CONTENTS

Chapter 1
THE STRUCTURE, PROPERTIES AND FUNCTIONS OF PROTEINS

1.1. BIOLOGICAL FUNCTIONS OF PROTEINS

The structure and function of cells determine a large number of different molecules: proteins, nucleic acids, carbohydrates. Proteins play a special role in the life of the cell. First of all, the specific features of the structure and functioning of the cell are determined by a set of proteins synthesized in it. More than half of its dry substance is attracted to the share of proteins inside the cell. In the human body there are more than 50,000 individual proteins. A set of proteins in the body determines individual specificity, the set of proteins in different cell types determines their morphological and functional features.

Protein functions

- **Structural function.** Proteins are directly involved in the construction of the cell membranes and cytoskeleton (integral, semi-integral and surface proteins). The substance of connective tissue and the extracellular matrix form proteins collagen, elastin, keratin, proteoglycans.
- **Enzymatic function**. All enzymes are proteins. Enzymes catalyze the transformations of various molecules in the cells of the body. Enzymes constitute more than 50% of all proteins.
- **Receptor function.** This function is the selective binding of hormones, biologically active substances and mediators on the surface of membranes or inside the cells.
- **Transport function.** Only proteins transport some substances in the blood. For example, lipoproteins (lipid transfer), hemoglobin (oxygen transport), transferrin (iron transport). Proteins transport cations of calcium, magnesium, sodium and other ions into the blood.

- **Regulatory function.** The regulation and coordination of metabolism in different cells of the body are carried out by hormones. Many hormones such as insulin and glucagon are proteins, all pituitary hormones are peptides or small proteins.
- **Storage function.** Animals and humans do not have specialized depots of proteins, but with prolonged fasting, muscle proteins, lymphoid organs, epithelial tissues and the liver are used.
- **Contractile function.** There are several intracellular proteins designed to change the shape of the cell and the movement of the cell itself or its organelles (tubulin, actin, myosin).
- **Protective function.** Immunoglobulins protect the body from the action of bacteria, toxins, foreign proteins, preventing the infectious process and maintaining the stability of the body. The factors of the complement system also take place in the body protection. Blood coagulation proteins work when the tissues are damaged (fibrinogen, prothrombin). Mechanical protection of mucous membranes and skin provide collagen and proteoglycans.

1.2. STRUCTURES, PROPERTIES AND CLASSIFICATION OF AMINO ACIDS

Proteins are high-molecular compounds and are polymers formed from α-amino acids linked together by peptide bonds. In nature, more than 300 different amino acids are known, but only 20 are part of the proteins of humans and animals. Each amino acid has a carboxyl group, an amino group in the alpha position (on the second carbon atom) and a radical (side chain) that is specific for each amino acid (Fig. 1.1).

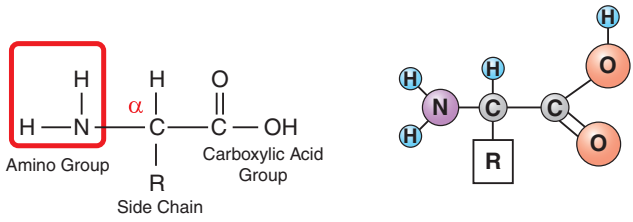


Fig. 1.1. Basic amino acid formula

In aqueous solutions at neutral pH, amino acids exist as bipolar ions. All amino acids (with the exception of glycine) contain an asymmetric carbon atom, therefore they can exist as L- and D-stereoisomers (Fig. 1.2). For the synthesis of human proteins only L-amino acids are used. In some proteins with a long lifetime, L-isomers can be converted to D-isomers.

All 20 amino acids in the human body differ in structure, size and physicochemical properties of radicals (side chains). Amino acid radicals are variable parts of a polypeptide backbone and may contain various functional groups.

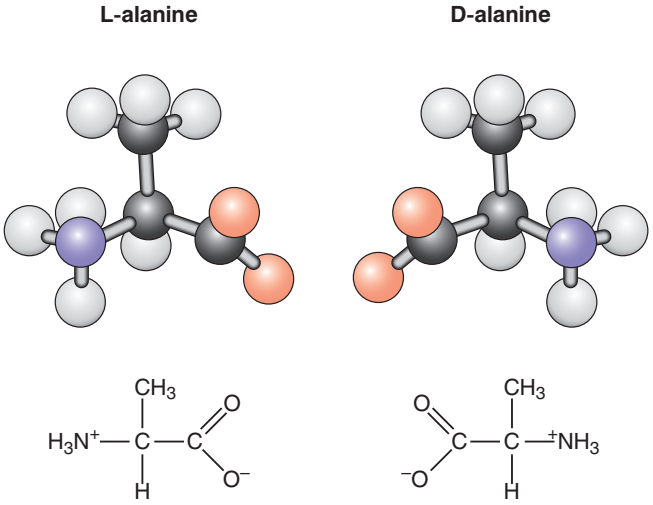


Fig. 1.2. Optical isomers of amino acids

- **Polar (hydrophilic):**
  - Hydroxyl group –OH;
  - Carboxyl group –COOH;
  - Amino group $-NH_2$;
  - Imino group $=NH_2$;
  - Amide group $-CO\text{-}NH_2$;
  - Thiol group –SH.
- **Non-polar (hydrophobic):**
  - Methyl group $-CH_3$;
  - Aromatic group.

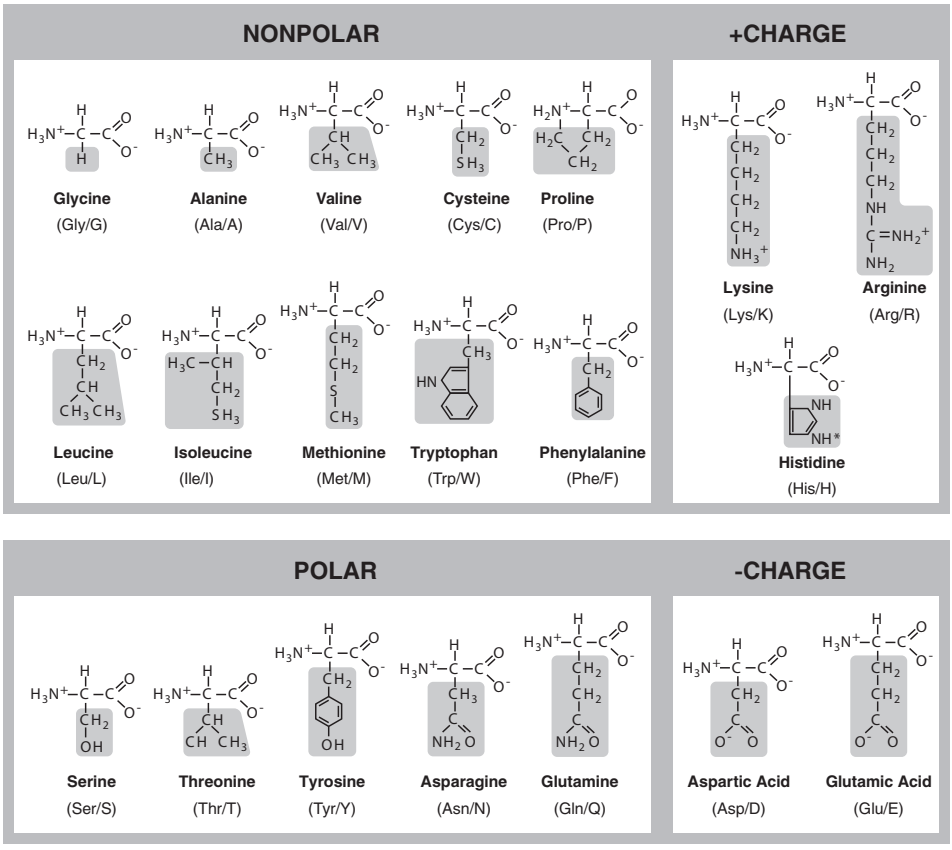
To designate amino acids in proteins, their trivial names are usually used. In addition, for the convenience of recording the amino acid sequence of peptides and proteins, their three-letter and one-letter designations are used (Table 1.1).

Amino acids are classified according to the physicochemical properties of their radicals. All amino acids can be divided into 4 groups

Amino acids can be divided into groups according to their ability to dissolve in water. The solubility of amino acid radicals is determined by the polarity of the functional groups. Polar groups attract water, non-polar repel it.

Amphotericity is the main physicochemical property of amino acids. Amphoteric means that the substance combines the properties of both acids and bases. In an aqueous solution, amino acids simultaneously behave like acids — proton donors and as bases — proton acceptors. Amino acids with polar negatively charged radicals have an additional carboxyl group in the radical. At a physiological pH of 7.0, it dissociates to form COO^- and H^+. The radicals of such proteins are anions. Amino acids with polar positively charged radicals have a second amino group in the radical. At physiological pH, these groups are positively charged. Radicals of such proteins are cations.

