



STRUCTURE OF PROTEINS AND APPLICATION IN THE FIELD OF BIOTECHNOLOGY

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ABSTRACT

In order to study the chemical structure of proteins, the possibilities of using them on an industrial scale with the help of methods of biological activity determination and methods of protein engineering were studied. On the basis of biotechnological approaches, scientific research is being conducted on the creation of modern technology using proteins in industry. Proteins are not only one of the foundations of modern biotechnology, but the application of developing physico-chemical methods is becoming a topical area today.

To determine the priorities for the development of biotechnology and the improvement of the country's biological safety system, to ensure the integration of science, education and production in these areas, in order to develop the economy and social sphere on the basis of advanced biotechnologies, as well as to consistently implement the tasks defined in the state program for the implementation of the Development Strategy in the seven priority directions of the Development Strategy of New Uzbekistan in 2022-2026:

organizing the implementation of a unified state policy in the field of ensuring the country's economic and biological security;

preparation, expert evaluation and review of recommendations and proposals aimed at protecting the population from the effects of dangerous biological factors and protecting the environment, preventing biological risks, creating and developing a biological risk monitoring system;

development of effective measures for the practical implementation of measures aimed at ensuring the biological safety of the country;

to ensure mutual cooperation of ministries, agencies, local state authorities, scientific research and educational institutions, civil society institutions in the performance of targeted tasks to ensure biological security of the country;



In accordance with the Decree of the President of the Republic of Uzbekistan dated November 25, 2020 No. PQ-4899 "On comprehensive measures to develop biotechnologies and improve the system of ensuring biological safety of the country"

ISO 20387:2018 "Biotechnology. Storage of biological samples. General requirements for storage", ISO 20391-1:2018

Biotechnology - Cell counting - Part 1: General guidelines for cell counting methods", ISO 20391-2:2019 "Biotechnology - Cell counting - Part 2: Experimental design and statistical analysis to determine the parameters of the counting method" ,

ISO 20395:2019 "Biotechnology - Performance evaluation of quantitative methods for nucleic acid target sequences. General requirements", ISO 20688-1:2020 "Biotechnology - nucleic acid synthesis - Part 1: Requirements for production and quality control of synthesized oligonucleotides General requirements" standards have been approved.

Ensuring the implementation of the provisions of the Convention on the prohibition of the development, production and stockpiling of bacteriological (biological) and toxic weapons and their elimination;

discussion of current issues and problems, including among the public, that are of significant national importance and arise during the implementation of measures to ensure the country's biological security.

In order to comply with international standards and modern requirements, to improve the procedure for the storage, use, and delivery of strains of microorganisms and viruses, toxins, and natural and plant-derived poisons used for veterinary procedures.

Protein structure, function and engineering is a science that studies the chemical structure of living matter and its role in metabolism. The structure, function and engineering of proteins is the bridge between biochemistry, biology and chemistry, and this field is also called the chemistry of life.

Vital processes in living beings are in constant contact with the external environment and occur as a result of energy exchange.

The main task of this science is to study the basic laws of biochemical processes, the structure and functions of biomolecules involved in them, and the exchange of substances, including their interrelated activities.

Despite the difference from individual chemical processes, it is a biochemical unit that unites species. Such a scientific view is called a general biochemical sphere.

Given the importance of protein in living nature. as well as the fact that protein makes up half of the body's mass and has a number of remarkable properties to understand the structure and function of proteins, to be the basis for solving important problems for biology and medicine, it is necessary to study the course of biochemistry in medical institutions - starting with this class. organic substances.

Proteins perform various and many functions typical for living organisms, some of which we will get to know during the course.

In 1938, the Swedish chemist I. Ya. Bcrsclius isolated a nitrogen-containing organic compound from plant and animal tissues and named it proleintar (Greek protos means primary, important).



In current literature, nitrogen-containing high-molecular compounds are called proteins. The term protein is based on the fact that egg whites turn white when heated.

Proteins are the main part of the tissue of any living organism and are important in various processes that take place in the tissue. Proteins are the basis of both structure and function of living organisms.

According to F. Crick, one of the founders of molecular biology, proteins are very important substances that can perform various functions very easily and delicately.

