attention should be paid to the cleanliness of chemical dishes and reagents.

- Chemical dishes should be washed immediately after finishing work, since wet contaminants are washed easier than dried ones.

- When performing some works, the dishes in which soil samples (water and other extracts) are placed should not only be clean, but also dry. In this case, it must be washed the day before, covered with paper and left standing so that it dries in the air.

- When drying dishes in the drying cabinet, you should stop the heating of the cabinet at the end of drying and allow the dishes to cool down in the cabinet to room temperature.

- Flasks with reagent solutions should be periodically wiped with a clean, damp cloth or towel to clean them from dust and NH_4Cl, which easily precipitates on glass objects if they are located near concentrated solutions of ammonia and hydrochloric acid.

2. Rules for the use of chemical reagents.

When using chemical reagents, the following rules should be observed:

- Reagents should be taken in such an amount that is required for its one-time use.

- Reagents should be weighed on analytical scales in clean, dry dishes – in a weighing bottle, a small chemical cup or on a watch glass (if the reagent is not hygroscopic). It is not allowed to pour the excessively taken reagent back into the jar.

- When taking large samples on technical scales, a chemical cup or a porcelain cup is used as a container. It is also possible to weigh non-hygroscopic salts (KCl, NaCl, Mohr's salt, etc.) on paper (wax or parchment).

- Powdered reagents are taken with porcelain spoons or spatulas. The use of metal spatulas is unacceptable, as this can lead to deterioration of the reagents, especially if the reagent has an acidic or highly alkaline reaction.

- Jars and flasks with reagents can not be left open, since reagents are contaminated with dust, absorb moisture or pollute the air with their vapors (acid vapors, ammonia, etc.).

2.1. Safety requirements at the end of laboratory works.

At the end of laboratory works, it is necessary:

- to put the workplace in order;

- to wash your hands thoroughly with soap, rinse your mouth with water, and in some cases brush your teeth;

- to place the used chemical dishes in a marked container for washing after finishing the work;

- to collect the spent flammable liquids in a special closing container, and as it is filled, take it to a specially designated place for storage;

- to turn off the fume hood, all electrical appliances and water taps.

3. Laboratory works

3.1 Laboratory work 1

Obtaining a vegetable protein solution and studying its physico-chemical properties.

Proteins are high–molecular nitrogen-containing organic compounds whose molecules are constructed from α, L-amino acids (simple proteins), as well as containing other additional components: metal ions, inorganic and organic prosthetic groups (complex proteins).

Proteins as the basis of all living things have long been in the focus of researchers' attention. The more than two hundred-year history of protein chemistry is filled with continuous improvement of experimental methods and is rich in various theoretical concepts.

Many scientists have made a great contribution to the development of protein chemistry: A. Ya. Danilevsky, N. D. Zelinsky, V. C. Sadikov, D. Ya. Talmud, A. S. Spirin, etc., as well as foreign researchers: E. Fischer, T. Curtius, M. Bergman, F. Shorm, F. Sanger et al.

Protein chemistry is a special field that combines the ideas and methods of biology, chemistry, physics, medicine. Proteins are the material basis of cell activity. The functions of proteins in nature are universal: catalytic, transport, protective, structural, contractile, respiratory, spare, regulatory, etc. It is impossible to imagine the work of the genetic apparatus of a cell without the participation of proteins that carry out replication, transcription, and translation of information.

Proteins form the basis of the structure and function of living organisms, it is estimated that in nature there are about 1010-1012 different proteins that ensure the existence of about 106 living organisms, from viruses to humans. Each organism is characterized by a unique set of proteins. The phenotypic features and the variety of functions are due to the peculiarities of the spatial structure of proteins, depending on the sequence of the same amino acid residues, as well as the specificity of the combination of proteins in many cases in the form of supramolecular and multimolecular structures, which in turn determine the ultrastructure of cells and their organelles.

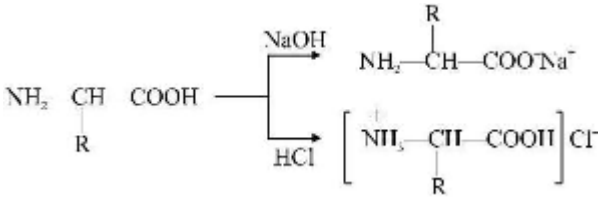
Amino acid composition of proteins

The whole variety of proteins is built from α-amino acids. The total number of α-amino acids included in their composition is close to 70. Among them, a group of 20 most important α-amino acids, are distinguished and constantly found in all proteins. Amino acids are crystalline substances soluble in water. In the solid state, α-amino acids exist as a bipolar ion. α-Amino acids are heterofunctional compounds containing a carboxyl group and an amino group in the same α–carbon atom.

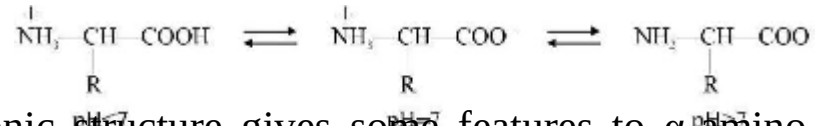
The principle of constructing α-amino acids, i.e. finding two different functional groups, a radical and a hydrogen atom, in the same carbon atom, determines the chirality (asymmetry) of the α-carbon atom (the exception is glycine). Almost all natural α-amino acids belong to the L-series (the location of the amino group in the Fischer projection formula on the left).

The use of only L-series enantiomers for the construction of proteins of living organisms has crucial importance for the formation of the spatial structure of proteins and their manifestation of biological activity.

α-Amino acids are amphoteric compounds, due to the presence of acidic and basic functional groups in their molecules. Therefore, α-amino acids form salts with both alkalis and acids:



In an aqueous solution, α-amino acids exist as an equilibrium mixture of bipolar ion, cationic and anionic forms of molecules. The equilibrium position depends on the pH of the medium:



The ionic structure gives some features to α-amino acids: high melting point (above 200 °C), non-volatility, solubility in water, which is an important factor in ensuring their biological functioning, their absorbability, transport in the body, etc.

The equilibrium position, i.e. the ratio of different forms of α-amino acids in an aqueous solution at certain pH values significantly depends on the structure of the radical, mainly on the presence of ionogenic groups in it that play the role of additional acidic or basic groups. Common to all α-amino acids is the predominance of cationic forms in strongly acidic (pH 1-2) and anionic — in highly alkaline (pH 13-14) media.

The pH value at which the concentration of bipolar ions is maximal is called the isoelectric point (IEP, pI). The value of pI is determined by the equation: pI = ½ (pK1 + pK2). The value of pK (negative decimal logarithm of the dissociation constant) characterizes the acidic and basic properties of the carboxyl and amino groups.

At the isoelectric point, the total charge of the ionic form of the α-amino acid molecule is zero, bipolar ions do not move in an electric field. With changes in the pH of the medium that lower than pI, the α-amino acid cation moves to the cathode, with a pH higher than pI, the α-amino acid anion moves to the anode.

In neutral α-amino acids, the pI value is not equal to 7, but lies somewhat lower (5.5–6.3) due to the high ability to dissociate the carboxyl group (pI of alanin is 6.02, pI of glycine is 5.97, pI of phenylalanine is 5.48, etc.).

The isoelectric point of amino acids containing additional acidic or basic groups depends on the acidity or basicity of the radicals of these amino acids. In acidic amino acids that have an additional carboxyl group in the radical, the isoelectric point is calculated as the arithmetic mean of the values of pK α-COOH and COOH-radical, pI of acidic amino acids is in a highly acidic medium (pI of asparagine is 2.74, pI of glutamine is 3.24). For the main α-amino acids having additional amino groups in the radical, pI is calculated from the half-

sum of the pK values for the α-NH2 and NH2 groups of the radical. The isoelectric point of basic amino acids is located at the pH above 7 (pI of histidine is 7.59, pI of lysine is 9.82, pI of arginine is 10.76).

In the body, at physiological pH values of 6.9–7.4, mostly all α-amino acids (with the exception of histidine) are in cationic or anionic form, and not in the form of a bipolar ion. Histidine has significant buffering properties at physiological pH. Hemoglobin contained in erythrocytes is characterized by a high content of histidine residues, which gives it a significant buffer capacity.

Effective and sensitive methods (electrophoresis and ion exchange chromatography) for identification and quantitative determination of each of the 20 α-amino acids have been developed based on differences in the acid-base properties of α-amino acids, i.e., on differences in the sign and magnitude of the total electric charge at a given pH value.

The purpose of the work. To obtain a solution of vegetable protein and animal protein, to study the physico-chemical properties of protein.

Obtaining of protein solutions

Protein solution for color reactions and precipitation reactions. Separate the protein of one chicken egg from the yolk, dissolve it in 15-20-fold volume of distilled water. Filter the solution through gauze folded in 3-4 layers. Store it in the refrigerator.

Egg white solution for salting and dialysis reactions. Separate the proteins of three chicken eggs from the yolks and dissolve it in 700 ml of distilled water. Add it to 300 ml of saturated sodium chloride solution. Filter the solution through gauze folded in 3-4 layers. Store it in the refrigerator.

A solution of milk albumins. Add an equal volume of saturated ammonium sulfate solution to 200 ml of skimmed milk. Mix and leave it for 10-15 minutes. Filter it through a folded paper filter. Albumins are in solution, globulins and casein are in precipitate.

A solution of vegetable proteins. Add 160 ml of distilled water to 40 g of wheat flour. Stir it and transfer the flask to the refrigerator (1-20 ^{0}C) for a day. Mix it again and filter first through hygroscopic cotton wool, and then through a folded paper filter. The solution contains mainly albumins. Store in the refrigerator.

A) Obtaining vegetable protein

The course of work:

Pour a suspension of pea flour (3-5 g) into a flask, add 30 ml of 10% ammonium sulfate solution, stir it for 3 minutes, left to settle for 30 minutes, then filter the mixture through a filter moistened with an ammonium sulfate solution into another flask. If the filtrate is cloudy, drain it back to the filter. The resulting solution contains protein. Explain which protein went into the solution.

B) Obtaining egg white

Egg white: the protein from 1 chicken egg is dissolved in 250 ml of water, filtered through a layer of gauze and stored in the refrigerator.

Aim. To obtain plant protein and animal protein solutions, to study the physico-chemical properties of protein.

A) Obtaining plant protein

Procedures:

A suspension of pea flour (3-5 g) is poured into a flask, 30 ml of 10% ammonium sulfate solution is added and stirred for 3 minutes, left to settle for 30 minutes, then filtered into another flask through a filter that was moistened with an ammonium sulfate solution. If the filtrate is cloudy, then it is drained back to the filter. The resulting solution contains protein. Explain which protein went into the solution.

B) Obtaining egg white

Egg white: the protein from 1 chicken egg is dissolved in 250 ml of water, filtered through a layer of gauze and stored in the refrigerator.

Qualitative reactions to amino acids and proteins.

The peculiarity of the chemistry of amino acids and proteins lies in the presence of numerous qualitative (color) reactions that at one time formed the chemical basis for the analysis of α-amino acids and proteins. Currently, when the study of α-amino acids and proteins is carried out by physico-chemical methods, many qualitative reactions retain their significance and are used to detect α-amino acids, peptides and proteins in electrophoresis and chromatographic analysis.

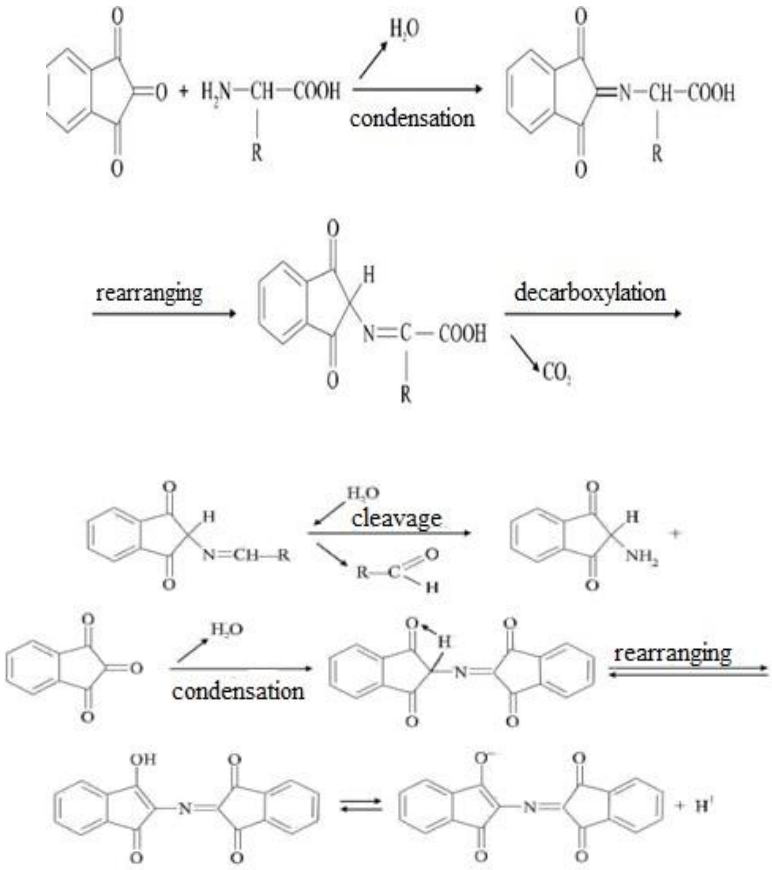
The general qualitative reaction of α-amino acids is a reaction with ninhydrin, and for proteins it is a biuret reaction. There are also a number of particular reactions to detection of individual α-amino acids or groups of related α-amino acids.

Reagents and equipment:

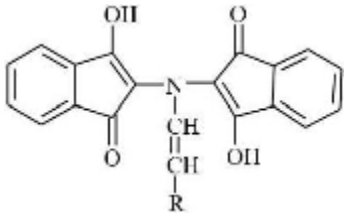
Sodium hydroxide (10% and 20%); nitric acid (conc.); sulfuric acid (conc.); hydrochloric acid (5%); sulfanylic acid (1%); glacial acetic acid; copper sulfate (1%); lead acetate (1%); sodium hypobromide (0.2 %); potassium nitrite (0.5%); sodium carbonate (10%); phenol (0.1%); ninhydrin (1% in 95% acetone); urea (cryst.), urea (40%); α-naphthol (0.2% of alcohol solution); gelatin (1%); 0.01% solutions of amino acids: glycine, proline, tyrosine, cysteine, arginine, histidine, tryptophan. Millon's reagent: 40 g of mercury is dissolved in 60 ml of conc. HNO3 (p 1.40 g /ml) at room temperature. Then it is placed in a warm water bath until the release of reddish brown vapors stops and then it should be mixed. Then 120 ml of H2O is added and the resulting solution is diluted with water 1:1. Tripod, test tubes, measuring pipettes (1 and 5 ml), thermostat.