Table 1.1. Classification of amino acids on the chemical structure of their radicals



Peptide bond. The structure and properties of peptides

Amino acids can covalently bind to each other using peptide bonds. A peptide bond is formed between the α-carboxyl group of one amino acid and the α-amino group of another, i.e. is an amide bond. When a peptide bond is formed, a water molecule is split off (Fig. 1.3).

The amount of amino acids in the composition of the peptide can vary. Peptides containing up to 10 amino acids are called **oligopeptides**. Peptides containing more than 10 amino acids are called **polypeptides**. Polypeptides containing more than 50 amino acid residues are called **proteins**.

The monomers of amino acids that make up the protein are called amino acid residues. Amino acid residue having a free amino group is called **N-terminal** and is written on the left. Amino acid residue having a free carboxyl group is called **C-terminal** and is written on the right (Fig. 1.4). Amino acid residues in a polypeptide chain are numerated from the N-terminus. A chain of repeating amino acid residues without radicals is called a **peptide backbone**.

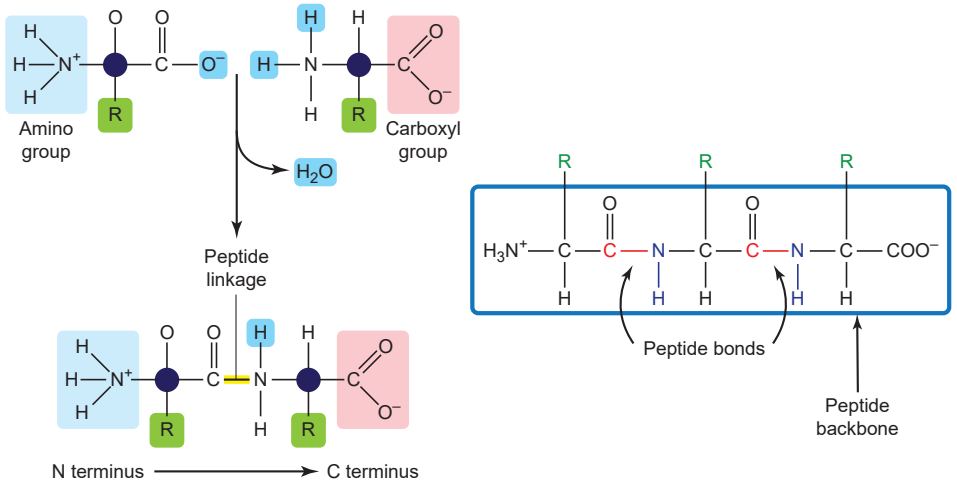


Fig. 1.3. Peptide bond formation

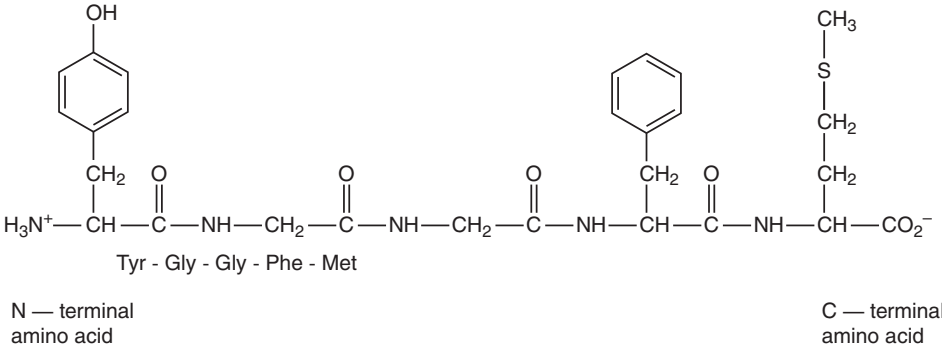


Fig. 1.4. Formula of pentapeptide Tyr-Gly-Gly-Phe-Met

The peptide bond formed by the imino group of the proline differs from other peptide bonds, since the nitrogen atom of the peptide group is associated not with hydrogen, but with a radical (Fig. 1.5).

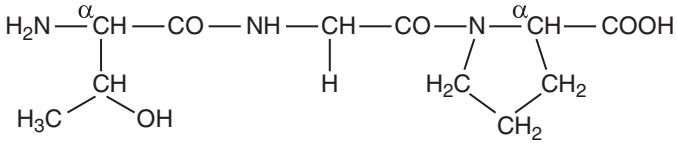


Fig. 1.5. Formation of a peptide bond between Threonine and Proline

The peptide bond is a strong covalent bond. It has a partial double bond character. The peptide bond's length is less than a single bond, it is a rigid (planar) structure, and rotation around it is difficult. But since, in addition to the peptide, there are other bonds in the protein, the chain of amino acids is able to rotate around the main axis, which gives proteins a different conformation (the spatial arrangement of atoms). All atoms in the peptide group are in the same plane, while the atoms H and O are located on opposite sides of the peptide bond (Fig. 1.6 A). The oxygen and hydrogen atoms in the peptide group have the ability to form hydrogen bonds with the oxygen and hydrogen atoms of other peptide groups (Fig. 1.6 B). Amino acid radicals in relation to the axis of the peptide C–N bonds are on opposite sides, in the trans-position (Fig. 1.6 C).

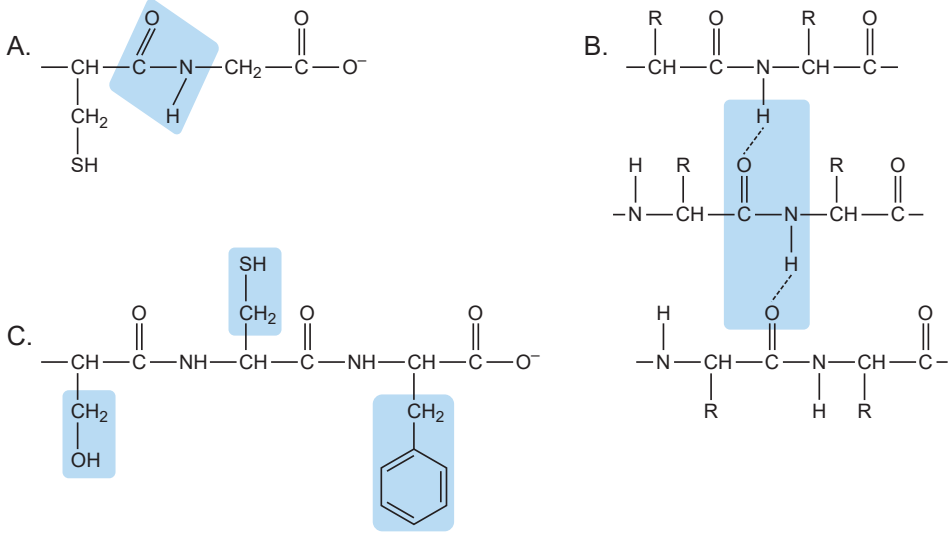


Fig. 1.6. Properties of the peptide bond

These properties of the peptide bond determine the ability of amino acids to interact with each other within one protein, as well as with other proteins. Peptide bonds are very strong and under physiological conditions they do not spontaneously break. In laboratory conditions, the peptide bonds are hydrolyzed in the presence of concentrated hydrochloric acid at 105° C within a day. In living organisms, peptide bonds in proteins are destroyed with the help of special proteolytic enzymes — proteases. To detect proteins and peptides in a solution, the color biuret reaction is used.

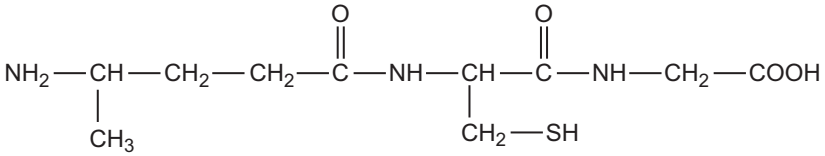
The biological role of amino acids and peptides

Amino acids are the building blocks of protein molecules, but their functions are not limited to this. Amino acids such as histidine, tryptophan, glutamic acid, tyrosine are the sources for the formation of neurotransmitters in the CNS (respectively,

histamine, serotonin, gamma-aminobutyric acid, dopamine and noradrenaline), and glycine and glutamic acid are neurotransmitters themselves. The amino acid methionine is necessary for the synthesis of phosphatidylcholine, one of the main components of cell membranes. Amino acid tyrosine is completely included in the composition of thyroid hormones (thyroxin, triiodothyronine) and adrenal medulla (adrenaline, norepinephrine). Certain amino acids are necessary for the synthesis of purine and pyrimidine which are the precursors of nucleic acids synthesis. Some amino acids are used for the synthesis of low molecular weight biologically important compounds (creatine, carnitine, carnosine, anserine, etc.).