There are approximately 10,000,000 different proteins in nature, which support the functioning of 10,000 different living organisms, from viruses to humans. Today, the composition and structure of very few of the large number of naturally occurring proteins is clear

Each organism is characterized by its own set of proteins. The variety of phenotypic signs and functions is due to the specificity of this protein, in most cases it has a multimolecular structure.

Each organism is characterized by its own set of proteins. The variety of phenolipic characters and functions is determined by the specificity of this protein, in most cases it has a multimolecular structure.

There are about 3,000 different proteins in the E.coli cell, and more than 100,000 in the human body. All natural proteins are composed of a few simple building blocks, the monomeric molecules of which are amino acids.

Amino acids are linked together in a polypeptide chain. Natural proteins are composed of 20 different amino acids. These amino acids can be connected in different sequences

Therefore, they produce a large number of different proteins. Various isomers can be formed by placing the specified number of amino acids in a polypeptide in different ways

If only 2 isomers can be formed from 2 amino acids, theoretically 24 isomers can be formed from 4 amino acids.

DNA. sequence of nucleotides in the molecule determines the sequence of amino acid residues in the polypeptide chain of the synthesized protein. The resulting polypeptide chain has functional information and, accordingly, has a stable tertiary structure.

Protein is up to 25% of the mass of the human body, and after drying it is 45-50%. The amount of protein in different organs and tissues is different (Table 1). Protein performs the following functions in human and animal organisms:

Structural function - all tissues, cells and organoids are made of protein. Fibrillar proteins (collagen, keratin, elastin, etc.) play an important role here.

Catalytic function - biocatalysts in the body - enzymes have a protein nature and control the occurrence of all biochemical reactions, that is, they allow the speed of reactions to proceed in a certain order and be controlled.

Energetic function - proteins are broken down in the gastrointestinal tract and absorbed in the form of simple amino acids. A certain part of amino acids is oxidized and generates energy.

Transport task. Proteins are well soluble in water and blood and form complexes with substances insoluble in water and blood to ensure the solubility and transport of the protein.



For example: blood plasma protein, albumin, fatty* acids, lipids, other proteins transport iron, copper, vitamins, hormones to target organs.

Contractile function - actin, myosin, troponins, which are part of muscle proteins, have the ability to contract.

These proteins enter the muscles and take part in mechanical work. The contraction function is also characteristic of cytoskeletal proteins, which ensure the processes of cell life (mitosis, chromosome separation).

Physico-chemical properties of proteins

	Amount of proteins, % Amount of proteins, %		Amount of proteins, % Amount of proteins, %		
	Dry by weight imbaian	In the body of protein General from the amount	Organs and tissues	Dry by weight imbaian	In the body of protein General from the amount
Organs and tissues					

High viscosity, low diffusion, swelling ability,

optical activity, movement in an electric field, low osmotic pressure and high oncotic pressure, physico-chemical properties such as absorption of light at 280 nm.

Since proteins have free NH²- and COOH-groups, they are amphoteric like amino acids. All the properties of acid and base are characteristic for them

Depending on the pH of the environment, the ratio of acidic and basic amino acids, proteins in solutions have a negative or positive charge and move toward the anode or cathode. This property is used to separate proteins by electrophoresis. Proteins also have hydrophilic properties.

Molecular mass of proteins

Proteins are high-molecular biopolymers with a molecular mass ranging from 6,000 to several million, depending on the number of polypeptide chains in the protein structure.

Serum albumin	69000
Serum globulin	176000
Human fibrinogen	450000
Actomyosin	5000000

The mass unit dalton (Da) corresponds to the atomic mass of hydrogen (1.0000). The mass unit kilodalton (kDa) corresponds to 1000 daltons. Most proteins have a mass of 10 to 100 kilodaltons.

The amino acid composition and sequence of several thousand proteins have been determined. Therefore, the molecular weight of the compound can be found with high accuracy.

Determining the molecular weight of proteins by the method of sedimentation analysis is carried out in ultracentrifuges, where it is possible to create a centrifugal acceleration (g) of more than 200,000, i.e. higher than the force of gravity.