1. Ninhydrin reaction

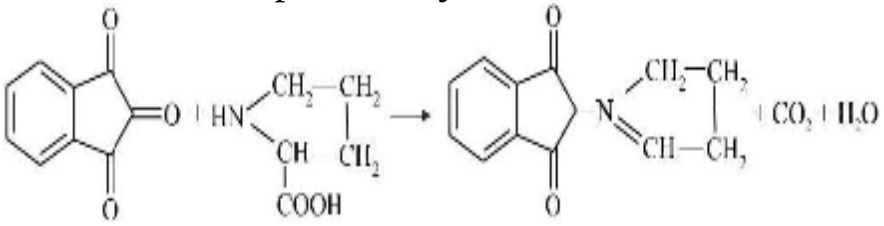
The ninhydrin reaction is characteristic of amino groups located in the α-position and included in proteins, peptides and free amino acids. α-Amino acids, peptides, proteins, when heated with a solution of ninhydrin, give a blue or blue-violet staining. Ninhydrin reaction with alcohol or acetone solutions is widely used in electrophoresis, chromatographic methods for the discovery of individual amino acids and determination of their amount. As a result of the interaction of α-amino acids with ninhydrin, the Schiff base is formed, which is rearranged, decarboxylated and cleaved into aldehyde and aminodiketohydrinden. Aminodiketohydrinden condenses with another ninhydrin molecule. The resulting compound, enolizing, turns into a colored form, called the "Rueman's blue-violet complex".



In the presence of organic solvents (acetone, ethanol), on which a solution of ninhydrin is prepared, a side reaction is possible with the formation of a compound containing an amino acid radical in its composition.



The presence of an amino acid radical in the composition of this compound causes a different color of the compounds (red, yellow, blue) that occur during the reaction of amino acids with ninhydrin. In the reaction of glycine with ninhydrin, a compound with a blue-violet color is formed, and with proline – yellow.



Procedures:

A. Add 1 ml of 1% glycine solution and 5 drops of 1% ninhydrin solution in 95% acetone solution into the test tube. The contents of the test tube are thoroughly mixed and placed for five minutes in a thermostat at a temperature of 70 °C.

B. Pour 3 ml of 0.01% proline solution and 5 drops of 1% ninhydrin solution in acetone into the test tube. The contents of the test tube are stirred and heated in a thermostat at 70 °C for 5 minutes.

C. Add 5 drops of 0.5% aqueous solution of ninhydrin to 1 ml of 1% protein solution, stir and place it into a thermostat for 5 minutes at a temperature of 70 °C. A pink-violet staining appears in the test tube, and the solution turns blue over time.

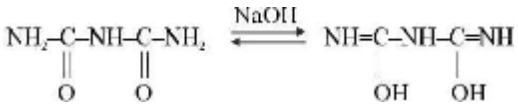
2. Biuret reaction

In an alkaline medium, the protein solution, when interacting with copper ions, acquires a blue-violet or red-violet staining. The biuret reaction can be given by substances that contain at least two peptide bonds, for example tri-, tetrapeptide, etc.

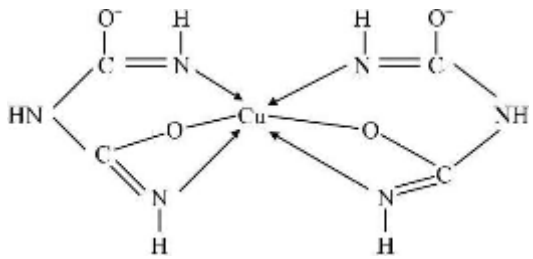
For the first time, the reaction of the formation of complex copper compounds was carried out for a biuret, hence the name of the reaction is biuret. Biuret is formed by heating dry urea:

$$2NH_2—CO—NH_2 \xrightarrow{180\ ^\circ C} NH_2—CO—NH—CO—NH_2 + NH_3$$

In an alkaline medium , the biuret turns into an enol form:



Two biuret molecules interact with copper hydroxide to form a biuret complex:



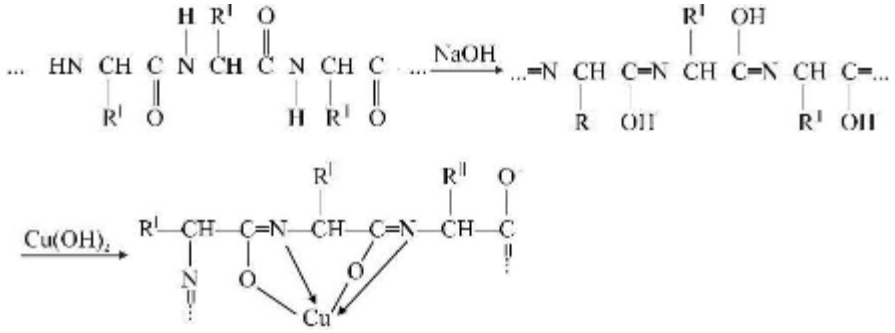
The biuret reaction is caused by the formation of a biuret complex as a result of the combination of copper ions with a protein peptide

bond. The color of the biuret complex depends on the amount of copper ions, the length of the polypeptide chain, the protein concentration and can vary from pink to blue-violet.

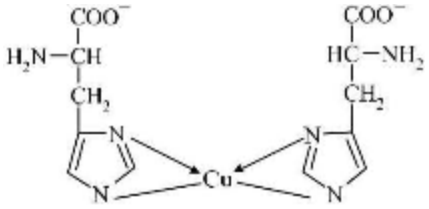
Copper (II) hydroxide for the biuret reaction is obtained by the interaction of sodium hydroxide and copper sulfate:

$$2NaOH + CuSO_4 = Na_2SO_4 + Cu(OH)_2\downarrow$$

In an alkaline medium, the peptide bonds of the protein from the keto form are transferred into the enol form, which interacts with copper (II) hydroxide:



Amino acids do not give a positive biuret reaction with the exception of histidine, serine, threonine, provided their concentrations are high in solution:



Biuret histidine complex

Procedures:

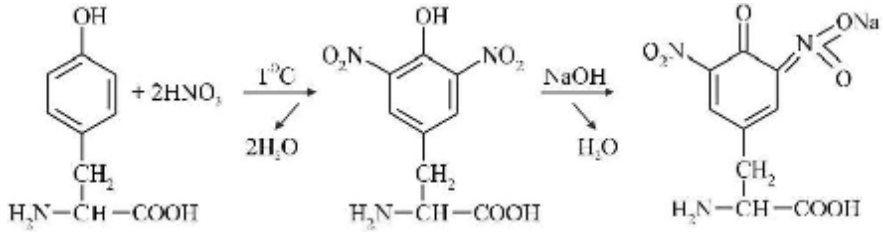
A. Add 2 ml of 10% sodium hydroxide solution and 2 drops of 1% copper sulfate solution to 1 ml of 1% egg white solution, and mix everything. The contents of the test tube become violet. Excess copper sulfate can not be added, since the blue precipitate of copper hydroxide masks the characteristic violet staining of the biuret protein complex.

B. Heat several urea crystals in a test tube until melting. Then, after cooling the test tube, add 2-4 drops of water and 1 drop of

sodium hydroxide and copper sulfate. The contents of the test tube turns pink.

3. Xanthoprotein reaction

The reaction was called xanthoprotein from the Greek xanthos — yellow. The reaction ensures the appearance of yellow staining when concentrated nitric acid gets on the skin, nails, etc. The xanthoprotein reaction proves the presence of aromatic amino acids in the protein: phenylalanine, tyrosine, tryptophan. Proteins: gelatin, salmin, clupein give a negative reaction to nitric acid, which indicates the absence of aromatic amino acids in the composition of these proteins. Tyrosine and tryptophan are easily nitrated, while phenylalanine is nitrated hardly.



Tyrosine Dinitrothyrosine Sodium salt of dinitrothyrosine

Procedures:

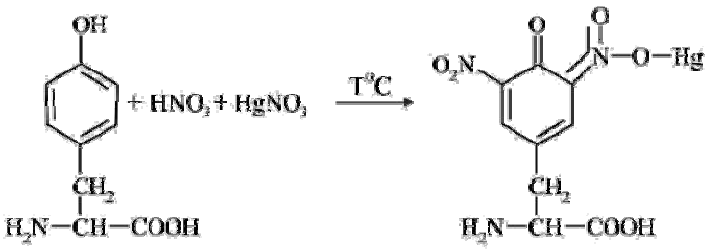
Take three test tubes. Pour 1 ml of 0.01% tyrosine solution in the first tube. Pour 1% egg white solution in the second tube. Pour 1% gelatin solution in the third tube. Then add 0.5 ml of concentrated nitric acid to all test tubes and heat carefully. The appearance of a yellow color in the first test tube is observed, a yellow precipitate is in the second, and a very weak staining is in the third, since gelatin contains almost no aromatic amino acids. In an alkaline meduim, the nitro derivatives of aromatic amino acids form salts of the quinoid structure, colored orange. After cooling, add 10-15 drops of 20% alkali solution into the first two test tubes until orange staining appears.

4. Millon's reaction

Millon's reaction is qualitative to the amino acid tyrosine. Therefore, it is used for the discovery of tyrosine in proteins. When Millon’s reagent is added to the protein solution, the protein

precipitates, which turns red when heated. The reaction proves the presence of a phenolic ring in the tyrosine radical which is capable of forming the mercury salt of dinitrothyrosine.

An excess of the Millon's reagent should not be added to the protein solution, since it contains nitric acid, which, when interacting with the protein, can give a yellow staining, masking the Millon's reaction.



Mercury salt of dinitrothyrosine

Procedures:

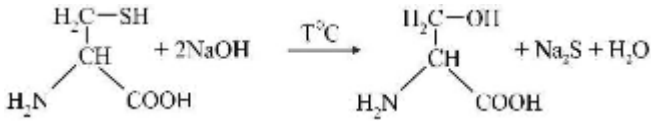
A. Add 0.5 ml of the Millon's reagent to 1 ml of 0.01% tyrosine solution, and mix thoroughly. After 10 minutes, the solution turns red.

B. Add 0.5 ml of the Millon's reagent to 1 ml of 1% egg white solution, and mix thoroughly the reaction mixture. After 10 minutes, heat the white precipitate carefully and observe its staining in red.

5. Sulfhydryl reaction

The Sulfhydryl reaction allows the discovery of cysteine containing a weakly bound sulfhydryl group in a protein or peptide. Although methionine is a sulfur-containing amino acid, it does not give this reaction, unlike cysteine, since sulfur is firmly bound in it.

When boiling cysteine in an alkaline medium, sulfur is cleaved in the form of hydrogen sulfide, which easily forms sodium sulfide in an alkaline medium. The formation of sodium sulfide can be detected with lead ions forming an insoluble black lead sulfide with sulfur ions.



$$Na_2S + Na_2PbO_2 + 2H_2O \rightarrow PbS\downarrow + 4NaOH$$

Lead acetate can be used to detect sodium sulfide, which interacts with sodium hydroxide to form sodium plumbite. The product of the reaction of sodium plumbite with sodium sulfide becomes lead sulfide.

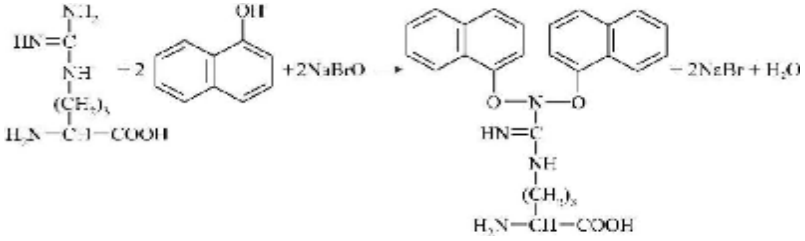
Procedures:

A. Add 2 ml of concentrated sodium hydroxide solution and 1 ml of Fol reagent (10% lead acetate solution with 10% alkali solution) to 1 ml of 0.01% aqueous solution of cysteine. Stir and boil the mixture for 2 minutes. After 5 minutes, a reddish brown or black precipitate falls out.

B. Add 1 ml of Fol reagent to 1 ml of 1% egg white solution. Mix and boil it for 2 minutes. After cooling, the formation of a reddish brown precipitate is observed, indicating the presence of cysteine residues in the protein molecule.

6. Sakaguchi reaction

The qualitative reaction to arginine is the Sakaguchi reaction. Arginine, having a guanidine moiety, in the presence of α-naphthol is oxidized by hypobromide in an alkaline medium and form a pink-red condensation product.



Procedures:

A. Pour 1 ml of 0.01% arginine solution, 1 ml of 10% sodium hydroxide solution, 3 drops of 0.2% alcohol solution of α-naphthol into a test tube, mix thoroughly and add 3 drops of 0.2% sodium hypobromide solution and mix again. To stabilize the rapidly developing red staining, 1 ml of 40% urea solution is immediately poured in.

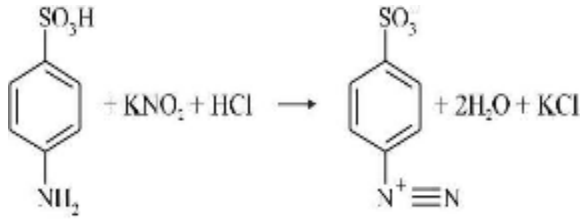
B. Add 1 ml of 10% alkali solution, 3 drops of α-naphthol solution to 1 ml of egg white. Mix thoroughly and add 3 drops of

sodium hypobromide solution, mix, and then 1 ml of 40% urea solution is quickly added.

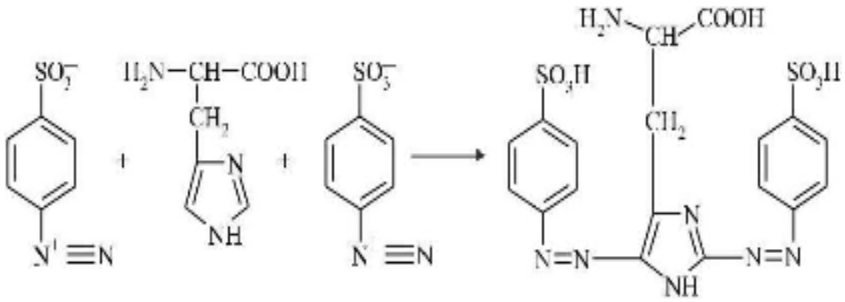
7. Pauly reaction

The Pauly reaction reveals the amino acids histidine and tyrosine in protein solutions and hydrolysates.

When an acidic solution of sulfanylic acid interacts with potassium nitrite, a diazotization reaction occurs and diazobenzenesulfonic acid is formed.



The reaction of diazobenzenesulfonic acid with histidine (or tyrosine) produces a complex cherry-red compound.