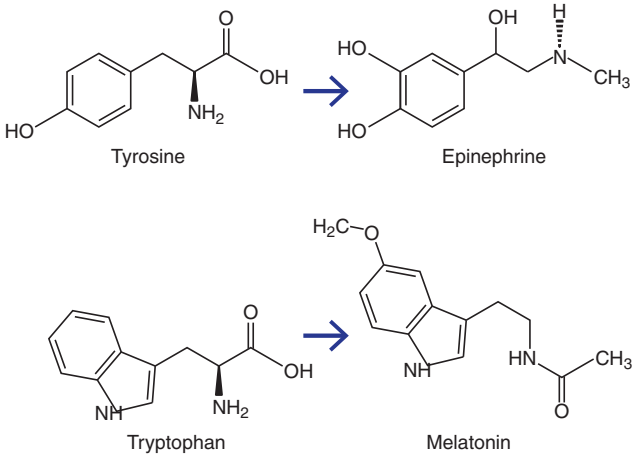
A number of hereditary and acquired diseases, accompanied by serious problems in the development of the organism, such as cystinosis, homocysteinemia, leucinosis, tyrosinemia, etc., are associated with metabolic disorders of amino acids. Phenylketonuria is the most famous example of metabolic disorders of amino acids. Phenylketonuria, also called PKU, is an inherited disorder caused by a defect in the gene that encodes the enzyme needed for phenylalanine metabolism. This eventually leads to serious health problems.

One of the most common peptides with protective properties is tripeptide glutathione. Glutathione (GSH) is often referred to as the body's master antioxidant. Reduced glutathione (GSH) is a linear tripeptide of L-glutamine, L-cysteine, and glycine. The molecule has a sulfhydryl (SH) group on the cysteinyl portion, which accounts for its strong electron-donating character. As electrons are lost, the molecule becomes oxidized, and two such molecules become linked (dimerized) by a disulfide bridge to form glutathione disulfide or oxidized glutathione (GSSG). This linkage is reversible upon re-reduction.



Some amino acids and several peptides are important human hormones. Hormones, in general, are biological molecules used in multicellular organisms to direct and regulate biological processes, such as growth, reproduction and metabolism. A peptide hormones are chains of amino acids, which serve as a biological communication molecules. Peptide hormones have a short half-life, meaning they break apart quickly. This allows organisms to use peptide hormones to direct processes quickly and efficiently, without the signal lingering for a long time.

The amino acid-derived hormones are relatively small molecules derived from the amino acids tyrosine and tryptophan. If a hormone is amino acid-derived, its chemical name will end in «-ine». Examples of amino acid-derived hormones include epinephrine and norepinephrine, which are synthesized in the medulla of the adrenal glands, and thyroxine, which is produced by the thyroid gland. The pineal gland in the brain makes and secretes melatonin, which regulates sleep cycles. The formulas amino acid-derived hormones are below:



The structure of peptide hormones is that of a polypeptide chain (chain of amino acids). The peptide hormones include molecules that are short polypeptide chains, such as antidiuretic hormone and oxytocin produced in the brain and released into the blood in the posterior pituitary gland. This class also includes small proteins, such as growth hormones produced by the pituitary. Secreted short proteins, such as insulin, are stored within vesicles in the cells which synthesize them. They are then released in response to stimuli (e.g., as high blood glucose levels in the case of insulin). Amino acid-derived and polypeptide hormones are water-soluble and insoluble in lipids. These hormones cannot pass through plasma membranes of cells; therefore, their receptors are found on the surface of the target cells.

The group of peptides that affect vascular tone (vasoactive) includes bradykinin, kallidin and angiotensin. The first peptide contains 9 amino acid residues, the second — 10, and the third — 8. All of them are synthesized from inactive protein precursors as a result of the post-translational modification process. Peptides can regulate the processes of digestion, for example, gastrin, cholecystokinin. Peptides that regulate appetite are, for example, leptin, b-endorphins.

Peptides, called enkephalins, or opiate peptides, are found in the brain tissue and perform an analgesic effect similar to that of opium substances. Another type of opiate (anesthetic) peptides has also been found in the brain — and they are called endorphins. These longer peptides (from 13 to 30 a.a.) got their names for the analgesic effect, similar to the effect of morphine. They have a more complex physiological effect and have not only an anesthetic effect, but also affect behavior.

Many toxins are peptides. For example, the toadstool (Amanita phalloides) contains peptide toxins amanitin and phallodin. They are contained in these mushrooms in high concentrations. The lethal dose for humans is about 5–7 mg, that is, one or two eaten by the fungus can cause death. All toxins of this type are cyclic peptides. Amatoxins cause a violation of RNA synthesis in cells, and phalloidin violates the integrity of the membrane of the liver cells — hepatocytes. Peptide toxin from bee venom (Apis melifera) apamin, a linear peptide of 18 a.a, affects the

functioning of calcium channels in membranes, mellitin — a peptide of 22 a.a. — causes ionic conductivity in membranes, and the third — MSD-peptide causes allergic and inflammatory reactions. Peptide toxins from snake venoms can be attributed to protein substances by the number of amino acids, but they traditionally suggest the presence of peptides. These toxins, as a rule, act on the membranes of nerve cells or axons, disrupt their normal functioning. However, in low concentrations, toxins and poisons are used as effective drugs against a number of diseases associated with neuromuscular disorders.

1.3. THE LEVELS OF PROTEIN STRUCTURES: PRIMARY, SECONDARY, TERTIARY

Peptide chains contain hundreds and thousands of amino acid residues linked by strong polypeptide bonds. Due to intramolecular interactions, proteins form a specific spatial structure, called **protein conformation**. The linear amino acid sequence in a protein, determines the construction of a three-dimensional spatial structure. There are 4 levels of the spatial organization of proteins: primary, secondary, tertiary and quaternary structures (Fig. 1.7). There are general rules by which they are formed.

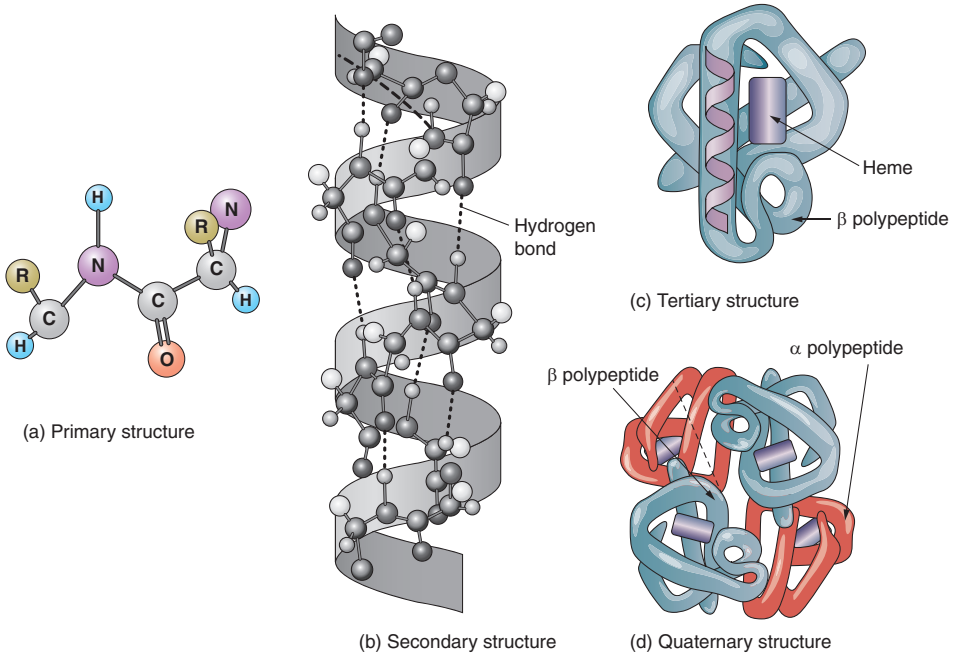


Fig. 1.7. Protein organization levels

Primary structure

Amino acid residues in the peptide chain are not randomly located, but arranged in a specific order. **The linear sequence of amino acid residues in a protein is called the primary structure of the protein** (Fig. 1.8).