Usually, the molecular weight is calculated according to the sedimentation rate or sedimentation equilibrium of the protein molecule. During the movement of molecules from the center to the periphery, a sharp boundary is formed between the solvent and the protein (automatically detected). The optical properties of the solvent and protein are used to determine the sedimentation rate, and it is determined by the sedimentation constant S , which depends on the mass and shape of the protein particle:

The shape of protein molecules is determined by ultracentrifugation, X-ray structural analysis or electron microscopy. Analysis showed that protein molecules are asymmetric substances in all three dimensions. The charge of amino acids can be neutralized in a certain pH environment. This pH is called the isoelectric point of amino acids.

Isoelectric points of some proteins.

The name of the protein	pH indicator	The name of the protein	pH indicator
Pepsin	1	Myoglobin	6,8
Egg albumin	4,6	Chymotrypsin	8,1
β -lactoglobulin	5,2	Ribonuclease	9,45
γ -lobulin	5,2	Chymotrypsinogen	9,5
Phosphorylase	5,8	Lysozyme	10,5
Hemoglobin	6,6	Cytochrome S	10,7

So, at a certain pH value of the solution, the total charge of the protein molecule can remain neutral. This electroneutral state is the isoelectric state of the protein, and this pH value is called the isoelectric point (IEN) of the protein.

Proteins are unstable at the isoelectric point and easily precipitate. But the protein does not settle by itself at the isoelectric point. For this, it is necessary to remove the water shell surrounding the protein molecule. Only then the protein molecules stick together, grow larger and slowly settle out of the solution.

Natural proteins are divided into 2 groups depending on the shape of the molecule: globular and fibrillar. The fibrillar protein molecule is thread-like, and its length is 100 times greater than its diameter.

A globular protein molecule has a spherical shape, its length is 3-10 times greater than its diameter. For example: the elastin protein molecule has an oval shape with a diameter of 70 nm, a hemoglobin protein has a slightly elongated shape with a diameter of 220 nm, a myosin molecule has a diameter of 100 nm and a length of one thousand angstroms. Thus, myosin protein is fibrous.

Fibrillar and globular proteins have different properties. Some are soluble in water and salt solutions. Most fibrillar proteins are insoluble in water. Fibrillar proteins include myosin, silk, fibrinogen, collagen and elastin.

Since proteins are optically active substances, they bend the plane of polarized light by a certain angle. Protein solutions have the ability to refract, scatter, and absorb ultraviolet rays.

This physical property of proteins can be used to determine their quantity, molecular mass and other parameters.



Due to the high molecular mass of protein, it forms colloidal solutions when dissolved. When proteins are dissolved in water, polar molecules of water are placed opposite to the protein charge and form a water shell

Since the diameter of protein particles in water is greater than $0.001\ \mu\text{m}$, a colloidal solution is formed and has the property of light scattering (Tyndal effect). Protein molecules cannot pass through the small pores of animal and human membranes.

By using this property of protein, it is possible to purify it from small molecular substances with the help of semiconducting membranes. This method is called dialysis.

Due to the presence of $-\text{NH}_2$ and $-\text{COOH}$ groups in the protein molecule, it exhibits amphoteric properties. In the protein molecule, the free carboxyl group is acidic, and the amino group is basic. It can be expressed as follows:

Russian scientist A.Ya.Danilevsky and German biochemist E.Fisher and others found out that the amino acids in the protein molecule combine to form a polypeptide with the help of mutual peptide bond ($-\text{CO}-\text{NH}-$). Based on this, a dipeptide is formed from two amino acid molecules.

Free amino- or carboxyl groups in the dipeptide molecule are attached to the third amino acid molecule to form a tripeptide:

Another amino acid molecule is added to the tripeptide to form a tetrapeptide, and the fifth amino acid to form a pentapeptide. In this way, hexapeptides and polypeptides can be formed.

Many hypotheses have been proposed about the structure of the protein molecule. Most of these biochemists accepted the polypeptide theory.