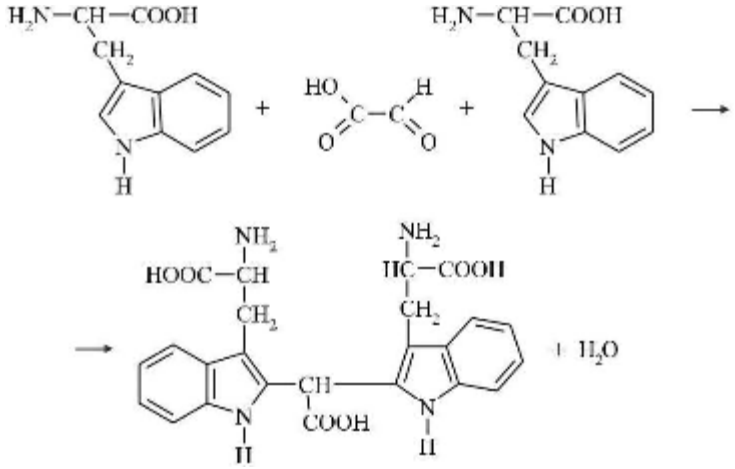
2, 5-bis-II-sulfo benzene histidine

Procedures: Pour into two test tubes 1 ml of 1% sulfanilic acid solution in 5% hydrochloric acid solution and 2 ml of 0.5% potassium nitrite solution. Shake the test tubes hard. Then, quickly pour 2 ml of 0.01% histidine solution into the first tube, and pour 2 ml of 1% egg white solution into the second. Shake the tubes thoroughly and add 6 ml of 10% sodium carbonate solution to both tubes. An intense cherry-red color develops.

8. Adamkiewicz reaction

The Adamkiewicz reaction is a specific reaction to tryptophan and is used to detect it in proteins. Tryptophan reacts with glyoxylic acid

(aldehydes) in an acidic medium, forming red-violet-colored condensation products.



Procedures:

A. Add 0.5 ml of glacial acetic acid, which contains a small amount of glyoxylic acid to 0.5 ml of 0.01% tryptophan solution. Heat the resulting mixture. Then cool it down and carefully along the wall drop by drop so that the liquids do not mix, add 1 ml of concentrated sulfuric acid. After 10 minutes, the formation of a red-violet ring is observed at the interface of the two layers.

B. Perform the Adamkiewicz reaction with 1 ml of 1% egg white solution.

It is recommended to make a report on this work in the form of a table [1].

Table 1. Qualitative reactions to proteins and amino acids

No.	Name of the reaction	Reagents	What does it discover	Chemism of the reaction

Physico-chemical properties of protein

Precipitation of proteins

Two main factors allow the retention of heavy protein molecules in solution. Firstly, it is the presence of a hydrate shell; secondly, the presence of a charge in a protein molecule. A hydrate shell is a layer

of water molecules oriented in a certain way on the surface of a protein molecule. The surface of most protein molecules is negatively charged, and the dipoles of water molecules are attracted to it by their positively charged poles. The more hydrophilic properties a protein molecule has, the more amino acids with polar (hydrophilic) radicals there are in its composition and on its surface, the more hydrate shell is defined and more firmly it is retained and the more layers there are in it. Hydrate shell water has special properties: it is not free; it is bound to a protein molecule – it is "bound" water. Surrounding each protein molecule, the hydrate shell does not allow these protein molecules to get closer, connect and precipitate. To precipitate the protein from the solution, it is necessary to deprive it of both stabilization factors: both the charge and the hydrate shell.

Protein deposition reactions are very diverse. Depending on the reagent used, they can be reversible or irreversible. Irreversible reactions include precipitation of proteins by heavy metal salts, heating, mineral and organic acids. Reversible protein precipitation reactions can be carried out using organic solvents (in the cold) or alkali metal salts as a precipitator. There are no profound changes in the protein molecule, so the resulting precipitate dissolves well in the original solvent, and the protein completely retains its native properties.

1. Salting out proteins. This is a reversible precipitation of proteins from the solution by adding neutral salts (especially sulfates) in high concentrations. During salting out, dehydration of protein molecules and elimination of charge occur. The precipitation process depends on the molecular weight and charge of the protein and the degree of hydrophilicity.

Procedures:

Add along the wall an equal volume of saturated ammonium sulfate solution to 20 drops of egg white solution. A white ring (a globulin precipitate) is formed at the boundary of liquids. Filter the contents of the test tube, and add ammonium sulfate powder to the filtrate. Stir with a glass stick, in small portions, until the solution is completely saturated. The solution becomes cloudy – albumins are precipitated. Transfer half of the contents of the test tube to a clean

test tube and add 20 drops of distilled water. The precipitate dissolves. Explain the obtained results.

2. Precipitation of protein with alcohol, acetone is based on dehydration of protein molecules. Short-term exposure of alcohol in the cold does not violate the protein structure, at room temperature, protein denaturation occurs.

Procedures:

Add 10 drops of ethanol to 10 drops of albumin solution. Note the protein precipitation. Then pour 20 drops of distilled water into the test tube – the precipitate does not dissolve.

2. Precipitation of protein by acids. Concentrated mineral and some organic acids cause denaturation of protein molecules. In addition, mineral acids dehydrate protein molecules.

Procedures:

Pour 10 drops of albumin solution into two test tubes. Then add 5 drops of 10% trichloroacetic acid to the first tube, and 5 drops of concentrated nitric acid to the second. Note the precipitation which does not disappear when water is added.

3. Precipitation of protein by heavy metal salts. Heavy metal ions, when interacting with proteins (especially with SH groups), form water insoluble complexes. The protein is denatured.

Procedures:

Pour 10 drops of albumin solution into two test tubes. Add 2 drops of a 2% solution of copper sulfate into the first tube, and 2 drops of a 5% solution of lead acetate into the second. Note the appearance of sediment that does not disappear when water is added.

4. Precipitation of protein during heating.

When the protein solution is heated to 60-70 °C, as a rule, protein precipitation occurs. This is due to deep violations of the structure of the protein molecule. The concentration of hydrogen ions (pH) plays an important role in the precipitation of denatured protein. The most complete and rapid precipitation occurs at the isoelectric point of the protein. In strongly acidic and highly alkaline solutions, the protein denatured during heating does not precipitate, since the protein

molecules carry a positive charge in the first case, and a negative charge in the second.

Procedures:

Pour 10 drops of albumin solution into four numbered test tubes. Then, add 1 drop of 1% acetic acid solution to the second tube, 5 drops of 10% acetic acid solution to the third, and 5 drops of 10% caustic soda solution to the fourth. Boil all test tubes and note the absence or precipitation, as well as its characteristics. Explain the results.

Enter the received data into the table.

Name of the precipitator groups	Reagents used	Characteristics of precipitation	Causes of precipitation
1.Neutral salt	а) $(NH_4)_2SO_4$ solution	White ring (globulin)	Dehydration of protein, removal of charge
	б) $(NH_4)_2SO_4$ crystal	Turbidity of the solution (albumin)	“ – “
	в) H_2O distilled	Dissolution of precipitation	Reversibility of precipitation
2. Etc.			

After drawing up the table, make conclusions:

a) about the possibility of reversible and irreversible protein precipitation;

b) about the main mechanisms of protein precipitation;

c) about the practical application of protein precipitation reactions.

Determination of the isoelectric point of the protein.

Protein molecules have an electric charge resulting from the ionization of free carboxyl groups and amino groups. The charge of the protein depends, firstly, on the amino acid composition of the protein, and secondly, on the pH of the medium. At a certain pH value, the total charge of the protein molecule can become zero. This pH value is called the isoelectric point of the protein (IEP). Different proteins have different IEP. In acidic proteins, IEP lies at pH <7, in basic proteins at pH > 7.

In the isoelectric state, the protein is maximally unstable and can be precipitated from the solution very easily. This feature of protein behavior is used for experimental determination of its IEP.

Procedures:

From burettes pour 2 ml of buffer solution with different pH values (3.0; 4.0; 5.0; 6.0) and 2 ml of gelatin solution into four numbered test tubes. Mix the contents of the test tubes, preventing the formation of foam, then layer carefully 1 ml of alcohol into each tube without stirring. After 30 minutes, mark the test tube, in which the most intense (due to protein precipitation) turbidity at the interface of liquids is observed. The pH of the solution in this test tube corresponds to the IEP of this protein.

The degree of turbidity is indicated as follows: (–) – absence of turbidity; (+) – weak turbidity; (++) – high turbidity; (+++) – maximum turbidity.

Enter the results of the experiment into the table.

Test tube No.	pH of the buffer solution	The degree of turbidity at the boundary of the media devision
1.		
Etc.		

Based on the results obtained, make a conclusion about the value of the isoelectric point and about the features of the amino acid composition of this protein.

Control questions

1. What are proteins?
2. What properties do they have?

3. What are qualitative reactions used for?

4. What factors allow heavy protein molecules to be retained in solution?

5. Describe specific reaction to tryptophan?

6. What is protein salting?

3.2 Laboratory work 2

Protein dialysis.

The dialysis method is based on the unequal ability of the solution components to diffuse through thin membrane films with selective permeability. The membrane is a porous film through the pores of which small molecules can penetrate. The dialysis method is used to purify high-molecular compounds from low-molecular compounds, as well as to concentrate polymer solutions.

Reagents: *aqueous protein solution (the solution is prepared as indicated in laboratory work No. 1); saturated sodium chloride solution; 0.5% silver nitrate solution; 10% nitric acid solution; 10% sodium hydroxide solution; 1% copper sulfate solution.*

Equipment: test tubes, cellophane bag; glass

Procedures:

Task 1. Dialysis

1. Mix equal volumes of protein solution and saturated sodium chloride solution in a test tube.

2. Pour the prepared protein solution into a cellophane bag, filling it up to half.

3. Hang the bag on a glass stick and immerse it in a glass of distilled water. Sodium ions and chloride ions freely penetrate through the walls of the bag and are evenly distributed throughout the entire volume of water. The protein molecules are larger than the cellophane pore sizes and remain in the bag. Dialysis is carried out at room temperature for 20 minutes.

Task 2. Analysis of water in a glass

1. Add 2 drops of nitric acid solution and 2-3 drops of silver nitrate solution to 1 cm^3 of liquid from a glass. A white precipitate of silver chloride appears.

2. Add 5 drops of alkali solution and 1-2 drops of copper sulfate solution to 1 cm^3 of liquid from a glass. There is no violet staining characteristic of proteins.

Task 3. Analysis of the contents of the pouch.

1. Add 5 drops of alkali solution and 1-2 drops of copper sulfate solution to 5 drops of solution from the bag. There is a characteristic staining caused by the formation of a complex copper salt with a polypeptide.

Recording of results:

Briefly describe the progress of the work. Make a schematic drawing of dialysis. Make a conclusion about the distribution of low- and high-molecular substances before and after dialysis [13].

Control questions:

1. What is dialysis? What is the principle of the protein dialysis method?
2. Which membranes are used during dialysis?
3. How to speed up dialysis?
4. How to precipitate proteins?
5. Name artificial semipermeable membranes
6. What is the amino acid composition of proteins?

3.3 Laboratory work 3

Isolation of deoxyribonucleoproteins from plant and animal material.

Materials and equipment:

- Banana
- Table salt
- Warm water
- Liquid soap
- Mixer
- Toothpicks
- Glass jar
- Medical alcohol
- Knife

Procedures:

1. Using a knife, cut the banana into small pieces to expose more cells.
2. Place the banana slices in a blender, add a teaspoon of salt and add a little warm water to the mixture. The salt will help the DNA stay together during the mashing process.
3. Mix in a blender for 5 to 10 seconds, making sure that the mixture is not too liquid.
4. Pour the mixture into a glass jar through a sieve so that the jar is half full.
5. Add approximately 2 teaspoons of liquid soap and gently stir the mixture. You should try not to create bubbles when stirring. Soap helps break down cell membranes to release DNA.
6. Carefully pour very cold alcohol down the side of the vessel.
7. Wait for 5 minutes to allow the DNA to separate from the solution.
8. Use a toothpick to extract the DNA that floats on the surface.

(https://learn.genetics.utah.edu/content/labs/extraction/howto/)




Fibers of deoxyribonucleoproteins

Hints:

1. When pouring alcohol, make sure that two separate layers are formed (the bottom layer is banana mixture and the top layer is alcohol).
2. When extracting DNA, twist the toothpick slowly. Make sure to remove only the DNA from the top layer.

3. Try to repeat this experiment again using other foods, such as onions or chicken liver.

A) Excretion from the liver or spleen

The liver, spleen, pancreas, kidneys, yeast are rich in nucleoproteins. They dissolve in dilute solutions of alkalis and precipitate during acidification of the solution. Deoxyribonucleoproteins also dissolve well in saline solutions.

Reagents and materials: *a) liver or spleen of cattle or pigs, fresh or frozen; b) sodium chloride, 5% solution; c) wooden stick with notches.*

Procedures:

Cut 2-2.5 g of liver or spleen into small pieces and then ground in a mortar with a 5% solution of sodium chloride, adding a little glass powder. Add the salt solution in small portions (10-15 ml each), a total of about 80 ml is consumed.

Rub the mixture for 12-15 minutes until a homogeneous mass is obtained. Pour the contents of the mortar into centrifuge tubes and centrifuge it for 10-15 minutes (at 2500 rpm), after which measure the volume of the centrifuge (draining it into a glass cylinder).

Pour distilled water into the glass (the volume of which should be six times the volume of the centrifuge) and, stir slowly in the glass with a wooden stick, pour the water into the centrifuge. Deoxyribonucleoproteins fall out in the form of threads that are wound on a stick.

B) Isolation of nucleoproteins from yeast

Reagents and materials: *a) pressed baking yeast; b) diethyl ether; c) caustic soda, 0.4% solution; d) acetic acid, 5% solution; e) glass powder or river sand, thoroughly washed and calcined.*

Procedures:

Moisten 5 g of yeast in a mortar with 1 ml of diethyl ether and 1 ml of water, add a little glass powder or sand and rub the mixture with a 0.4% solution of caustic soda, pouring it in small portions (5-10 ml each). In total, up to 50 ml of an alkali solution is consumed; continue rubbing for 15-20 minutes. Filter the contents of the mortar through a folded filter or centrifuged for 10 minutes (at 2500 rpm). Pour the filtrate or centrifuge into a glass and add a 5% solution of acetic acid

to it drop by drop until the nucleoprotein is completely precipitated (usually 10-15 ml of the solution is consumed).

Separate the precipitate by centrifugation.

Qualitative reaction to DNA

The presence of DNA is determined by the color reactions characteristic of deoxyribose. A reaction with diphenylamine (C_6H_5–NH-C_6H_5) is often used. Diphenylamine with deoxyribose or DNA forms a blue compound. Ribose and RNA give green staining with diphenylamine.

Reagents and materials: a) deoxyribonucleoprotein precipitate (see previous work); b) diphenylamine reagent: 1 g of diphenylamine is dissolved in 100 ml of glacial acetic acid. 2.75 g of concentrated sulfuric acid (p_{20} = 1.836) is added to the solution; c) caustic soda, 0.4% solution.

Transfer the part of the deoxyribonucleoprotein precipitate to a test tube and add 0.5-1 ml of caustic soda solution (before dissolution). Add an equal volume of diphenylamine reagent to the solution. The precipitate that falls out at the beginning will dissolve in subsequent portions of the reagent. Then heat it for 15-20 minutes in a boiling water bath. A blue staining appears [3].