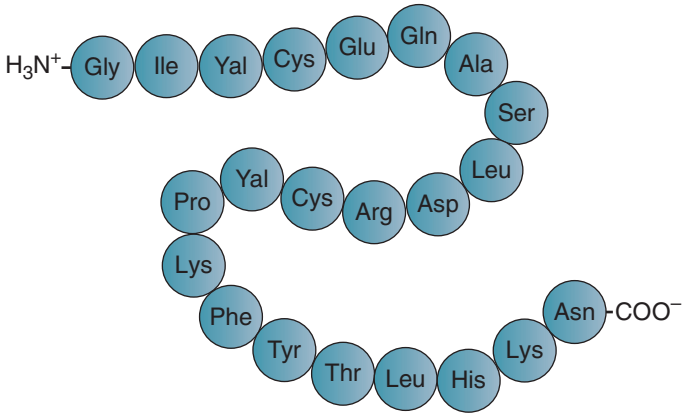


Fig. 1.8. Primary structure of the protein

The primary structure of proteins, i.e. the amino acid sequence in it is programmed by the nucleotide sequence in the DNA. The deletion, insertion, replacement of a nucleotide in DNA leads to a change in the amino acid composition and, therefore, the structure of the synthesized protein. If a change in the amino acid sequence is not lethal, but adaptive or at least neutral, then the new protein can be inherited and remain in the population. As a result, new proteins appear with similar functions. This phenomenon is called protein polymorphism.

For example, there are about 300 different types of hemoglobin, some of them are necessary at different stages of ontogenesis: for example, HbP — embryonic, formed in the first month of development, HbF — fetal, necessary in the later stages of fetal development, HbA and HbA2 — adult hemoglobin. The diversity is provided by the polymorphism of globin chains: there are 2ξ and 2ε chains in hemoglobin P, 2α and 2γ chains in HbF, 2α and 2β chains in HbA, and 2α and 2δ chains in HbA2. Proteins of the main histocompatibility complex provide tissue transplantation incompatibility. They have extremely high polymorphism, in general, there are several million alleles of these proteins. Due to this diversity, each person has an almost unique set of alleles.

In addition, many genetic diseases result from the amino acid sequence violation of proteins. Information about the primary structure of a normal and mutant protein is needed to diagnose and predict the development of a disease.

Secondary structure

The secondary structure of a protein is a **spatial structure resulting from interactions between the functional groups that make up the peptide backbone**. The secondary structure

is formed only with the participation of hydrogen bonds between peptide groups: the oxygen atom of one group reacts with the hydrogen atom of the second, while the oxygen of the second peptide group is bound to the third hydrogen, etc. (Fig. 1.9).

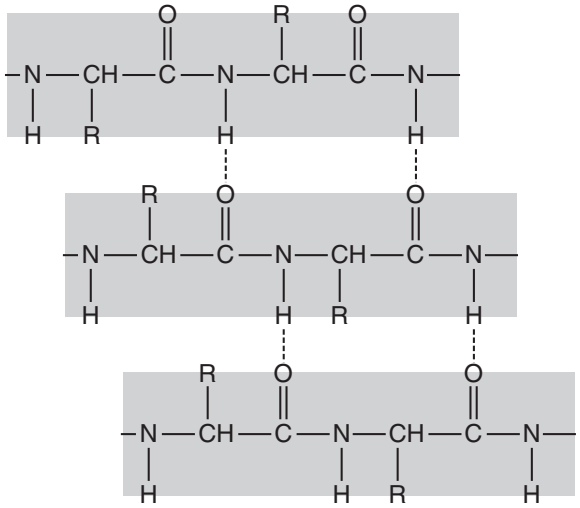


Fig. 1.9. Hydrogen bonds between peptide backbone groups form a secondary protein structure

When the secondary structure is formed, the peptide accepts the conformation with the largest number of bonds between the peptide groups. The type of secondary structure depends on the stability of the peptide bond, the mobility of the bond between the central carbon atom and the carbon of the peptide group, the size of the amino acid radical. All of this, together with the amino acid sequence, will subsequently lead to a strictly defined configuration of the protein. In this case, peptide chains can form regular structures of two types: α-helix and the β-sheet.

In one protein, as a rule, both structures are simultaneously present, but in different proportions. In globular proteins, the α-helix predominates, in fibrillar proteins, the β-structure prevails.

α-Helix

The most common form of the secondary structure is the α-helix (the polypeptide chain seems to be twisted clockwise on an imaginary cylinder, which is due to the L-amino acid composition of natural proteins). At each turn (step) of the helix there are 3.6 amino acid residues, the helix pitch is 0.54 nm per turn, and one amino acid residue is 0.15 nm (Fig. 1.10).

Not all globular proteins are helical across the entire length of the polypeptide chain. In the protein molecule, the α-helical regions alternate with the linear ones. Practically all atoms of the oxygen and hydrogen peptide groups are involved in the formation of hydrogen bonds. Since all the hydrophilic groups of the peptide core are occupied, the hydrophilicity α-helix (the ability to form hydrogen bonds with water) decreases, and the hydrophobicity increases.

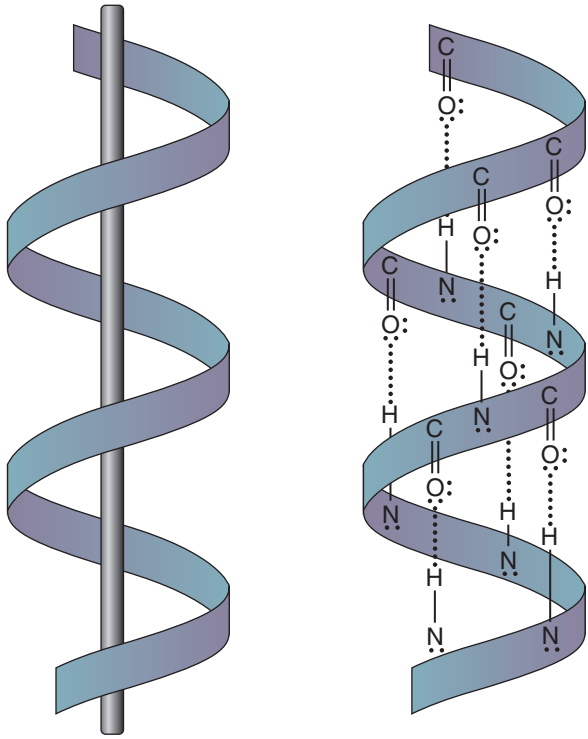


Fig. 1.10. The secondary structure of proteins in the form of α-helix

The α-helix is a very stable conformation of the peptide backbone. The amino acid radicals are on the outside of the α-helix and are directed away from the peptide backbone. They do not participate in the formation of hydrogen bonds characteristic of the α-helix, but some may disrupt its formation, proline and hydroxyproline cause chain bending, for example, in collagen.

β-Sheet

A β-sheet is formed by the formation of hydrogen bonds between the atoms of the peptide groups of the linear regions of one polypeptide chain, making bends or between many different polypeptide chains (Fig. 1.11). It looks like a folded sheet. In this way of laying the protein the molecule makes a snake-shaped form, the remote segments of the chain are going close to each other. As a result, peptide groups that previously were remote amino acids of the protein chain are able to interact by hydrogen bonds.

The orientation of the reactive sites may be parallel (i.e. the direction of N-terminal to C-terminal ends is the same) or antiparallel where chains go in the opposite direction (Fig. 1.12). Such sites of one protein interacting with each other can be from two to five.