Polypeptide theory was the first, in 1902, Russian scientist A.YA. Created by Danilevsky and German scientist E. Fischer. According to this theory, the amino acids in the protein molecule form a polypeptide chain by joining together with the help of a mutual peptide bond

Amino acid residues contain free amino, carboxyl, hydroxyl, phenol, amide and other groups. Therefore, these groups have a great influence on the spatial configuration (formation) of polypeptide chains.

Disruption (loss) of natural properties of proteins (solubility, electrophoresis, enzymatic, hormonal, immunoactivity) as a result of various physical and chemical effects is called denaturation.

As a result of denaturation, the spatial conformation of the protein molecule, i.e. the secondary, tertiary and quaternary structure is disturbed, but the primary structure remains. As a result of denaturation, the peptide chain of the protein is not broken, mainly disulfide and hydrogen bonds are broken.

Denaturation is divided into two types according to its direction: reversible and irreversible.

Protein is extracted from animal tissues and macroorganisms using special methods.

The extraction method is used to extract protein from the homogenate obtained by the above methods.

The obtained homogenate is dissolved in 8-10% salt solution. Buffer solutions with a certain pH, organic solvents and non-ionic detergents are often used for protein extraction. For this purpose, solutions of organic substances, which have been used for a long time -



glycerin solution in water, sucrose solution, citric acid and borate buffer mixtures, tris-buffer solutions are used.

After protein extraction, the extract is purified from tissue elements by centrifugation, and the protein that has passed into the solution is separated by fractionation.

Currently, proteins are separated into fractions by the following methods: precipitation under the influence of salts, denaturation under the influence of heat, precipitation using organic solvents, chromatography, gel filtration, electrophoresis, ultracentrifugation methods.

The protein mixture is separated into fractions using ion exchange, adsorption chromatography, gel filtration, and affinity chromatography.

In this method, activated carbon and aluminum oxide are used as adsorbents. The adsorbent is placed in the column, the solvent is poured and the protein solution is added, in which the protein binds with the adsorbent. Then, protein fractions are isolated using buffer solutions with different pH.

Separation of proteins into fractions is done using the method of partition chromatography. Partition chromatography is a variant of adsorption chromatography, and chromatography paper, starch, silica gel, etc. are used as adsorbents.

Various gels are used in this method, for example: sephadex of various brands, made of dextran, dextran is a polymeric substance consisting of high molecular weight glucose residues, which, when reacted with epichlorhydrin in an alkaline medium, forms a gel. Water-soluble monomer to form polyacrylamide gel. Currently, proteins are divided into the following groups depending on their biological function:

Catalytic function. All biological catalysts - enzymes - have protein nature. Currently, more than 2100 enzymes are known. This function of proteins is unique and determines the speed of chemical reactions in biological systems.

Feed (reserve) function. This function is performed by reserve proteins, which are a source of nutrients for the development of the fetus, for example, albumin is an example of this.

Casein, the main protein of milk, also acts as a nutrient. Undoubtedly, the body uses a number of other proteins as a source of amino acids, which in turn are the precursors of biologically active substances that control the metabolism.

Proteins make up 70-80% of the dry mass of muscles, lungs, spleen, and kidneys, and 45% of the dry weight of the human body. Unlike animal tissues, proteins are stored less in plants.

To study the chemical composition, structure and properties of proteins, their color reactions are usually considered characteristic for liquid tissues or protein-rich amino acids. In the ninhydrin reaction, an amino acid reacts with ninhydrin to produce a blue color and CO₂

glycylalanine from the combination of the carboxyl group of glycine with the amino group of alanine; carboxyl group of alanine combines with amino group of glycine to form alanyl glycine i. Two different dipeptides were formed from the combination of two amino acids.



Such a dipeptide has free amine and carboxyl groups and can react with other amino acids. As a result, an additional peptide bond is formed, and a new 3-amino acid compound is formed, which is called a tripeptide.

Conclusion In addition to the growing demand for food products, the demand for protein-rich products is also increasing, and the field of production of high-quality biotechnological products is also developing.

On the basis of biotechnological approaches, scientific research is also being conducted on the creation of modern technology using proteins in industry. Many innovations are being created in the study of the structure, function and engineering of proteins, the chemical structure of living matter.

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