Control questions:

1. How to get a solution of vegetable protein?
2. In which solutions do nucleoproteins dissolve well?
3. How to get a solution of animal protein?
4. Describe qualitative reaction to DNA?
5. Why is soap added to the banana mixture?

3.4 Laboratory work 4

Hydrolysis of nucleoproteins and study of properties of hydrolysis products

Hydrolysis of nucleoproteins occurs during boiling with dilute sulfuric acid.

Reagents and materials: *a) nucleoprotein precipitate (see previous work); b) sulfuric acid, 5% solution; c) concentrated sulfuric*

acid ; d) caustic soda, 10% solution; e) ammonia, concentrated solution (20-25%); f) copper sulphate, 1%-solution; g) thymol, 1% alcohol solution; h) ammonia solution of silver oxide: a concentrated solution of ammonia is added to a 1-2% solution of silver nitrate until the precipitate that was formed at the beginning, is dissolved; i) molybdenum reagent: 3.75 g of ammonium molybdenum acid is dissolved in 50 ml of water and 50 ml of 32% nitric acid is added (p_{20} = 1,200). Complete dissolution of the salt occurs only after the addition of nitric acid.

Transfer the nucleoprotein precipitate to a round-bottomed flask, wash it off with 5% sulfuric acid. Pour the remainder of the acid into a flask with a precipitate. In total, no more than 20-25 ml of the solution is consumed. Close the flask with a stopper with a reverse refrigerator, put on an asbestos mesh, boil at low heat for 1-1.5 hours, then cool it down and the filter the hydrolysate through a paper filter. Reactions to polypeptides, purine bases, pentoses and phosphoric acid are carried out with the filtrate.

Weigh 1 g of compressed yeast, transfer the suspension to a round-bottomed flask, pour 30-40 ml of a 5% solution of sulfuric acid, close the flask with a stopper with an air refrigerator, boil it at low heat, placing an asbestos mesh under the flask. Boil for 1-1.5 hours, then cool down the flask, filter its contents through a paper filter. The following reactions are carried out with the filtrate.

Study of nucleoprotein hydrolysis products

The discovery of polypeptides. A biuretic reaction is carried out with a part of the filtrate (1-2 ml).

Discovery of purine bases.

Procedures:

Add a concentrated ammonia solution to 2 ml of filtrate before an alkaline reaction to litmus and add 1 ml of an ammonia solution of silver oxide. After a few minutes, flakes of precipitate of silver salts of purine bases fall out.

Procedures:

The discovery of pentoses. The discovery of pentoses is based on a reaction with thymol and concentrated sulfuric acid. Sulfuric acid causes dehydration of pentoses and the formation of furfural, which with thymol gives red compounds (condensation products).

Add 2-3 drops of 1% alcohol solution of thymol to 1 ml of filtrate and layer carefully 1 ml of concentrated sulfuric acid along the tube wall. The liquid turns red. The coloration is more shown at the boundary of the layers.Pentoses can also be detected by reactions with orcine or phloroglucine or pheling fluid.

Discovery of phosphoric acid. Phosphoric acid forms with the molybdenum reagent a yellow crystalline precipitate of ammonium phosphorous-molybdenum acid.

Procedures:

Add an equal volume of molybdenum reagent to 1-2 ml of filtrate, boil it for 2-3 minutes. Yellow staining appears due to the formation of ammonium phosphorous-molybdenum acid. During standing, a yellow precipitate falls out.

Control questions:

1. What is the biological significance of nucleoproteins?
2. Which proteins are part of the nucleoproteins?
3. Describe products of hydrolysis of nucleic acids?
4. What are proteins in composition?
5. What contains nucleic acids?

3.5 Laboratory work 5

Purification and separation of nucleic acids

Reagents and materials: *a) spleen, liver, kidneys; b) trichloroacetic acid, 5-10-50% solutions; c) caustic potassium (KOH, 0.5 n solution; d) ethyl alcohol, 95%; e) diethyl ether; f) a mixture of ethyl alcohol with diethyl ether (3:1); g) a mixture of ethyl alcohol with chloroform (3:1); h) hydrochloric acid ($HClO_4$), 5 n solution. Prepare the solution carefully, as perchloric acid is unstable and can explode during storage (especially at elevated temperatures), as well as in contact with organic substances.*

Procedures:

The process of isolation, purification and separation of nucleic acids consists of a number of successive stages:

1. Crush 1 g of tissue (raw weight) in a glass homogenizer with a 5% solution of trichloroacetic acid in the cold until a homogeneous thin gruel is obtained (the homogenizer is cooled with ice), then quantitatively wash the mixture off the walls of the homogenizer with 5 ml of a 10% cold solution of acetic acid, transfer it to a centrifuge tube. Centrifuge it for 7 min. (3000-4000 rpm) in a cooled centrifuge or a low-temperature room. Drain the liquid above the precipitate, and pour 5-10 ml of a 10% cold trichloroacetic acid solution back into the precipitate and centrifuge again. Repeat the operation for the third time. Connect the washing waters.

Nucleic acids, proteins and lipids remain in the precipitate, low molecular weight derivatives of nitrogenous bases (including nucleotides) and inorganic phosphoric compounds pass into the washing water.

Note. All operations must be carried out at a low temperature in order to avoid cleavage of purines (apurinization) and subsequent rupture of DNA chains during alkaline hydrolysis.

2. Free the precipitate from lipids by extraction with organic solvents, for which rub it with a glass stick and sequentially centrifuge it with ethyl alcohol, twice with a mixture of alcohol with chloroform (3:1), then with a mixture of alcohol with ether (3:1) and finally with ether. Each time take 10 ml of solvent. Dry the washed precipitate in air; to accelerate drying, rubbed it with a glass stick.

Nucleic acids and proteins remain in the precipitate.

Separation of RNA and DNA.

Procedures:

With mild alkaline hydrolysis, RNA is cleaved to nucleotides, while DNA remains polymeric and precipitates when acid (chloric or trichloroacetic) is added.

Add 5-10 ml of 0.5 n KOH solution to the air-dried and powdered precipitate and place it in a thermostat at 37^0C for 15-18 hours, then cool it down to 0-2^0 and add a cold 5 n solution of perchloric acid ($HClO_4$) at the rate of 0.2 ml of solution per millimeter of hydrolysate.

Note. Instead of explosive concentrated perchloric acid, it is better to use a 50% trichloroacetic solution, which is added to the hydrolysate in such a way as to bring the concentration of acid in it (after neutralizing KOH) to 5%.

After adding the acid, keep the hydrolysate for 5 minutes in the refrigerator at 0^0C and then centrifuge it.

The supraplastic fluid contains nucleotides that were released during the hydrolytic cleavage of RNA. Drain the filler liquid, combine it with the washing waters obtained in the first stage. The RNA content is determined in the liquid fraction.

DNA and proteins remain in the precipitate.

4. Dissolve the precipitate in 0.5 N KOH solution at 37^0C, then cool it down to 0^0C. Drain the solution and determine the DNA content in it. Throw out the precipitate of insoluble substances [3].

Control questions:

1. What are the methods of nucleic acid separation?
2. Why do all nucleic acid separation operations need to be carried out at a low temperature?
3. How is a charged DNA molecule formed?
4. What are the methods of purification of nucleic acids?
5. What is the starting material for purification and separation of nucleic acids?

3.6 Laboratory work 6

PCR protein analysis

Polymerase chain reaction (PCR) is a method of molecular biology that allows you to create copies of a certain DNA fragment from the original sample, increasing its content in the sample by several orders of magnitude.

The principle of the method.

We all know that DNA is a double-stranded molecule, where each strand consists of links-nucleotides. Nucleotides are made up of three molecules: phosphoric acid residue, sugar and nitrogenous base. If sugar and phosphate are the same for all nucleotides in DNA (there is another sugar in RNA), then there are four nitrogenous bases (except for rare modifications): adenine, thymine, cytosine and guanine, denoted A, T, C and G, respectively. In RNA molecules, thymine is replaced by uracil. Nucleotides are connected in a strand, forming bonds between the phosphate group of one nucleotide and the hydroxyl group of the other. As a result, a phosphate group (5′-the

end) "hangs" at one end of each DNA strand, and a hydroxyl group (3′- the end) at the other. Two strands of nucleotides are located in the DNA molecule antiparallel, that is, opposite the 3′-end of one is the 5′- end of the other. In order for the molecule to be stable, the strands must somehow interact with each other. This is provided by hydrogen bonds formed between the nitrogenous bases of opposite strands on the principle of complementarity: A connects only with T (or U in RNA), and G connects with C. And therefore, in accordance with this rule having one DNA strand it is easy to build a pair of it. Actually, this is what PCR is based on.

A typical reaction mixture.

1. Analyzed DNA sample. It can be either a separate piece of a molecule, or a plasmid, a chromosome, or the entire genome of a cell. For a rough assessment, even a suspension of cells is acceptable. DNA can be a template for multiple copying of the desired site.

2. Primers. A primer is an artificially synthesized short strand of nucleotides (15-30 pieces), complementary to the selected section of one of the strands of the analyzed DNA. One of the primers usually corresponds to the beginning of the amplified segment, the other to its end, but on the opposite strand. Primers, like any oligo- or polynucleotide, have 3′- and 5′-ends.

3. Nucleotides. Deoxynucleotide triphosphates are four types of “building blocks” for the for new DNA strands: dATP, dTTP, dCTP and dGTP.

4. DNA polymerase. An enzyme that builds a DNA complementary template. It can start synthesis only from the 3′-end of the primer. Thermally stable polymerases originally isolated from thermophilic bacteria and archaea are usually used: Thermus aquaticus (TaqDNA polymerase), Pyrococcus furiosus (PfuDNA polymerase) and Pyrococcus woesei (Pwo DNA polymerase).

5. Buffer solution. A solution containing various ions to maintain the desired pH, magnesium salts necessary for the work of polymerase, and a non-ionic detergent Tween-20 in combination with BSA (bovine serum albumin) to prevent the components of the reaction from sticking to the walls of the test tube. For the templates with high GC content, an enhancer DMSO (dimethyl sulfoxide) is often added to the mixture, preventing undesirable interactions between complementary template elements.

Reagents for PCR from "Diaem".

The main difficulty in setting up PCR of any variation is to create the “right cocktail” of ingredients: isolated DNA, enzymes, triphosphates and buffer in a strictly defined optimal ratio. Maintaining the correct ratio is a big problem for laboratories where several thousand PCRs are performed per day: it is enough to slightly change the concentration of magnesium salts, and the results of PCR will not be perfect.

Ready-to-use mixes for PCR: "Extramixes".

To simplify the life of laboratory assistants, Diaem produces ready-to-use mixes — "Extramixes" for PCR - both for the classical variation and for PCR with real-time detection.

In fact, "Extramixes" are kits containing all the necessary ingredients for PCR: DNA polymerase, reaction buffer, Mg^{2+} salt solution, as well as deoxynucleotide-5-triphosphates. To perform PCR using “Extramix” from “Diaem”, you only need to mix the isolated DNA, PCR mix and primers in one test tube.

“Extramixes” for standard PCR with HS-Taq polymerase are for high-performance of hot start PCR, which are recommended for rapid development of PCR products for subsequent cloning or sequencing.

• “Extramixes” for RT-PCR — for the reaction of reverse transcription of RNA and subsequent PCR on a cDNA template by a one-step method.

• “Extramixes” for quantitative PCR with intercalating dye SYBR and normalization by ROX are recommended for amplifiers requiring signal normalization by passive reference dye ROX.

• “Extramixes” for quantitative real-time PCR with fluorescent probes. Real-time PCR is recommended for multiplex PCR and genotyping using oligonucleotide probes.

• “Extramixes” for amplification of long fragments (up to 25,000 bp), as well as for obtaining products for TA-cloning and amplification of GC-rich templates and complex templates.

• Mix all components in the required volume of deionized water in special tubes for PCR and place them in an amplifier (or PCR cycler) (Fig. 1).

Figure 1. PCR consumables and equipment. a — PCR-test tubes. b — C1000 Touch™ amplifier manufactured by Bio-Rad.

Reaction stages

The purpose of PCR is to obtain a set of identical double-stranded pieces of DNA of a strictly defined length (usually no more than 2-3 thousand pairs of nucleotides). For this, 20-30 reaction cycles are carried out.

Each cycle consists of three stages.

1. Denaturation

In order for the polymerase to work, the two DNA template strands need to be separated. Heat the reaction mixture to 94-98 °C. Under such conditions, hydrogen bonds between the nitrogenous bases of parallel chains are destroyed.

2. Annealing of primers

At this stage, the primers specifically bind to the denaturated DNA template strands from different sides of the copied section by 3′-ends to each other (Fig.2). In order for primers to be able to complementarily bind (anneal) only with the necessary areas. It is necessary to take into account such an important characteristic as the melting temperature (T_m) when designing the primers. This is the calculated temperature at which half of the primers bind to the target DNA site. Annealing is carried out at a temperature 1-5 °C below T_m,

but not above the optimal temperature of the polymerase, that is, within 40-72 °C.

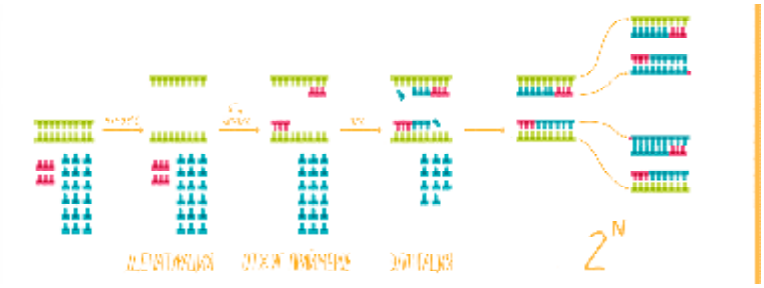


Figure 2. Polymerase chain reaction

At the denaturation stage, the DNA chains are separated, at the next stage (annealing) primers bind to them, and then the polymerase begins its work — the synthesis of new DNA chains (elongation). And this cycle repeats many times.

To see the figure in full size, click on it.

Ideally, primers should meet the following criteria:

• the melting temperatures of the two primers should not differ by more than 5 °C;

• the GC content of a primer should be between 40% and 60%

• there should be no hairpins in the structure of oligonucleotides (areas complementary to each other);

• primers should not form duplexes (pair) with each other.

Even better, if there is guanine or cytosine at the 3′-end of the primer: they form three hydrogen bonds with complementary bases (two are formed between A and T), which makes the primer-template complex more stable.

In reality, it is rarely possible to meet all the conditions due to a variety of reasons. However, the more criteria are met when designing primers, the higher the probability of their correct operation.

In order to develop effective primers, it is necessary to know the DNA sequence at the ends of the target site, and, guided by the mentioned criteria, select suitable fragments to which future primers will be complementary. All this is convenient to do in special computer programs — for example, Primer Select: they will calculate the T_m, depict all sorts of pairings, and generally make a verdict whether this is a successful pair of primers or not.