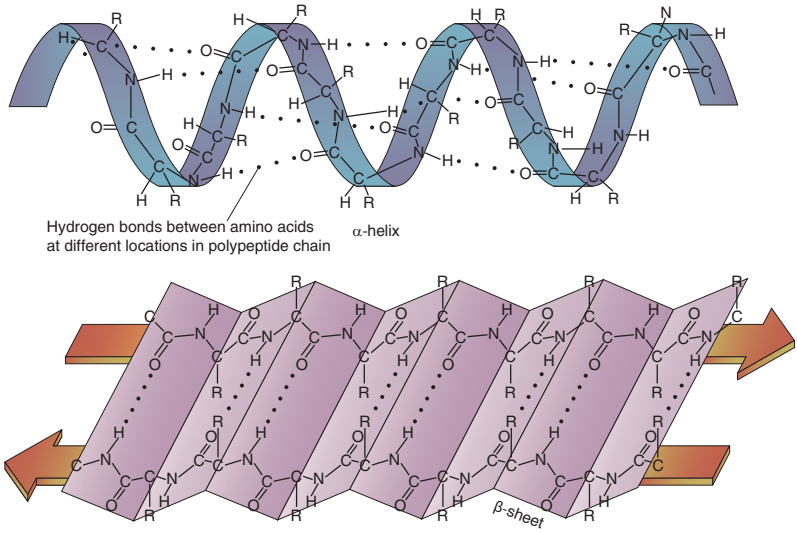


Fig. 1.11. The secondary structure of proteins in the form of β-sheet

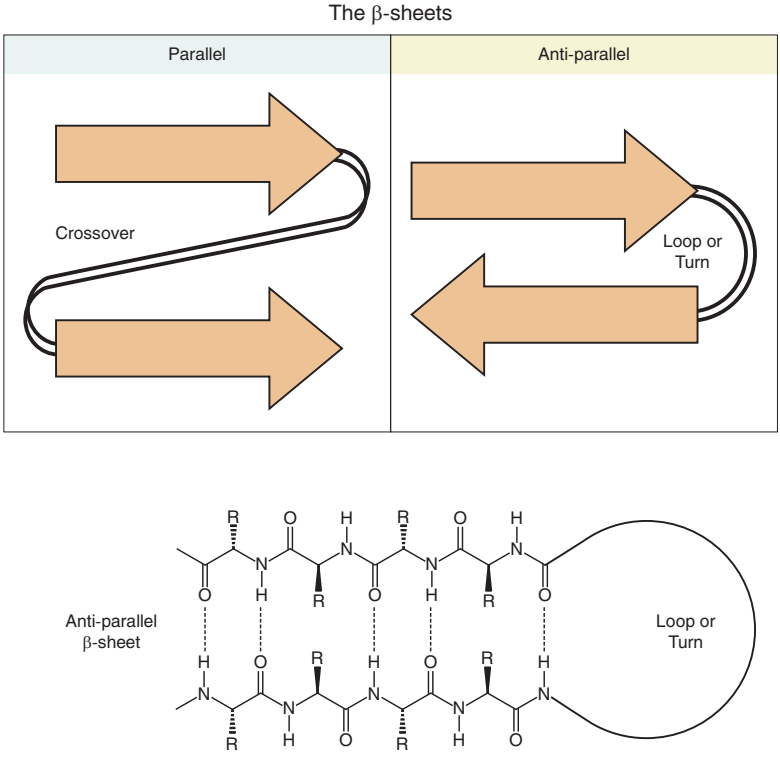


Fig. 1.12. Parallel and antiparallel β-sheets

Irregular secondary structures

Some parts of the protein are ordered but do not form any regular structures. They should not be confused with a random coil, an unfolded polypeptide chain lacking any fixed three-dimensional structure. They are represented by loop-like and ring-shaped structures having a less regular packing than the α-helix and β-sheet described above. However, they do not vary so much from one protein molecule to another. In each individual protein, they have their fixed conformation, determined by the amino acid composition of this chain segment and its surrounding regions.

Tertiary structure

The tertiary structure of proteins is a **three-dimensional spatial structure formed due to interactions between amino acid radicals,** which can be located at a considerable distance from each other in the polypeptide chain. Secondary structures of proteins often constitute distinct domains. A domain is the basic unit of structure and function. Tertiary structure describes the relationship of different domains to one another within a protein. Four types of chemical bonds are involved in the formation of the tertiary structure: hydrophobic, ionic, hydrogen and disulfide (Fig. 1.13).

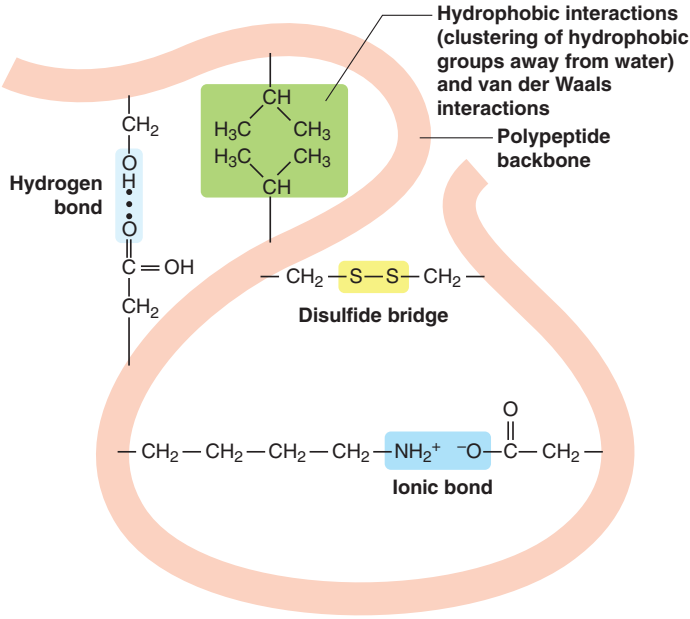
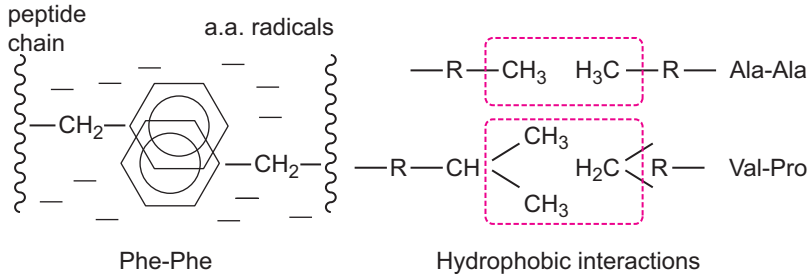


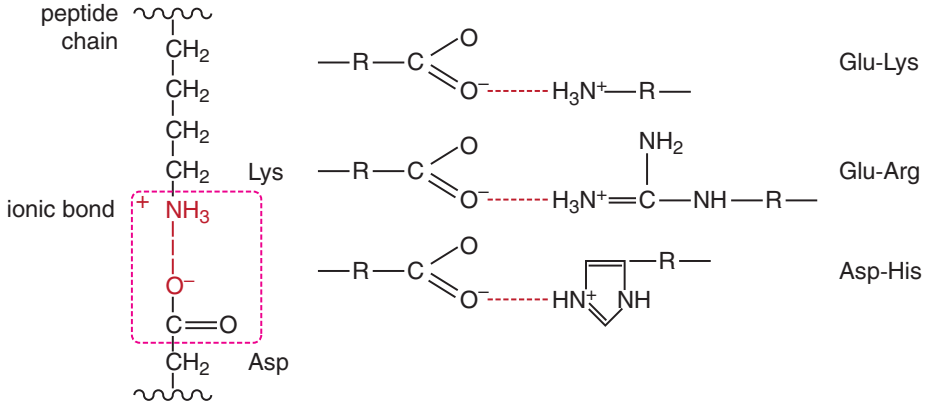
Fig. 1.13. Types of chemical bonds are involved in the formation of the tertiary structure

- **Hydrophobic interactions:** Hydrophobic interactions occur between non-polar hydrophobic radicals of amino acids that formed the protein. The polypeptide chain of a protein tends to take an energetically stable form, characterized by a minimum of free energy. Therefore, hydrophobic amino acid radicals tend to unite

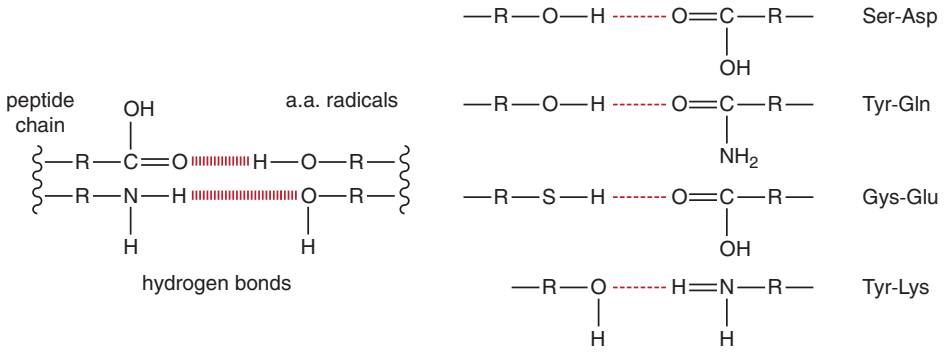
within the globular structure of water-soluble proteins. Between them, so-called hydrophobic interactions arise, as well as van der Waals forces between closely adjacent atoms. As a result, a hydrophobic core forms inside the protein globule.



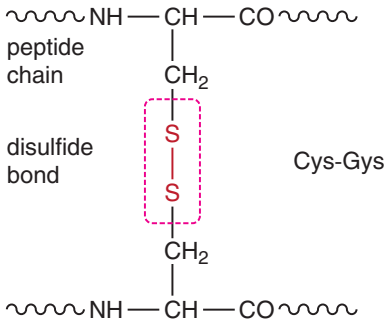
- **Ionic bonds:** Ionic bonding can occur between negatively charged (anionic) carboxyl groups of aspartic and glutamic acid radicals and positively charged (cationic) groups of lysine, arginine and histidine radicals.



- **Hydrogen bonds:** Hydrogen bonds occur between hydrophilic uncharged groups —OH, —$CONH_2$, —SH, and any other hydrophilic groups.



- **Disulfide bonds:** The covalent disulfide bond is formed between two -SH groups of cysteine radicals located in different places of the polypeptide chain. Disulfide bonds can stabilize the spatial structure of a single polypeptide chain or link two chains together, such as in an insulin molecule.



All proteins with the same primary structure and under the same conditions acquire the same conformation, which determines their function. The functionally active protein conformation is called the **native structure.** Hydrophobic, ionic and hydrogen bonds are weak bonds, therefore their breaking is possible even under physiological conditions. This fact ensures the conformational lability of proteins, i.e. they are capable of small changes due to the breaking of some weak bonds and the formation of others. Protein conformation can change with changing the chemical and physical properties of the medium, as well as when interacting with other molecules. Conformational changes play a huge role in the functioning of proteins in a living cell.

The breaking of a large number of weak bonds leads to the destruction of the native conformation of the protein. The loss of the native conformation is accompanied by the loss of the specific function of the protein. This process is called **protein denaturation**. When denaturation occurs, an occasional break of weak bonds happen and protein molecules acquire a random conformation.

Initially the weakest bonds are torn and when conditions are tightened, stronger ones are also broken. Therefore, at first, the quaternary, then the tertiary and secondary structures are lost. Denaturation does not break the peptide bonds, i.e. the primary structure of the protein is not disturbed.

Denaturation may be reversible, if restoration of the protein-characteristic structure is possible (for example, membrane receptors); or irreversible, if the restoration of the spatial configuration of the protein is impossible. Irrevesible denaturation usually occurs when a large number of bonds are broken, for example, when eggs are boiled. If the protein has undergone reversible denaturation, then when normal conditions of the environment are restored, it is able to completely restore its structure and, accordingly, its properties and functions. The process of restoring the protein structure after denaturation is called **renaturation** (Fig. 1.14).

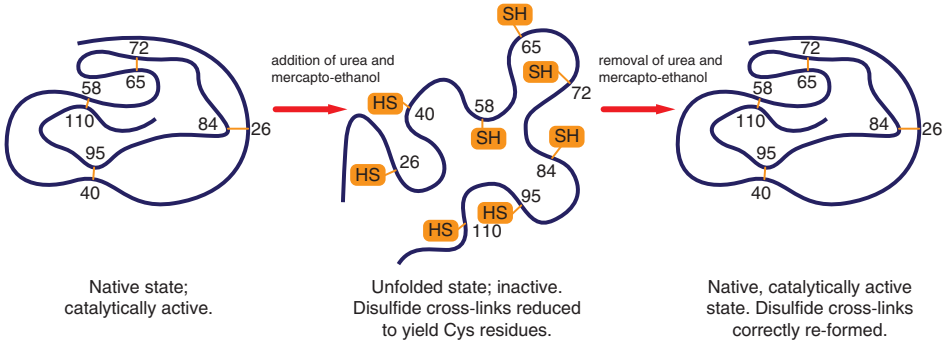


Fig. 1.14. Denaturation and renaturation of ribonuclease

Proteins can be denatured at high temperatures (over 50 °C), by vigorous shaking of the protein solution, organic substances (alcohol, phenol, urea), acids and alkalis, heavy metal salts, detergents. The most famous detergent is soap. In medicine, denaturing agents are used to sterilize instruments, materials, and as antiseptics.

A **protein domain** is an element of the tertiary structure of a protein, which is a fairly stable and independent substructure of a protein, the folding of which passes independently of the other parts. The domain usually includes several elements of the secondary structure. Domains similar in structure are found not only in related proteins (for example, hemoglobins of different animals), but also in completely different proteins. Domains can perform different functions and undergo folding into independent compact globular structural units interconnected by flexible sections within a protein molecule (Fig. 1.15).

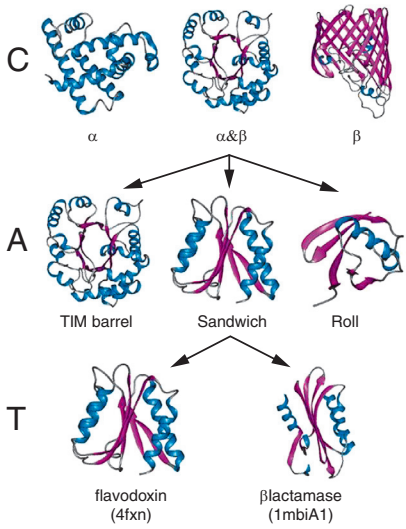


Fig. 1.15. Examples of protein domains classified by CATH (class, architecture, topology, homology)

Quite often, domains are assigned separate names, since their presence directly affects the biological functions performed by the protein, for example, the $Ca2^{+}$ binding domain of calmodulin, a homeodomain responsible for binding to DNA in various transcription factors. Different domains in the protein can move relative to each other when interacting with the ligand, which facilitates the further functioning of the protein.

The formation of the three-dimensional structure of the protein in the cell is the most important process, since its biological function depends on the spatial structure of the protein. The process of packing the polypeptide chain into the correct spatial structure is called protein folding. The concentration of proteins in a cell is very high, so an abnormal protein conformation may occur. For many proteins with high molecular weight and complex spatial structure, folding occurs with the help of special chaperone the participation proteins. Chaperones isolate the protein from the environment and allow it to accept the native conformation.

Protein functioning

Each protein with a unique primary structure and conformation has a unique function. Proteins perform many different functions in a cell. A prerequisite for the functioning of a protein is the binding of another substance called a **ligand**. Ligands can be both low molecular weight substances, such as metal ions, small organic molecules and macromolecular substances, such as other protein molecules. The interaction of the protein with the ligand is highly specific, which is determined by the structure of the protein site, called the **active site (active center)** of the protein.

The **active site (active center)** of proteins is a specific part of a protein molecule, usually located in its recess («pocket»), formed by the amino acid radicals collected in a certain spatial region during the formation of the tertiary structure and capable of complementary binding to the ligand (Fig. 1.16).

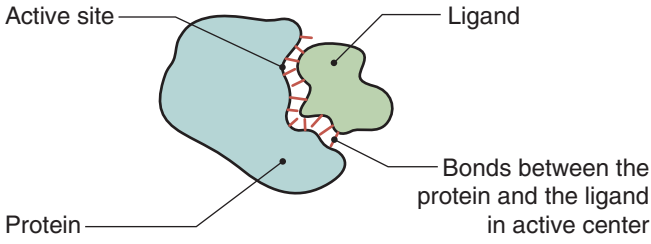


Fig. 1.16. The active site of the protein and its interaction with the ligand

In the linear sequence of the polypeptide chain, the radicals forming the active center can be located at a considerable distance from each other. The high specificity of protein binding to the ligand is ensured by the complementary structure of the active center of the protein to the ligand structure.

Complementarity means the spatial and chemical correspondence of interacting molecules. The ligand must have the ability to enter and spatially coincide with the conformation of the active center. This coincidence may be incomplete, but due to the conformational lability of the protein, the active center is capable of small changes and «fits» into the ligand. In addition, between the functional groups of the ligand and the radicals of the amino acids forming the active center, bonds must arise that hold the ligand in the active center. The bonds between the ligand and the active center of the protein can be both non-covalent (ionic, hydrogen, hydrophobic) and covalent (Fig. 1.17)

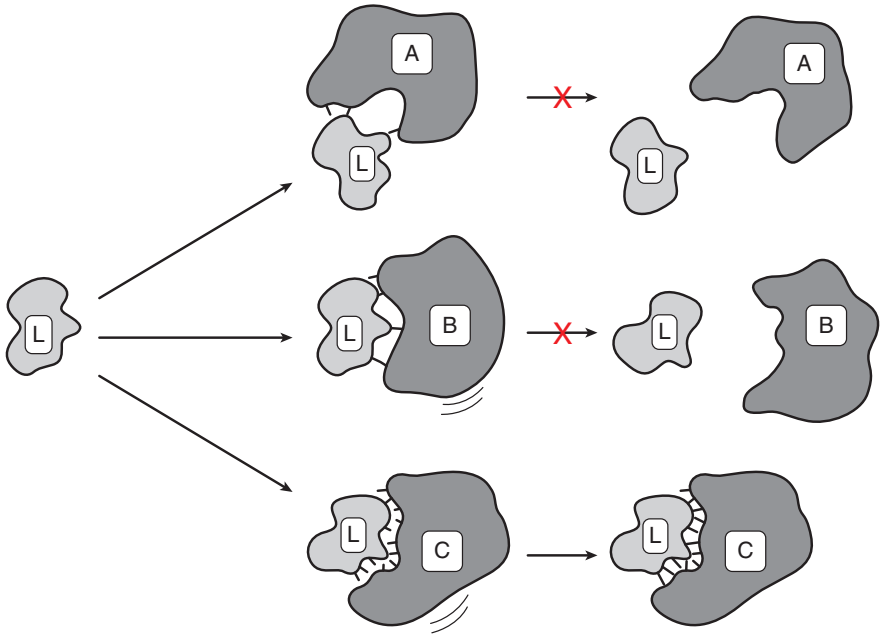


Fig. 1.17. The interaction of protein with a ligand in the active site. A and B are incomplete interaction. C is complementary interaction, L-ligand

The unique properties of the active center depend not only on the chemical properties of the amino acids forming it, but also on their exact mutual orientation in space. Therefore, even minor violations of the general conformation of the protein as a result of point changes in its primary structure or environmental conditions can lead to a change in the chemical and functional properties of the radicals that form the active center, disrupt the binding of the protein to the ligand and its function. During denaturation, the active center of proteins is destroyed, and their biological activity is lost. Often the active center is formed in such a way that the access of water to the functional groups of its radicals is limited, i.e. conditions are created for the binding of the ligand to amino acid radicals. In some cases, the ligand is attached to only one

of the atoms with a certain reactivity, for example, the addition of O_2 to the iron ion of myoglobin or hemoglobin.