3. Elongation, or DNA synthesis

This polymerase reaction is due to the fact that during its course the DNA polymerase enzyme sequentially builds a DNA chain (polymer) from nucleotides (monomers), i.e. polymerizes them. And it does it at the third stage of PCR.

This stage is more often carried out at a temperature of 72 °C which is optimal for the operation of Taq polymerase. The enzyme binds the primer-template complexes and, snatching nucleotides from the solution, begins to add them to the 3′-end of the primer according to the principle of complementarity (Fig. 7). Elongation of the new DNA strand goes at a maximum speed of 50-60 nucleotides per second (that is, about 3000 per minute). However, when programming the PCR cycler, the time is set with a margin: one minute for every thousand pairs of nucleotides.

Each newly synthesized DNA strand, along with the old one, becomes the template for synthesis in the next cycle. Thus, the amount of the desired product increases exponentially during the reaction. After passing through all the cycles, so many specific double–stranded products are formed in the reaction mixture that their "array" can be seen with the naked eye by conducting gel electrophoresis, which we will describe below.

Unfortunately, exponential amplification cannot last forever. After 25-30 cycles, the number of functional polymerase molecules in the reaction mixture is depleted. But in order to achieve even greater product yield, the contents of the test tube can be diluted, for example, 1000 times and used again for amplification with new components.

Visualization of PCR products.

In order to see if the necessary DNA sections have multiplied, after the end of PCR, the contents of the test tubes are subjected to electrophoresis in agarose or polyacrylamide gel, followed by staining. DNA molecules of different lengths are separated spatially and become visible to the naked eye. Polyacrylamide gel is much denser, therefore it is more suitable for separating very short fragments (several dozen pairs of nucleotides), while you can see the difference even in one nucleotide!

Pour the gel melted at 65 °C into a special mold (round die/cutter) with a comb installed in it, forming wells (Fig.3). When the gel hardens, remove the comb, place the mold in the electrophoresis chamber and fill with a special buffer. Then add a solution from PCR tubes, mixed with paint (often bromophenol blue), into the wells with a micropipette. In order to determine the size of the amplified fragments later, insert a molecular weight marker (ladder) containing a set of DNA pieces of known sizes into a separate well. Connect the camera to a power source and observe the magic bubbles running from the electrodes for tens of minutes or several hours. It dependis on the size of the DNA fragments, the density of the gel and the applied voltage.

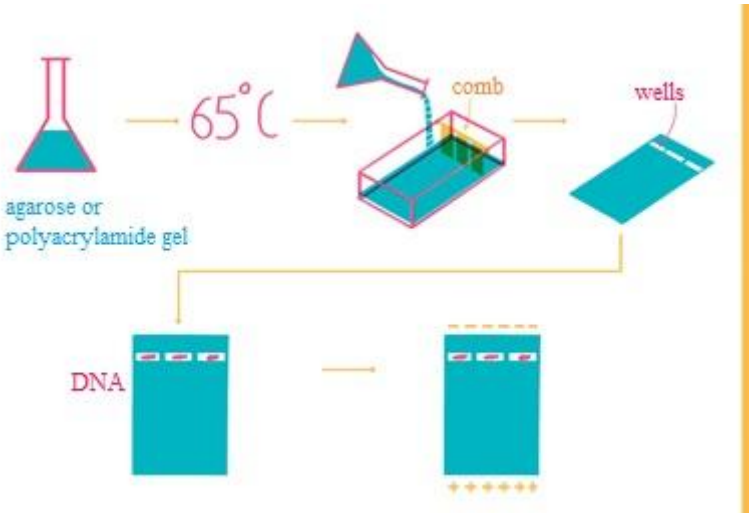


Figure 3. Preparation of gel for horizontal electrophoresis.

Due to the negatively charged sugar phosphate backbone of the DNA, fragments move in the gel under the action of an electric field from the negative cathode to the positive anode. Shorter molecules do this faster than longer ones. Bromophenol blue is needed in order to monitor the progress of the sample front in the gel and prevent them from going beyond it.

After the end of electrophoresis, remove the gel from the round die/cutter and, in order to see the location of the fragments, soak it in a solution of a fluorescent dye that binds firmly to DNA. Sometimes it is injected into the gel even before the round die/cutter is filled. If the dye is ethidium bromide, which is embedded between DNA nucleotides, visualization is carried out under ultraviolet light (Fig.4).

If the experimenter's goal was simply to understand whether there is the right sequence of nucleotides in the DNA template, the gel is

thrown away after visualization. But it is not difficult to isolate the necessary fragments from the gel for further work: to cut them into pieces for comparison with other fragments, to insert into plasmids for further study, to sequence, etc.

“Diaem”: Gel documentation systems and electrophoresis.

To visualize the result of gel electrophoresis of nucleic acids stained with dye, special devices - transilluminators are used. At the same time, detection is carried out both visually and with the help of automated image capture and processing systems – Gel documentation systems.

To increase the contrast of the image obtained by detecting amplified fragments in agarose gel, Vilber company has developed transilluminators with Super-Bright technology. The effectiveness of this technology in comparison with a conventional UV transilluminator is clearly visible in the photo below. A special light filter completely removes the visible glow range of the lamps, which significantly increases the contrast of the resulting image, allowing detect even small amounts of nucleic acids in the gel.

This technology is implemented on the basis of autonomous Vilber transilluminators, and as part of automatic gel-documentation systems E-Box, universal Fusion FX systems and highly sensitive Infinity systems.

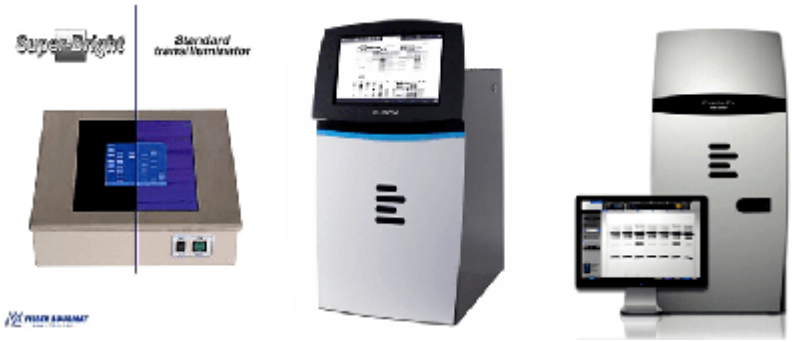


Figure 4. “Diaem”: equipment for classical PCR:

• Gel documentation systems and transilluminators.

• Chambers and current sources for DNA electrophoresis in agarose gels.

• Reagents for nucleic acid electrophoresis.

• Markers of DNA molecular weight [29].

Control questions:

1. What is the principle of the PCR method?
2. List the components of the composition of a typical reaction mixture?
3. What is a buffer?
4. Types of electrophoresis, their comparison, advantages and disadvantages.
5. What is the main difficulty in setting up PCR?

Review questions and tasks:

1. What does molecular biology study?
2. Characterize nucleic acids as material carriers of hereditary features of biological systems?
3. What are the fundamental discoveries of molecular biology?
4. What are the methods of molecular biology?
5. What is the structure of DNA and how to record genetic information in accordance with the Chargaff's rules?
6. Give the definition of the nucleic acid. What nucleotides do DNA and RNA consist of?
7. How is the DNA supercoil formed and which enzymes are involved in this process?
8. Look at an X-ray of a DNA molecule. How did the analysis of this image affect the establishment of the DNA structure?
9. How was the mechanism of DNA replication proved during the experiments of Meselson and Stahl?
10. Describe the reaction of DNA biosynthesis involving DNA polymerase. What experimental facts have confirmed the process of this reaction? What determines the sequence of newly synthesized DNA?
11. What experiments helped to establish the fact of RNA mediation in the transmition of information in the DNA-protein system? What are the three main structural differences between DNA and RNA?
12. How is the central dogma of molecular biology formulated? How absolute is this dogma?
13. What is meant by adaptive RNA (F. Crick assumption)? What is the general structure and diversity of adaptor RNAs?

14. How to obtain rRNA, mRNA and tRNA preparations from a certain cell type based on differences in their physicochemical properties (quantity, size, complexity and HZ content)?

15. Some diseases that cause disorders of the nervous system are caused by prion proteins and are spread using proteins, not nucleic acids. How can we prove the absence of the effect of nucleic acids in the transmission of infection based on the knowledge of the Avery-MacLeod-McCarty experiment in this case?

16. What is DNA replication? What is the biological meaning and medical significance of DNA replication?

17. Describe the replication fork, define the terms "leading and lagging strand", "replication primer", "Okazaki fragment". Show in which direction the replicative fork is moving and the DNA strands are growing.

18. Why is the DNA of the SV40 virus considered a convenient model object for replication?

19. What ingredients are needed for DNA polymerase reaction?

20. What does an enzymatic complex for linear DNA replication look like?

21. What is the significance of the formation of temporary DNA-protein complexes?

22. Name the founders of the physiological period of the development of molecular biology?

23. Name the structural features of prokaryotes.

24. Describe the features of chromosomes in bacteria?

25. What features are peculiar to the nucleoid of bacteria?

26. The concept of a gene and genetic information in molecular biology. The genetic role of nucleic acids.

27. The DNA structure. Watson and Crick's model. Proof of the double helix structure of DNA.

28. Modern ideas about the molecular mechanisms of DNA replication.

29. The general mechanism and stages of transcription, participants in this process. Modern ideas about the mechanisms of transcription. Regulation of transcription.

30. Processing of primary transcripts. Processing and maturation of mRNA in eukaryotes. Splicing. Gene transcription in mitochondria.

Tests

Topic: «The genetic role of nucleic acids»

1. The monomers of DNA and RNA are:
a) nucleosides
b) nucleotides
c) purines
d) pentoses

2. In nucleic acids, monomer residues are bound together by:
a) hydrogen bonds
b) peptide bonds
c) phosphodiester bonds
d) N-glycosidic bonds

3. Uracil is:
a) 6-amino-2-oxypyrimidine
b) 2-amino-6-oxypurine
c) 6-aminopurine
d) 2,6-oxypyrimidine

4. According to the Chargaff's rules, the content of adenine in the DNA molecule is equal to:
a) the content of thymine
b) cytosine content
c) guanine content
d) the content of uracil

5. A pentose sugar, which is part of RNA is:
a) glucose
b) phosphotriose
c) ribose
d) 2-deoxyribose

6. According to the Watson and Crick's model, the length of the spiral coil is:
a) 0.34 nm
b) 3.4 nm -
c) 10 nm

d) 100 nm

7. The secondary structure of DNA was established by:
a) E. Chargaff
b) J. Watson and F. Crick
c) M. Wilkins
d) M. Chase

8. Purines include:
a) cytosine and uracil
b) thymine and adenine
c) adenine and guanine
d) uracil and guanine

9. A strong chemical bond in the DNA molecule occurs between:
a) nucleotides
b) deoxyriboses of adjacent nucleotides
c) residues of phosphoric acid and sugar of adjacent nucleotides
d) nitrogenous bases

10. In which case are all the differences between i-RNA and DNA correctly named?
a) single-stranded, contains deoxyribose, stores information
b) double-stranded, contains ribose, transmits information
c) single-stranded, contains ribose, transmits information
d) double-stranded, contains deoxyribose, transmits information

Topic: «DNA replication»

11. The hypothesis of a semi-conservative replication mechanism was proven experimentally by:
a) M. Meselson and F. Stahl
b) J. Taylor
c) J. Watson
d) M Chase

12. An enzyme that unwinds the DNA double strands during replication, by destroying the hydrogen bonds between complementary bases, is called:

a) ligase
b) DNA polymerase
c) helicase
d) primase

13. Replication is the process of:
a) protein synthesis
b) synthesis of i-RNA
c) DNA self-replication
d) damage repair

14. Complementary copying of the template during replication is carried out by the enzyme:
a) DNA ligase
b) DNA topoisomerase
c) RNA primer
d) DNA polymerase

15. The first stage of DNA replication:
a) elongation
b) initiation
c) termination
d) activation

16. Stabilization of the unwound super-twisted DNA structure is carried out by the enzyme:
a) ligase
b) primase
c) SSB protein
d) DNA polymerase

17. Okazaki fragments are stitched into one covalently continuous DNA chain:
a) DNA ligase
b) primase
c) DNA polymerase
d) helicase

18. A replicative fork is formed in the process of:

a) initiation
b) protein biosynthesis
c) elongation
d) termination

19. What is replicated by rolling circle replication?
a) RNA
b) Eukaryotic DNA
c) DNA of some phages, viruses, mitochondria, plasmids
d) Human DNA
20. Why does DNA replication occur in the 5'-3' direction?
a) 5'-3' polymerase activity of DNA polymerase is associated with 5'-3' exonuclease activity
b) DNA polymerase in the 5'-3' direction does not exhibit exonuclease activity
c) the primer 3'-OH is needed for the polymerase to manifest
d) the RNA primer is synthesized only in the 5'-3' direction.

Topic: «Transcription»

21. The process of iRNA synthesis on the DNA template is called:
a) replication
b) transcription
c) protein biosynthesis
d) splicing

22. Which enzyme synthesizes tRNA in eukaryotes:
a) RNA polymerase- III
b) DNA ligase
c) DNA polymerase-1
d) RNA polymerase-1

23. What is a strictly defined region of DNA where RNA synthesis begins:
a) terminator
b) promoter
c) operon
d) transcriptone

24. Which part of a gene does not contain information about the protein:

a) exon
b) intron
c) operon
d) transcriptone

25. Splicing is:

a) stitching of exons, removal of introns
b) DNA synthesis
c) protein synthesis
d) the process of repairing damaged DNA sites

26. The transcription process takes place in:

a) the nucleus
b) cytoplasm
c) ribosomes
d) mitochondria

27. By which factor does the RNA polymerase recognize the promoter:

a) p-factor
b) g-factor
c) σ-factors
d) p-factor

28. A set of biochemical reactions, as a result of which the molecular weight of the precursor RNA decreases with the formation of mature RNA molecules:

a) translocation
b) transcription
c) broadcast
d) processing

29. Which part of a gene contains information about the protein:

a) intron
b) exon
c) transcript
d) terminator

30. The region of DNA between the promoter and the terminator is called:

a) intron
b) transcriptone
c) exon
d) p-factor

31. The structure of the clover leaf:

a) the secondary structure of the RNA molecule
b) the primary structure of the rRNA molecule
c) the secondary structure of the viral RNA molecule
d) the tertiary structure of tRNA

Topic: «Translation»

32. Translation of information from the 4-letter alphabet of nucleic acids to the 20-letter alphabet of amino acids:

a) transcription
b) translation
c) termination
d) splicing

33. The structure of the "clover leaf" has:

a) tRNA
b) rRNA
c) iRNA
d) mRNA

34. Protein synthesis takes place in:

a) ribosomes
b) endoplasmic reticulum
c) mitochondria
d) nucleus

35. Nonsense codons. Find the wrong answer:

a) UAA
b) UAG
c) UGA
d) AUG

36. The codon from which protein synthesis begins:
a) UUU
b) UAG
c) AUG
d) AAA

37. The process of amino acid activation is:
a) single-phase
b) two-phase
c) three-phase
d) multi-phase

Topic: «Genetic code»

38. How the genetic code is read:
a) every two nucleotides
b) in groups of five nucleotides
c) in groups of three nucleotides
d) through one nucleotide pair

39. The genetic code is degenerate because:
a) one nucleotide pair corresponds to 1 amino acid
b) several codons can correspond to one amino acid
c) two nucleotides correspond to one amino acid
d) it is triplet

40. Mutation, as a result of which one nucleotide is delegated, leads to:
a) overlap of nucleotides
b) shift of the reading frame
c) shift of one amino acid and pyrimidine
d) destruction of DNA

Amino acids, peptides and proteins

1. Write 1 formula from each group of amino acids that differ in the electrochemical nature of the radical. Explain why they belong to this particular group.