The main property of proteins that underlies their functions is the selectivity of specific ligands to attach to certain sections of the protein molecule.

There are ligands that can change their chemical structure upon attachment to the active center of the protein, for example, changes in the substrate in the active center of the enzyme. There are ligands that can attach to the protein only at the moment of functioning, for example, O_2 transported by hemoglobin. Ligands can be constantly associated with the protein, which plays an auxiliary role in the functioning of proteins, for example, iron which is part of hemoglobin. The connection of protomers in an oligomeric protein is an example of the interaction of high molecular weight ligands. Each protomer connected to other protomers serves as a ligand for them, just as they do for it. Sometimes the attachment of a ligand changes the conformation of the protein, resulting in the formation of a binding center with other ligands. For example, the protein calmodulin, after binding to four Ca^{2+} ions in specific areas, acquires the ability to interact with some enzymes, changing their activity.

Protein classification

Protein classification is based on chemical composition, structure, functions, and solubility in different solvents. Based on their chemical composition, proteins may be divided into two classes: **simple** and **complex**. Simple proteins also known as homoproteins, they are made up of only amino acids. For examples, are plasma albumin, collagen, and keratin. Complex proteins sometimes also called heteroproteins, except amino acids, have non-protein components. The non-protein part is called the **prosthetic group**, and the protein part — **apoprotein**. Complex protein — **holoprotein** — can dissociate into components:

holoprotein ↔ apoprotein + prosthetic group

The direction of the reaction depends on the bond strength of the holoprotein components. The prosthetic group can be organic substances, metal ions, nucleic acids, carbohydrates, lipids and other substances (Table 1.2).

Table 1.2. Complex protein examples

Proteins	Prosthetic groups
Metal Proteins	Metal Ions
Phosphoproteins	H_3PO_4
Hemoproteins	Hemes
Glycoproteins	Monosaccharides, oligosaccharides
Flavoproteins	Flavinnucleotides
Proteoglycans	Glycosaminoglycans
Lipoproteins	Triacylglycerols and Complex Lipids
Nucleoproteins: ribonucleoproteins (ribosomes, etc.); deoxyribonucleoproteins (chromatin)	 RNA DNA

Glycoproteins are proteins that covalently bind one or more carbohydrate units to the polypeptide backbone. Typically, the branches consist of not more than 15–20 carbohydrate units. Examples of glycoproteins are: glycophorin, the best known among erythrocyte membrane glycoproteins; fibronectin, that anchors cells to the extracellular matrix through interactions on one side with collagen or other fibrous proteins, while on the other side with cell membranes; all blood plasma proteins, except albumin; immunoglobulins or antibodies. Phosphoproteins are proteins that bind phosphoric acid to serine and threonine residues. Generally, they have a structural function, such as tooth dentin, or reserve function, such as milk caseins (alpha, beta, gamma and delta), and egg yolk phosvitin.

Based on their shape, proteins may be divided into two classes: **fibrous** and **globular**. Fibrous proteins have primarily mechanical and structural functions, providing support to the cells as well as the whole organism. These proteins are insoluble in water as they contain, internally as well as on their surface, many hydrophobic amino acids. The presence on their surface of hydrophobic amino acids facilitates their packaging into very complex supramolecular structures. In this regard, it should be noted that their polypeptide chains form long filaments or sheets, where in most cases only one type of secondary structure, that repeats itself, is found.

Here are some examples, collagen which constitutes the main protein component of connective tissue, and more generally, the extracellular scaffolding of multicellular organisms. α-Keratins constitute almost the entire dry weight of nails, claws, beak, hooves, horns, hair, wool, and a large part of the outer layer of the skin. Most of the proteins belong to the class of globular proteins.

They have a compact and more or less spherical structure, more complex than fibrous proteins. In this regard, motifs, domains, tertiary and quaternary structures are found, in addition to the secondary structures. They are generally soluble in water but can also be found inserted into biological membranes (transmembrane proteins), thus in a hydrophobic environment. Unlike fibrous proteins, that have structural and mechanical functions, they act as:

- enzymes;
- hormones;
- membrane transporters and receptors;
- transporters of triglycerides, fatty acids and oxygen in the blood;
- immunoglobulins or antibodies;

Substances that affect the functioning of the protein

Ligands interact with proteins and are able to change their conformation in such a way that new active centers are formed for communication with other ligands. As mentioned above, the interaction of a protein with a ligand has high specificity. Nevertheless, it is always possible to choose a substance (natural or synthetic), which is a structural analog of the ligand and can be complementary to the active center of the protein. If this substance binds to a protein instead of a natural ligand, protein function will be blocked. A substance that interacts with the active center of a protein and blocks the action of a protein is called a **protein inhibitor** or antagonist. If this substance is similar in structure to a ligand, it is called a structural analog of the

ligand. It also interacts with the active center of a protein. An analog that replaces a natural ligand in an active center protein and reduces its function is called a **competitive protein inhibitor**.

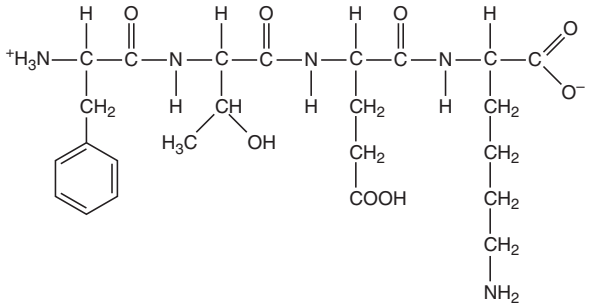
Analogs of natural protein ligands are used as medicines. Such drugs are widely used in the regulation of transmission of excitation through synapses. The neurotransmitter secreted during the passage of the pulse by the nerve endings should interact highly with receptor proteins on the postsynaptic membrane. However, by modifying the chemical structure of the neurotransmitter, it is possible to obtain substances that would also bind to the receptor, but the physiological effect changed, decreased or intensified. In pharmacology, such substances are called «antagonists» and «agonists», respectively.

Dithilin (a pharmacological substance) is an analog of acetylcholine, which ensures the transmission of a nerve impulse to a muscle, being a synapse mediator. When dithilin is introduced into the body, it binds to the N-cholinergic receptors of cholinergic synapses, being an antagonist of acetylcholine, a neurotransmitter that causes muscle contraction. As a result, the transmission is blocked and muscle relaxation (paralysis) occurs. Therefore, dithilin is used as a muscle relaxant in operations and endoscopic examinations. Another drug that acts as a protein inhibitor is atropine, an alkaloid of plant origin, which is also a natural analog of acetylcholine, but interacts with M-cholinergic receptors of the membrane of effector cells. Being an acetylcholine antagonist, it eliminates the irritation of parasympathetic nerves, and instead of smooth muscle contraction, which is stimulated by acetylcholine, relieves their spasm.

Some poisons, getting into the human body, firmly bind to certain proteins, inhibit them and thereby cause disturbances in biological functions. For example, cobra neurotoxins specifically interact with cholinergic receptors of postsynaptic membranes, blocking their work. The affinity of neurotoxins for cholinergic receptors causes to the formation of many bonds between the toxin and the receptor, which leads to their practically irreversible connection. It must be remembered that there is often a transparent border between drugs and poisons, and the effect of their action depends on the dose of the substance administered. Thus, drugs prescribed in doses greater than therapeutic can act as poisons, i.e. cause serious metabolic disorders and disruptions of body functions, and poisons in microdoses are often used as medications. For example, atropine, which is widely used to relieve spasms of smooth muscles, in high doses causes CNS excitement, and in even higher doses, it causes a sleep that goes into a coma. Known hypotensive agent clonidine in the case of an overdose causes collapse.