2. Write the formula of leucine in an aqueous solution and specify the total charge of this amino acid:

a) at pH 6-7;

b) at pH 4;

c) at pH 12.

3. Write the formula of glutamine in an aqueous solution in a neutral medium. Specify the total charge. How will the charge of this amino acid change:

a) with gradual acidification of the medium (up to pH 2);

b) with alkalinization of the medium (up to pH 9).

4. Write the formula of glutamate in an aqueous solution in a neutral medium. Specify the total charge. How will the charge of this amino acid change:

a) with gradual acidification of the medium (up to pH 2);

b) with alkalinization of the medium (up to pH 9).

5. Write the formula of lysine (in ionized form) in an aqueous solution and specify the total charge of this amino acid:

a) at pH 6-7;

b) at pH 4;

c) at pH 12.

6. Write the formula of the tripeptide: asp-val-phe; underline the peptide bonds; specify the name of the peptide; circle the peptide groups; specify the N- and C-ends of the molecule.

7. Write the formula of a hexapeptide containing 2 amino acid residues with hydrophobic radicals, 2 with hydrophilic uncharged radicals, one each with cationic and anionic radicals.

8. Determine the total charge of the pentapeptide glu-arg-lys-val-asp in a neutral medium. How will the total charge of this peptide change:

a) at pH << 7;

b) at pH >> 7?

9. Write the formula of the glutathione tripeptide, which has the composition: glu-cys-gly, taking into account that the peptide bond

between glu and cys is formed due to the carboxyl group of the glutamate radical. Determine the total charge of this peptide:

a) at pH 7;

b) at pH < < 7;

c) at pH >> 7.

10. Write the formulas of two pentapeptides and compare their solubility at pH 7:

a) ser-cys-glu-tyr-asp;

b) val-arg-met-phe-tyr.

11. Write the formulas and names of dipeptides that can be obtained from amino acids:

a) threonine and cysteine;

b) phenylalanine and asparagine;

c) arginine and leucine.

12. A protein globule contains a fragment -cys-ala-glu-asn-cys-asp-leu-phe-ser-met-trp-gly-thr-gln-arg-:

a) specify the hydrogen bonds stabilizing the secondary structure;

b) name the types of bonds stabilizing the tertiary structure of the molecule, between which radicals of amino acids are they formed?

13.The following amino acids are essential:

a) alanine, asparagine, proline;

b) phenylalanine, methionine, valine;

c) glycine, glutamate, serine.

14. Name the amino acids whose radicals contain:

a) hydroxyl group;

b) sulfur.

15. Classify amino acids (1-4) according to the electrochemical nature of the radical (a–g):

Amino Acid: Radical:

isoleucine; a) with a cationic radical;

asparagine; b) with an anionic radical;

glutamate; c) with a polar uncharged radical;

arginine. d) with a non-polar radical.

16. Nuclear histone proteins contain a large amount of arginine and lysine. In what medium is the isoelectric point of these proteins located?

a) pH > 7;

b) pH < 7;

c) pH 7.

17. Albumin (blood protein) contains many residues of glutamic and aspartic acids. In what medium is the isoelectric point of this protein located:

a) pH > 7;

b) pH < 7;

c) pH 7.

18. The method based on the separation of molecules by electric charge is:

a) distribution chromatography on paper;

b) ion exchange chromatography;

c) centrifugation;

d) gel filtration.

19. The method of separation of substances differing in molecular weight is:

a) ion exchange chromatography;

b) distribution chromatography on paper;

c) gel filtration;

d) electrophoresis.

20. The method of separation of substances based on differences in their solubility is:

a) ion exchange chromatography;

b) distribution chromatography on paper;

c) gel filtration;

d) electrophoresis.

21. Specify the correct sequence of operations that are carried out during protein isolation procedure:

a) fine (deep) purification;

b) extraction;

c) fractionation;

d) homogenization;

e) purification from other proteins.

22. At the isoelectric point, the protein:

a) has the greatest solubility;

b) has the lowest solubility;

c) is a cation;

d) is an anion.

23. Specify the correct sequence of operations that are carried out when deciphering the primary structure of the protein (the Edman method):

a) attachment of the peptide to an inert carrier;

b) attachment of the peptide to the isothiocyanate;

c) reconstruction of the primary structure of the peptide;

d) partial hydrolysis of a protein molecule;

e) reconstruction of the primary structure of the complete protein chain;

f) cleavage of an amino acid from a peptide and its identification.

24. Albumins are:

a) simple proteins soluble in water;

b) simple proteins soluble in alkaline solutions;

c) complex proteins soluble in water;

d) complex proteins soluble in alkaline solutions;

e) simple proteins soluble in 70-80% ethyl alcohol solution.

25. Globulins are:

a) simple proteins soluble in weak solutions of salts;

b) simple proteins soluble in water;

c) complex proteins soluble in water;

d) complex proteins soluble in weak alkaline solutions;

e) simple proteins soluble in 70-80% ethyl alcohol solution.

26. Prolamins are:

a) simple proteins soluble in weak salt solutions;

b) simple proteins soluble in 70-80% ethyl alcohol solution;

c) complex proteins soluble in alkaline solutions;

d) complex proteins soluble in weak saline solutions.

27. Glutelins are:

a) simple proteins soluble in weak saline solutions;

b) simple proteins soluble in 70-80% ethyl alcohol solution;

c) complex proteins soluble in weak alkaline solutions;

d) simple proteins soluble in weak alkaline solutions.

Amino acid and protein metabolism

1. List the reactions of the ornithine cycle in the correct sequence:

a) hydrolysis of arginine;

b) formation of carbamoyl phosphate;

c) synthesis of argininosuccinate;

d) formation of citrulline;

e) cleavage of argininosuccinate to arginine and fumarate.

2. The amino acid acting as an amino group acceptor in the urea cycle is:

a) citrulline;

b) glutamate;

c) ornithine;

d) arginine.

3. The enzyme arginase (one of the enzymes of the ornithine cycle) belongs to the class:

a) ligases;

b) hydrolases;

c) oxidoreductase;

d) transferase;

e) isomerases.

4. In animal organisms characterized by the neutralization of ammonia by converting it into urea, this process is carried out:

a) in all cells;

b) in liver cells;

c) in muscle fibers;

d) in the kidneys.

5. Specify the ornithine cycle enzymes that are localized in the mitochondria:

a) carbamoyl phosphate synthetase;

b) ornithine carbamoyltransferase;

c) argininosuccinate synthase;

d) argininosuccinate lyase;

e) arginase

6. Specify which of the listed enzymes belongs to proteinases:

a) carboxypeptidase;

b) pepsin;

c) aminopeptidase;

d) dipeptidase.

7. Hydrolysis of proteins entering the human body with food begins:

a) in the oral cavity;

b) in the esophagus;

c) in the stomach;

d) in the duodenum.

8. Hydrolysis of tissue (own) proteins in the animal body is carried out mainly:

a) on ribosomes;

b) in lysosomes;

c) in the mitochondria;

d) in the nucleus.

9. Amino acids that are nitrogen reserves for most plants include:

a) glutamate and aspartate;

b) alanine and leucine;

c) ornithine and citrulline;

d) glutamine and asparagine.

10. Most amino acid conversion reactions are associated with the participation of a cofactor:

a) pyridoxal phosphate;

b) thiamine pyrophosphate;

c) biotin;

d) flavinadenine dinucleotide

e) nicotinamide adenine dinucleotide.

11. As a product of deamination of α-amino acids in the cells of living organisms, the most widely represented:

a) unsaturated acids;

b) saturated acids;

c) α-keto acids;

d) aldonic acid.

12. Which amino acid is the acceptor of ammonia during its formation in the cell:

a) leucine;

b) glutamate;

c) glycine;

d) tryptophan.

13. Glutathione is:

a) a simple protein;

b) a dipeptide consisting of glutamate and aspartate;

c) a tripeptide consisting of glycine, glutamate and cysteine;

d) a tripeptide consisting of glutamate, aspartate and methionine

14. Name the products that are formed during transamination between:

a) glutamic and pyruvic acids;

b) 2-oxoglutaric acid and alanine;

c) glutamate and oxaloacetate.

Mono-, di- and polynucleotides

1. Write down the formula of adenosine triphosphate (ATP). Specify its role in living organisms.

2. Write down the formula of guanosine triphosphate (GTP). Specify its role in living organisms.

3. Write down the formula of uridine triphosphate (UTF). Specify its role in living organisms.

4. Write the formula of the dinucleotide that is part of deoxyribonucleic acid (DNA), in which adenine and cytosine would be as nitrogenous bases.

5. Write in two tautomeric forms each of the following nitrogenous bases: guanine, uracil, thymine and cytosine.

6. Write the formula of the dinucleotide that is part of ribonucleic acid (RNA), in which guanine and uracil would be the bases.

7. Define the concept of "complementarity". Explain the reason for the formation of pairs in DNA molecules: adenine — thymine, guanine — cytosine and indicate the number of bonds formed between them.

8. Specify the characteristic features of the prokaryotic genome that distinguish it from the eukaryotic genome.

9. Name the main stages of transcription in the correct sequence.

10. Specify the basic principles on which DNA replication is based.

11. Name the main stages of translation in the correct sequence.

Mono-, di- and polynucleotides (tests)

1. What are the properties of purine and pyrimidine bases:
a) hydrophilicity;
b) hydrophobicity;
c) amphipolarity.
2. The functions of NAD and NADP are due to the fact that these compounds:
a) contain macroergic bonds;
b) are able to be reversibly reduced and oxidized;
c) capable of irreversible recovery.
3. Nucleic acids are linear polymers in which nucleotide residues are connected by:
a) hydrogen bonds;
b) ionic bonds;
c) phosphodiester bonds;
d) coordination bonds.
4. The DNA parts that carry information about the protein structure and are part of the corresponding RNA and protein in eukaryotes are:
a) operons;
b) introns;
c) exons.
5. In a DNA molecule, the number of adenine residues is always equal to the number of residues:
a) uracil;
b) guanine;
c) cytosine;
d) thymine.
6. Specify the order of nucleotides in the DNA chain formed during replication by self-copying the chain: TCAAGTAT-TATTCGGTCA.
7. Write the sequence of nucleotides in the mRNA molecule in prokaryotes, which will be synthesized using the following DNA chain as a matrix: GATCCTTAGGATCAA.
8. The information of one DNA triplet corresponds to:
a) gene;
b) protein;
c) amino acid.

9. A nucleoside consisting of uracil and ribose is called _____ , and from cytosine and ribose is called _____ .

10. Specify the name of the transcribed but not translated DNA sequence (in eukaryotes):

a) exon;

b) intron;

c) operon.

11. A nucleoside consisting of guanine and deoxyribose is called _____, and from thymine and deoxyribose is called ____ .

12. Specify the name of the transcribed and translated DNA sequence (in eukaryotes):

a) exon;

b) intron;

c) operon.

13. The hypochromic effect of DNA is:

a) increasing in buoyant density;

b) increasing the negative angle of rotation of the plane of polarized light;

c) an increase in light absorption at 260 nm;

d) a decrease in viscosity.

14. Choose the correct statements:

a) DNA and RNA contain the same purine bases in their composition;

b) DNA and RNA contain the same pyrimidine bases;

c) only in the composition of DNA there are minor pyrimidine and purine bases.

Carbohydrates and their metabolism

1. Write formulas reflecting the elemental composition of oligosaccharides:

a) tetrasaccharide, consisting of hexose;

b) pentasaccharide consisting of pentose;

c) hexasaccharide, consisting of hexose;

d) heptasaccharide consisting of pentose.

2. Write the formulas of D-ribose and D-deoxyribose (linear and cyclic).

3. Write the linear formulas of D- and L-galactose.

4. Write the linear formulas of D- and L-fructose

5. Write linear formulas of two D-fructose epimers.

6. Write the linear formulas of D-mannose and its two epimers.

7. Specify the localization of glycolysis enzymes and the main functions of this process. What processes is glycolysis associated with (what processes can precede it and what processes does it precede)?

8. Write the reaction equations (with formulas and enzymes) of the second (exergonic) stage of glycolysis.

9. Specify the localization of the enzymes of the citrate cycle and the main functions of this process. What processes is this cycle associated with (which processes can precede it and which processes does it precede)?

10. Write the equations of transketolase reactions between the following compounds: a) xylulose-5-phosphate and ribose-5-phosphate; b) ribulose-5-phosphate and erythroso-5-phosphate; c) fructose-6-phosphate and phosphoglycerin aldehyde.

11. Write the equation of the transaldolase reaction between sedoheptulose-7-phosphate and phosphoglycerin aldehyde.

12. Write the equations of aldol condensation reactions between the following compounds:

a) phosphoglycerin aldehyde and dihydroxyacetone phosphate;

b) erythroso-4-phosphate and dihydroxyacetone phosphate.

Lipids and their metabolism

1. Write the structural formulas of tripalmitin, palmitodilaurin, palmitostearolein. Which triacylglycerols are in the group of simple, and which are in the group of mixed triacylglycerols?

2. Give a diagram of the hydrolysis of triolein and determine the number of ATP molecules that can be formed when its components are completely split.

3. Give a diagram of the hydrolysis of palmitodilaurine and determine the number of ATP molecules formed when its components are completely broken down.

4. Write the first cycle of β-oxidation of myristic acid ($C_{14}H_{28}O_2$).Calculate the energy balance of its complete oxidation.

5. Write a scheme for the complete cleavage of glycerol, calculate the number of ATP molecules formed.

6. Calculate the energy balance of the complete cleavage of one trimyristin molecule.

7. Calculate how many elongation cycles, as well as how many molecules of acetyl coenzyme A, ATP and restored NADP will be required for the synthesis of one molecule of lignocerinic acid ($C_{24}H_{48}O_2$).

8. Write the first cycle of elongation of the chain of higher fatty acid.

9. Calculate how many elongation cycles, as well as how many molecules of acetyl coenzyme A, ATP and restored NADP will be required for the synthesis of one molecule of arachinic acid ($C_{20}H_{40}O_2$).

Amino acid and protein metabolism

1. List the ways to neutralize ammonia in the body of animals (for example, mammals).

2. List the ways to neutralize ammonia in plant organisms.

3. Name the processes that use nitrogen-free amino acid residues formed during their oxidative deamination.

4. Write the equation of the reaction of the reductive amination of oxaloacetic acid (oxaloacetate).

5. Write the equations of transamination reactions between 2-oxoglutarate and alanine; between glutamate and pyruvate; between glutamate and oxaloacetate.

6. Write the equation of the reactions of oxidative deamination of alanine (direct and indirect).

7. Write the equation of decarboxylation reactions of aspartate and glutamate.

8. Give a diagram of the ornithine cycle and specify the enzymes that catalyze its reactions.

Tasks

Nucleic acids are high–molecular organic compounds, biopolymers, whose monomers are nucleotides. They are present in the cells of all living organisms and perform the most important functions of storing, transmitting and implementing hereditary information. There are 2 types of nucleic acids – DNA (deoxyribonucleic acid) and RNA (ribonucleic acid). The difference in the names is explained by the fact that the DNA molecule contains the five–carbon saccharide deoxyribose, and the RNA molecule contains ribose. Currently, a large number of varieties of DNA and RNA are known, differing from each other in structure and significance in metabolism.

Nucleotides are complex substances, each of them includes a nitric base, a five-carbon sugar (ribose or deoxyribose) and a phosphoric acid residue. There are five main nitrogenous bases: adenine, guanine, uracil, thymine and cytosine. The names of nucleotides come from the names of the corresponding nitrogenous bases; both are indicated by capital letters: adenine – adenylate (A), guanine – guanylate (G), cytosine – cytidylate (C), thymine – thymidylate (T), uracil – uridylate (U).

The DNA molecule consists of two polynucleotide chains spirally twisted around each other. The nucleotides of the DNA molecule include four types of nitrogenous bases: adenine, guanine, thymine and cytosine.

The DNA polynucleotide chain is twisted in a spiral like a ladder and is connected to another, complementary chain with the help of hydrogen bonds formed between adenine and thymine, as well as guanine and cytosine. Nucleotides A and T, G and C are called complementary. As a result, in every organism, the number of adenyl nucleotides is equal to the number of thymidyl nucleotides, and the number of guanyl nucleotides is equal to the number of cytidyl nucleotides. This pattern has been called the "Chargaff’s rule". Due to this property, the sequence of nucleotides in one chain determines their sequence in another.

The structure of RNA molecules is in many ways similar to the structure of DNA molecules. However, there are a number of significant differences. In the RNA molecule, instead of deoxyribose, ribose is included in the nucleotides, instead of thymidyl nucleotide

(T) there is uridyl (U). The main difference from DNA is that the RNA molecule is a single chain.

Some parameters of DNA and protein molecules:

One pitch is a full turn of the DNA helix – a 360^0 turn
One pitch consists of 10 pairs of nucleotides
One pitch is 3.4 nm in length
The distance between two nucleotides is 0.34 nm.
The molecular weight of one nucleotide is 345 g/mol
In the DNA molecule: A+G=T+C (Chargaff's rule)
Complementarity of nucleotides: A=T; G=C
DNA chains are held by hydrogen bonds, which are formed between complementary nitrogenous bases: adenine and thymine are connected by 2 hydrogen bonds, and guanine with cytosine by three bonds.

Examples of problem solutions
1. A fragment of a DNA molecule consists of 3000 nucleotides, 650 cytidyl nucleotides out of them. Determine the length of this fragment and the number of adenyl, thymidyl and guanyl nucleotides.

Solution:
Using the Chargaff rule (A = T; G = C), we determine the number of adenyl, thymidyl and guanyl nucleotides:
C = G = 650
A + T = 3000 – (G + C) = 3000 – 1300 = 1700 nucleotides
A = T = 1700:2 = 850
Determine the length of this DNA fragment. 3000 nucleotides are contained in a double-stranded DNA molecule, therefore, in one DNA chain there are 1500 nucleotides. The distance between the two nucleotides is 0.34 nm, hence:
1500 x 0,34 nm = 510 nm.
Answer: A = 850; T = 850; G = 650.
The length of this DNA fragment = 510 nm.
The length of the DNA molecule section is 272 nm, there are 31% of adenylic nucleotides in the molecule.

Determine the molecular weight of the molecule, the percentage of other nucleotides.

Solution:

Knowing the distance between two neighboring nucleotides and the length of a given DNA fragment, we calculate the number of nucleotides in one DNA chain:

272 : 0,34 = 800 nucleotides. Consequently, the two chains of the DNA section contain 1600 nucleotides.

Knowing the molecular weight of one nucleotide, we calculate the molecular weight of this DNA fragment:

x 345 g/mol = 552000 g/mol.

Using the Chargaff's rule, where A = T and G = C, we determine the percentage of nucleotides:

A = T = 31%

G + C = 100% - (A + T) = 100% - 62% = 38%

G = C = 38% : 2 = 19%

Answer: The molecular weight of the DNA molecule = 552000 g/mol; T = 31%, G = 19%, C = 19%.

A section of the DNA chain has a sequence of AGCGTTACGTAG nucleotides. Determine the sequence of t-RNA anticodons.

Solution:

T-RNA anticodons correspond to mRNA codons. We find out the sequence of nucleotides in mRNA using the law of complementarity:

DNA A G C G T T A C G T A G

mRNA U C G C A A U G C A U C

Determine the anticodons of t-RNA:

mRNA U C G C A A U G C A U C

t-RNA A G C G U U A C G U A G

Answer: the sequence of anticodons in t-RNA is AGCGUUACGUAG.

Determine the number of hydrogen bonds in the DNA fragment: GTCATGGATAGTCCTAT.

Solution:

As is known, in a double–stranded DNA molecule, there are two hydrogen bonds between complementary A – T, and three bonds between G – C.

In this DNA chain: GTCATGGATAGTCCTAT, there are 10 pairs of A – T (T – A) and 7 pairs of C – G (G – C). Count the number of hydrogen bonds in a given DNA molecule:

(10 x 2) + (7 x 3) = 41 hydrogen bonds.

Answer: 41 hydrogen bonding.

The DNA molecule consists of 3500 nucleotides. Determine the number of complete helical turns in a given molecule.

Solution:

In the problem statement, this DNA molecule contains 3500 nucleotides, which means 1750 pairs. The complete helix turn contains 10 pairs of nucleotides.

1750 : 10 = 175 complete turns.

Answer: This DNA molecule contains 175 complete helix turn.

Independent tasks:

1. A fragment of a DNA molecule consists of 5250 nucleotides. Determine the length of this DNA fragment.
2. A fragment of a DNA molecule consists of 468 pairs of nucleotides. Determine the length of this DNA fragment.
3. A fragment of a DNA molecule consists of 640 pairs of nucleotides, 325 are thymidyl nucleotides out of them. Determine the length of this fragment and the number of adenylic, guanylic and cytidyl nucleotides.
4. The DNA molecule consists of 860 pairs of nucleotides. Determine the number of complete helix turns in a given molecule.
5. The DNA molecule consists of 1660 nucleotides. Determine the number of complete helix turns in a given molecule.
6. The length of the segment of the DNA molecule is 850 nm. Determine the number of nucleotides in one DNA chain.
7. The length of the segment of the DNA molecule is 544 nm. Determine the number of nucleotides in the DNA.
8. The DNA molecule contains 35% of guanyl nucleotides. Determine the number of cytidyl nucleotides.
9. The DNA molecule contains 28% of thymidyl nucleotides. Determine the number of guanyl nucleotides.
10. The DNA molecule contains 17% of guanyl nucleotides. Determine the number of adenyl, cytidyl, thymidyl nucleotides.
11. A fragment of a DNA molecule consists of 2000 nucleotides, 24% out of them are adenylic nucleotides. Determine the number of guanyl, thymidyl and cytidyl nucleotides.
12. Determine the molecular weight of a DNA fragment if it consists of 630 nucleotides.
13. A fragment of a DNA molecule contains 220 adenylic nucleotides, which is 20% of the total number of nucleotides. Determine how many guanyl, thymidyl, cytidyl nucleotides are in this fragment and its molecular weight.
14. The length of the DNA molecule fragment is 544 nm. Determine the number of nucleotides in DNA and its molecular weight.
15. The length of the DNA molecule fragment is 245.48 nm, thymidyl nucleotides in the molecule are 12%. Determine the molecular weight of the molecule, the percentage of other nucleotides.

16. 880 guanyl nucleotides were found in the DNA molecule, which make up 22% of the total number of nucleotides in this DNA. Determine how many other nucleotides are in this DNA and what is the length of this fragment?

17. A DNA molecule with a relative molecular weight of 69,000 is given, of which 8625 are adenylic nucleotides. Find the number of all the nucleotides in this DNA. Determine the length of this fragment.

18. The length of the DNA molecule section is 68 nm, adenylic nucleotides in the molecule are 15%. Determine the molecular weight of the molecule, the percentage of other nucleotides and the number of hydrogen bonds in the DNA section.

19. The molecular weight of the DNA molecule is 17250 g/mol. Determine the number of nucleotides in the molecule and its length.

20. The molecular weight of the DNA molecule is 20700 g / mol, the adenylic nucleotide in it is 25%. Determine the number of other nucleotides in the molecule and its length.

21. A fragment of the coding DNA chain has the following sequence: TGAACTGAGGTCGAC. Determine the sequence of nucleotides of the mRNA transcribed from this fragment.

22. A fragment of the coding DNA chain has the following sequence: AGACTTAGCTCAGTC. Restore the second DNA chain and determine the sequence of nucleotides of mRNA transcribed from this fragment.

23. The mRNA fragment has the following sequence of nucleotides: UGAGCAUCAGACUGU. Determine the sequence of nucleotides of the fragment of the DNA molecule from which this fragment of mRNA is transcribed.

24. The fragment of mRNA has the following sequence of nucleotides: UAUCGAGUCACGC. Determine the sequence of nucleotides and the number of hydrogen bonds in the fragment of the DNA molecule from which this fragment of mRNA is transcribed.

25. The mRNA fragment has the following sequence of nucleotides: UAUGACUAGCAG. Determine the sequence of t-RNA anticodons corresponding to the mRNA codons.

26. A section of the DNA chain has a sequence of nucleotides: ACGATCTTAGCT. Determine the sequence of t-RNA anticodons.

27. The sequence of anticodons of t-RNA: AUG GCG UAU GUC. Determine the sequence of nucleotides of a DNA fragment that corresponds to t-RNA.

28. The section of the mRNA molecule consists of 420 nucleotides. Determine its length.

29. The section of the mRNA molecule consists of 222 nucleotides. Determine its length and molecular weight.

30. The length of the section of the mRNA molecule is 510 nm. Determine the number of nucleotides contained in this section of the molecule.

31. The mRNA molecule contains 19% of uracyl nucleotides, how many adenylic nucleotides are contained in the coding chain of the DNA section?

32. If the chain of the DNA molecule, from which the genetic information was transcribed, contained 18% adenylic nucleotides, how many uracyl nucleotides will be contained in the corresponding segment of mRNA?

33. The right DNA chain has the following structure ATGGTCATC. Determine the structure of the mRNA transcription that occurred from the left DNA chain.

34. The mRNA molecule contains 14% adenylic, 26% guanylic and 40% uracyl nucleotides. Determine the ratio of all types of nucleotides in the DNA from which this mRNA was transcribed.

35. The molecular weight of the DNA gene is 103500 g/mol. Determine the number of nucleotides in the mRNA transcribed from this gene.

Protein biosynthesis

Proteins are high–molecular compounds, biopolymers, whose monomers are amino acids. 20 amino acids are involved in the creation of proteins. They bind to each other in long chains that form the basis of a protein molecule of large molecular weight.

The genetic code is a unified system for recording hereditary information in nucleic acid molecules in the form of a sequence of nucleotides. Basic properties of the genetic code:

Tripletness. A triplet (codon) is a sequence of three nucleotides encoding one amino acid.

The redundancy (degeneracy) of the code is a consequence of its triplet nature and means that one amino acid can be encoded by several triplets (since there are 20 amino acids and 64 triplets)

Simultaneously with redundancy, the code has the property of unambiguity, which means that only one specific amino acid corresponds to each codon.

The code is collinear, i.e. the sequence of nucleotides in a gene exactly corresponds to the sequence of amino acids in a protein.

The genetic code is nonoverlapping and compact, i.e. one nucleotide belongs to only one.

The genetic code is universal, i.e. the nuclear genes of all organisms encode information about proteins in the same way, regardless of the level of organization and systematic position of these organisms.

Some protein parameters:

On average, one protein contains 400 amino acids

One amino acid encodes three (triplet) nucleotides.

The molecular weight of one amino acid is 100 g/mol

To determine the amino acid composition of a protein, a table of the genetic code is used:

Examples of problem solutions

A fragment of a DNA gene has the following sequence of nucleotides TCGGTCAACTTAGCT. Determine the sequence of mRNA nucleotides and amino acids in the protein polypeptide chain.

Solution:

Knowing the sequence of nucleotides in the DNA chain, we can determine the sequence of nucleotides in mRNA using the principle of complementarity:

DNA T C G G T C A A C T T A G C T

mRNA A G C C A G U U G A A U C G A

There are 5 triplets: AGC CAG UUG AAU CGA. Using the table of the genetic code, we determine the sequence of amino acids in this fragment of the DNA gene:

Ser – Gln – Phe – Asn – Arg.

Answer: mRNA: AGCCAGUUGAAUCGA; amino acid sequence: Ile – Gln – Phe – Asn – Arg.

The section of the protein molecule has the following sequence of amino acids: alanine-cysteine-valine-serine-glycine-threonine. Determine one of the possible sequences of nucleotides in the mRNA molecule.

Solution:

In this problem, the solution will not be the only correct one, because several triplets can encode one amino acid. Let us consider one of the options using the genetic code table.

GCC UGU GUG AGC GGU ACA.

Answer: GCCUGUGUGAGCGGUACA (other options are possible)

The site of the protein molecule has the following sequence of amino acids: glycine-tyrosine-arginine-alanine-cysteine. Determine one of the possible sequences of nucleotides in the DNA molecule.

Solution:

In this problem, the solution will not be the only correct one, because several triplets can encode one amino acid. Let us consider one of the options using the genetic code table.

GGA TAT CGA TCG TGC

Answer: GGA TAT CGA TCG TGC (other options are possible)

The fragment of the mRNA molecule has the following sequence of nucleotides: GCAUGUAGCAAGCGC. Determine the sequence of amino acids in the protein molecule and its molecular weight.

Solution:

According to the table of the genetic code, we determine the sequence of amino acids: Ala – Cys – Ser – Lys – Arg

Knowing the molecular weight of one amino acid, we determine the molecular weight of this protein molecule:

5 x 100 g / mol = 500 g / mol.

Answer: amino acid sequence: Ala – Cys – Ser – Lys – Arg; molecular weight of the protein molecule = 500 g/ mol.

The part of the DNA coding chain has a molecular weight of 217350g/mol. Determine the number of amino acids encoded in it.

Solution:

We determine the number of nucleotides in a given DNA chain:

217350g/mol : 345 g/mol = 630 nucleotides. 3 nucleotides encode one amino acid, hence: 630 : 3 = 210 amino acids.

Answer: 210 amino acids.

How many nucleotides does a DNA gene contain if 135 amino acids are encoded in it. What is the molecular weight of this gene and its length?

Solution:

Find out the number of nucleotides encoding 135 amino acids in mRNA: 135 x 3 = 405 nucleotides.

Calculate the molecular weight of the gene: 405 x 345 g / mol = 139725 g/ mol The length of the gene is calculated by the number of nucleotides in one DNA chain: 405 x 0.34 nm = 137.7 nm.

Answer: 405 nucleotides

molecular weight of the gene = 279450 g/mol;

gene length = 137.7 nm

A fragment of the coding DNA chain contains 3000 nucleotides, introns in it make up 50%. Determine the number of nucleotides in a mature mRNA molecule.

Solution:

An intron is a section of DNA that is part of a gene, but does not contain information about the sequence of amino acids of a protein. Therefore, if the DNA chain contains 3000 nucleotides, and 50% of their number are introns, i.e. 1500 nucleotides. mRNA consists of half as many nucleotides as DNA. Consequently, a mature mRNA molecule will contain 1500: 2 = 750 nucleotides.

Answer: 750 nucleotides.

Independent tasks:

1. A fragment of a DNA gene has the following sequence of nucleotides TTTGTCCTAACCGGA. Determine the sequence of mRNA nucleotides and amino acids in the protein polypeptide chain.
2. The section of the protein molecule has the following sequence of amino acids: glutamine-phenylalanine-leucine-tyrosine-arginine. Determine one of the possible sequences of nucleotides in the DNA molecule.
3. The section of the protein molecule has the following sequence of amino acids: proline-glutamine-valine-tryptophan. Determine the possible sequences of nucleotides in the DNA molecule.

4. The section of the protein molecule has the following sequence of amino acids: serine-glutamine-asparagine-tryptophan. Determine the possible sequences of nucleotides in the mRNA molecule.

5. The fragment of the mRNA molecule has the following sequence of nucleotides: UGCAAGCUGUUUAUA. Determine the sequence of amino acids in the protein molecule.

6. The fragment of the mRNA molecule has the following sequence of nucleotides: GAGCCAAAUACUUUA. Determine the sequence of amino acids in the protein molecule and its molecular weight.

7. The DNA gene includes 300 pairs of nucleotides. What is the length, molecular weight of the gene and how many amino acids are encoded in it?

8. The DNA gene includes 720 pairs of nucleotides. What is the length, molecular weight of the gene and how many amino acids are encoded in it?

9. The DNA fragment has a molecular weight of 414,000 g/mol. Determine the length of the DNA fragment and the number of amino acids encoded in it.

10. The part of the DNA coding chain has a molecular weight of 182160g/mol. Determine the number of amino acids encoded in it.

11. The right chain of DNA has the following sequence of nucleotides: CTATAGTAACAA. Determine the structure of the protein fragment synthesized along the left DNA chain.

12. The left chain of DNA has the following sequence of nucleotides: TGGAAGCTCTAT. Determine the structure of the protein fragment synthesized along the right DNA chain.

13. A fragment of one DNA chain has the following structure: GGTACGATGTCAAGA. Determine the primary structure of the protein encoded in this chain, the number (%) of different types of nucleotides in the two chains of the fragment and its length.

14. How many nucleotides does a DNA gene contain if 111 amino acids are encoded in it. What is the molecular weight of this gene and its length?

15. What is the molecular weight of a gene and its length, if a protein with a molecular weight of 42,000 g/mol is encoded in it?

16. 145 t-RNA molecules took part in the synthesis of the protein molecule. Determine the number of nucleotides in the mRNA, the

DNA gene and the number of amino acids in the synthesized protein molecule.

17. The fragment of the mRNA chain has the following sequence: GGGUGGUAUCCCAACUGU. Determine the sequence of nucleotides on DNA, anti-codons of t-RNA, and the sequence of amino acids corresponding to a fragment of the DNA gene.

18. A fragment of a DNA chain has the following sequence: GGTACGATGTCAAGA. Determine the sequence of nucleotides on mRNA, anti-codons of t-RNA, and the sequence of amino acids corresponding to a fragment of the DNA gene

19. t-RNA molecules with anticodons CAG, UAA, CCA, GGG, CUA took part in protein synthesis. Determine the nucleotide sequence in the DNA gene fragment and the sequence of amino acids in the section of the synthesized protein.

20. t-RNA molecules with anticodons GUC, CGU, UUC, GAU, AUG took part in protein synthesis. Determine the nucleotide sequence in the DNA gene fragment, the sequence of amino acids in the section of the synthesized protein and the number of nucleotides containing thymine, adenine, guanine and cytosine in the DNA fragment.

21. A fragment of the coding DNA chain contains 6000 nucleotides, introns in it make up 40%. Determine the number of nucleotides in a mature mRNA molecule.

22. The coding chain of DNA has a sequence of nucleotides: TAGCGTTTCTCGGTA. How will the structure of the protein molecule change if there is a doubling of the sixth nucleotide in the DNA chain. Explain the results.

23. The coding chain of DNA has a sequence of nucleotides: AGATAGGTACGTTCG. How will the structure of the protein molecule change if the tenth nucleotide in the DNA chain falls out? Explain the results.

24. During the replication of the DNA molecule on the coding chain: TTCAGACTCTAAGAT, a doubling of the fourth triplet occurred. Explain how the structure of the protein molecule will change.

25. Under the influence of mutagenic factors, the seventh nucleotide was replaced by an adenylic one in the fragment of the gene: GACCAGATTCAGCTA. Explain how the structure of the protein molecule will change.

Answers to independent tasks.
Nucleic acids.
892.5 nm
159, 12 nm
A = 325
G = 315
C = 315
217.6 nm.
86 complete helical turns.
83 complete helical turns.
2,500 nucleotides.
3,200 nucleotides.
C = 35%.
T = 22%.
C = 17%
A = 33%
T = 33%
T = 480 nucleotides
G = 520 nucleotides
C = 520 nucleotides.
217 350g/mol.
379 500g/mol
T = 220 nucleotides
C = 330 nucleotides
G = 330 nucleotides.
3,200 nucleotides
1 104 000g/mol
498 180g/mol
A = 12%
G = 38%
C = 38%
C = 22% = 880 nucleotides
A = 38% = 1200 nucleotides
T = 38% = 1200 nucleotides.
680 nm
T = 25 nucleotides
G = 75 nucleotides
C = 75 nucleotides
34 nm

T = 60 nucleotides
G = 140 nucleotides
C = 140 nucleotides
540 hydrogen bonds.
50 nucleotides
8.5 nm
T = 15 nucleotides
G = 15 nucleotides
C = 15 nucleotides
10, 2 nm
ACUUGA CUCCAGCUG mRNA
TCTGAATCGAGTCAG DNA
UCUGAAUCGAGUCAG mRNA
ACTCGTAGTCTGACA. DNA
ATAGCTCAGTGCG. DNA
33 hydrogen bonds
AUACUGAUCGUC t-RNA
ACGAUCUUGCGU t-RNA
ATGGCGTATGTC DNA
142, 8 nm
75, 48 nm
76 590 g/mol
1 500 nucleotides
A = 19%
U = 18%
UACCAGUAG.
A = 27%
T = 27%
G = 23%
C = 23%
150 nucleotides

Protein biosynthesis.

AAACAGGAUUGGCCU
lys-glu-asn-tyr-pro
GTTAAAGACATGGCT (other options are possible)

pro	gln	val	trp
GGA	GGT	CAA	ACC
GGG	GTC	CAG	
GGT		CAT	
GGC		CAC	

ser	gln	asn	trp
UCU	CAA	AAU	UGG
UCC	CAG	AAC	
UCA			
UCG			

Cys – lys – leu – phe – ile
glu – pro – asn – thr – leu
102 nm
207000 g/mol
100 amino acids
244 nm
496800 g/mol
240 amino acids
204 nm
200 amino acids
176 amino acids
Leu-Cys-Tyr-Gln.
Trp-Lys-Leu-Tyr
Pro-Cys- Tyr-Ser-Ser
G = 23%; C = 23%; A = 22%; T = 22%
5,1 nm
279450 g/mol
137, 2 nm
869400 g/mol
428, 4 nm
mRNA – 435 nucleotides

DNA – 870 nucleotides
t-RNA – 145 nucleotides

DNA	CCC	ACC	ATA	GGG	TTT	ACA
t-RNA	CCC	ACC	AUA	GGG	UUU	ACA
protein	gly	trp	tyr	pro	lys	cys

mRNA	CCA	UGC	UAC	AGU	UCU
t-RNA	GGU	ACG	AUG	UCA	AGA
protein	pro	cys	tyr	ser	ser

19.

DNA	CAG	TAA	CCA	GGG	CTA
protein	val	ile	gly	pro	asp

20.

DNA	GTC	CGT	TTC	GAT	ATG
protein	gln	ala	lys	leu	tyr

3600 nucleotides

normal protein – ile – ala – lys – ser – gln
altered protein – ile – ala – lys – ser – pro
normal protein – ser – ile – gys – ala – ser
altered protein – ser – ile – gys – gln and it will decrease by one amino acid
will increase by one amino acid – leu
the third amino acid tyrosine will be replaced by valine

Glossary

Activator - 1. A substance that stimulates the transcription of a specific gene or operon. 2. A protein that binds to the operator and accelerates transcription.

Anticodon - a triplet of nucleotides in a tRNA molecule complementary to nucleotides of a specific codon in an mRNA molecule.

cDNA Library - Collection of cDNA clones synthesized in vitro on mRNA templates originating from a single tissue or cell population.

Binary vector system - two-plasmid Agrobacterium system, designed to transfer the T-DNA region carrying cloned genes to plant cells. Virulence genes are localized on one plasmid, and the embedded T-DNA region is localized on another.

Vector - a self-replicating DNA molecule (for example, a bacterial plasmid) used in genetic engineering to transfer genes from a donor organism to a recipient organism, as well as to clone nucleotide sequences.

Vir genes - are a group of Ti plasmids genes that ensure the transfer of T-DNA into a plant cell.

Gene - transcribed portion of a chromosome encoding a functional protein either tRNA or rRNA.

A genetic code - a system for recording genetic information in the form of a nucleotide sequence, in which every three nucleotides that make up the codon, encode one amino acid. It consists of 64 codons encoding all 20 amino acids.

The regulator gene - a gene encoding a repressor protein that binds to an operator and regulates the transcription of "its" operon.

Derepression - induction of gene transcription as a result of suppression of repressor functions - blocking its binding to the promoter.

Inducer - a small molecule that binds to the regulatory repressor protein, which leads to derepression of the corresponding genes.

Intron - a transcribed region of the gene that does not contain codons and is excised from the primary transcript during processing to form a functional (mature) RNA.

Codon - A sequence of three adjacent nucleotides that encode a specific amino acid. In total, there are 64 combinations of nucleotides in codons; 61 of them encode 20 amino acids, 3 are nonsense (stop) – codons.

Complementary DNA, cDNA - a DNA molecule synthesized on an

RNA template with the participation of RNA-dependent RNA polymerase (RdRp) (reverse transcriptase, revertase).

Ligation - the joining of two DNA molecules using phosphodiester bonds. In vitro catalyzed by the enzyme DNA ligase.

Sticky ends - mutually complementary single-stranded DNA sections protruding at the ends of a double-stranded molecule; formed as a result of staggered cuts of double-stranded DNA.

Messenger RNA, mRNA - a RNA molecule that contains information about the amino acid sequence of a certain protein molecule.

Mutation - spontaneous or induced change in gene structure.

A frameshift mutation - a genetic mutation caused by indels (insertions or deletions) of one or a number of nucleotides in a DNA sequence that is not divisible by three. Leads to a violation of the triplet code and the synthesis of a completely different protein (unless the synthesis is blocked at all).

Negative regulation - a type of regulation in which gene transcription is suppressed by a regulatory protein (repressor); accordingly, when the regulator protein is inactivated, the structural genes remain in the active state.

Reverse transcriptase (revertase) - A RIC-dependent DNA polymerase using a RNA molecule as a template for the synthesis of a complementary DNA strand.

Operator - a section of DNA directly adjacent to the structural gene and regulating its transcription with the participation of a repressor or activator.

Operon - a section of DNA containing several structural genes transcribed to form a single polycistronic mRNA.

Peptide - a short chain of amino acids connected by peptide bonds.

A peptide bond - a covalent bond between a free carboxyl group at the o—carbon atom of one amino acid and a free carboxyl group at the same atom of an adjacent amino acid in a polypeptide chain.

Plasmid - an extrachromosomal genetic element capable of long-term autonomous existence and replication. This is usually a double-stranded ring DNA with a length of 1-200 kbp.

Positive regulation - a type of regulation in which a regulated gene is transcribed only in the presence of an activator protein.

Post-translational modifications - a change in the structure of protein molecules after the completion of their synthesis by

ribosomes. Such modifications include: phosphorylation, glycosylation, oxidation of cysteine, cleavage of signal sequences, etc.

Processing - a set of processes for the formation of mature RNA molecules and proteins in a cell. Includes a series of sequential cleavages of the precursor molecule by endonuclease or proteinases.

Replication - the process of self-replication (synthesis) of DNA.

Repression - one of two alternative (along with induction) mechanisms for gene regulation. Consists of inhibiting transcription or translation by binding a repressor protein to an operator.

Restriction endonuclease - a bacterial enzyme that cleaves a double-stranded DNA molecule at specific sites.

Restriction map - diagram of the restriction recognition sites located on the DNA molecule.

Ribosome - a cellular organelle, a ribonucleoprotein unit, with the participation of which protein synthesis (translation) is carried out. Consists of two subunits, large and small.

Ribonucleic acid, RNA - is a nucleic acid consisting of ribonucleotides in which ribose is a sugar and uracil is one of the pyrimidines (instead of thymine).

RNA polymerase - an enzyme that synthesizes RNA from ribonucleoside triphosphates. The template can be DNA or RNA, the corresponding RNA polymerases are called DNA or RNA-dependent.

Splicing - cutting out introns from the mRNA precursor and covalent connection of exons to form mature mRNA molecules.

T-DNA - a fragment of Ti-plasmid that is inserted into the nuclear DNA of the host cell and is stably inherited by it. Causes tumour formation in plants (crown gall).

Transgenic organism - an organism whose genome contains foreign genetic material using genetic engineering methods.

Transcription - a RNA synthesis process catalyzed by RNA polymerase, which uses one of the DNA strands as a template.

Ti plasmid - a plasmid of the soil bacterium Agrobacterium tumefaciens, the T-region of which is able to be included in the nuclear DNA of the host cell, which leads to the formation of tumors.

Exon - is a section of the gene that is part of the primary transcript that remains in it after processing (cutting out introns). Together with other exons, it forms a mature mRNA.

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