Review tests

1. Choose one correct answer. The peptide contains:



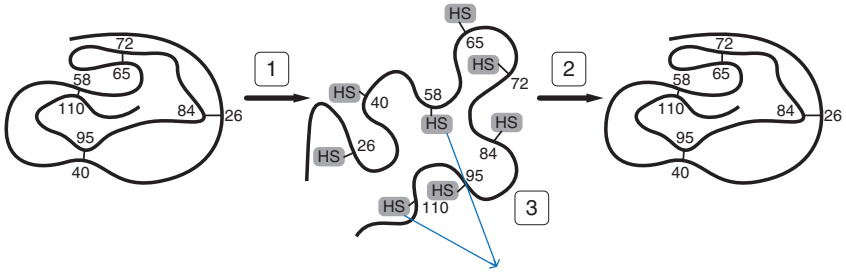
A. Three peptide bonds.
B. Lysine with a free α-amino group.
C. Tyrosine with a free α-carboxyl group.
D. Only polar radicals.
E. Tyrosine with a free α-amino group.

2. Choose one correct answer. The primary structure of the protein:
A. Is formed by hydrogen bonds between adjacent amino acids.
B. Is destroyed at extreme pH values.
C. Encoded in a DNA molecule.
D. Depends on the type of cells synthesizing it.
E. Capable of denaturation.

3. Which one of the following bonds is involved in the formation of the secondary structure of the protein:
A. Peptide.
B. Hydrogen.
C. Disulfide.
D. Ionic.
E. Hydrophobic.

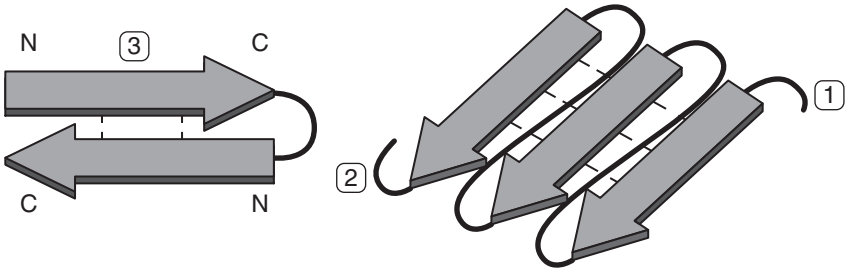
4. Choose one correct answer. A peptide containing only hydrophobic amino acid radicals:
A. Val-Pro-Thr-Trp.
B. Ile-Leu-Trp-Ala.
C. Met-Ser-Leu-Arg.
D. Pro-Tyr-Val-Gly.
E. Ile-Trp-Gln-Met.

5. Denaturation and renaturation of ribonuclease. Match the figure with the letter:



A. Renaturation.
B. Disulfide bonds.
C. Native protein.
D. Denaturation.
E. Cysteine radicals.

6. Match the figure with the letter. Secondary structure of protein:

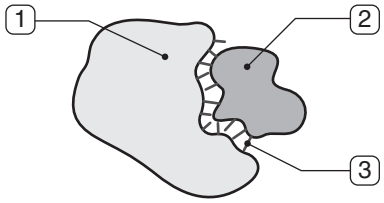


A. N-terminal end.
B. Parallel β-sheet.
C. C-terminal end.
D. Antiparallel β-sheet.
E. Hydrogen bonds between the atoms of the peptide backbone.

7. Match the figure with the letter. Levels of structural organization of proteins:

1. The primary structure.
2. The secondary structure.
3. Tertiary structure:
   a) spatial folding of the polypeptide chain;
   b) the order of alternation of amino acids;
   c) a structure formed by interradical interactions;
   d) spatial stacking of the peptide backbone;
   e) the specific arrangement of secondary structures.

8. Match the figure with the letter. Protein-ligand interaction:



A. Protein.
B. A ligand.
C. Peptide bonds.
D. Active center.
E. Weak connections.

Situational Problems

1. The use of natural proteins as drugs requires compliance with certain storage and use conditions, which are clearly prescribed in the instructions. So, most protein preparations must be stored in a refrigerator at a temperature not exceeding 10 °C, and dry preparations should be dissolved in boiled water cooled to room temperature. Some protein preparations are stored in hermetically sealed ampoules, from which air is removed. Why do protein drugs require strict adherence to all the conditions prescribed in the instructions, and what can happen if these conditions are violated?
2. Atropine is an alkaloid found in some plants: belladonna, dope, and bleach. It is part of the drugs belonging to the group of antispasmodics that relieve smooth muscle spasms. Such drugs are used for spastic pain and stomach, intestines, to relax the smooth muscles of the bile ducts. What is the mechanism of action of this substance?

1.4. QUATERNARY STRUCTURE OF PROTEINS. HEMOGLOBIN

If proteins consist of two or more polypeptide chains linked by non-covalent bonds, then they are said to have a **quaternary structure**. Such aggregates are stabilized by hydrogen bonds, ionic bonds and electrostatic interactions between oxygen residues located on the surface of the globules. Such proteins are called **oligomers**, and their individual chains are called **protomers (monomers, subunits).** Proteins with identical subunits are termed **homo-oligomers**. Proteins containing several distinct polypeptide chains are termed **hetero-oligomers**.

The formation of proteins with a quaternary structural organization allows organism to expand its capabilities in the field of qualitative diversity of proteins with a slight increase in the quantity of the genetic material. For example, the lactate dehydrogenase (LDH) enzyme, consisting of 4 subunits, is formed from 2 genetically determined polypeptide chains H and M. Their different combinations (HHHH, HHHM, HHMM, HMMM, MMMM) allow 5 LDH enzymes to exist in the body, catalyzing the same reaction in different organs and tissues: LDH1, LDH2, LDH3, LDH4 and LDH5. Such proteins with identical functions, but differing in physicochemical properties, are called **isoproteins**.

Oligomeric proteins exhibit properties absent in monomeric proteins. The influence of the quaternary structure on the functional properties of the protein can be considered by comparing the structure and functions of two related heme containing proteins: myoglobin and hemoglobin. Both proteins have a common evolutionary origin, a similar conformation of individual polypeptide chains and a similar function (participate in oxygen transport), but myoglobin is a monomeric protein and hemoglobin is a tetramer. The presence of a quaternary structure in hemoglobin gives this protein properties that are absent in myoglobin.

Myoglobin

Myoglobin is classified as heme-containing proteins, i.e. it contains a prosthetic group — heme, quite firmly connected with the protein part. Myoglobin is classified

as a globular protein; it has only one polypeptide chain. Myoglobin is found in red muscles and is involved in oxygen storage. In conditions of intense muscular work, when the partial pressure of oxygen in the tissue drops, O_2 is released from the complex with myoglobin and is used in the mitochondria of cells to obtain the energy necessary for muscle work.

Heme is a molecule with the structure of cyclic tetrapyrrole, where 4 pyrrole rings are connected by methylene bridges. This organic part of the heme is called protoporphyrin.

In heme, 4 nitrogen atoms of the pyrrole rings of protoporphyrin IX are linked by four coordination bonds with Fe^{2+} located in the center of the molecule (Fig. 1.18).

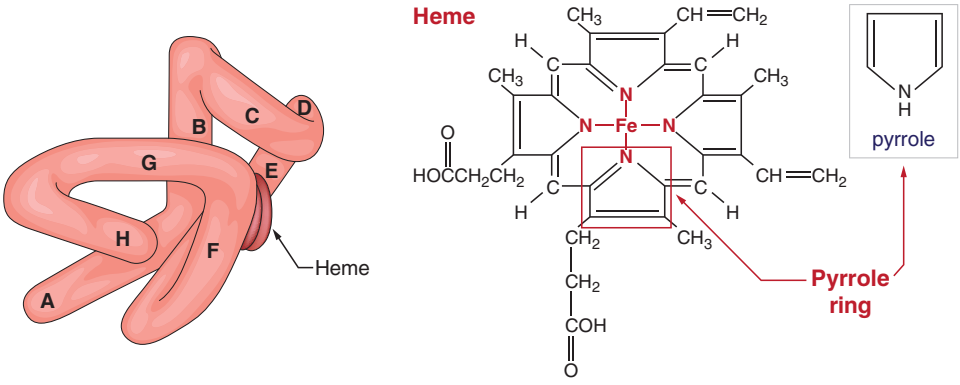


Fig. 1.18. Myoglobin structure. Heme is a complex of porphyrin and ferrous iron (Fe^{2+})

Apomyoglobin — the protein part of myoglobin; the primary structure is represented by a sequence of 153 amino acids, which are arranged in 8 α-helices in the secondary structure. The α-helices are denoted in Latin letters from A to H, starting from the N-terminus of the polypeptide chain. The tertiary structure has the form of a compact globule (there is practically no free space inside), formed due to loops and turns in the region of non-helical sections of the protein. The inner part of the molecule consists almost entirely of hydrophobic radicals, with the exception of two histidine residues located in the active center.

Heme is a specific apomyoglobin ligand that joins the protein part in the pocket between the two α-helices F and E. The heme binding site is formed mainly by hydrophobic amino acid residues surrounding the hydrophobic pyrrole rings of the heme.

In addition to hydrophobic amino acids, the active center of apomyoglobin also includes 2 histidine residues (His E7 and His F8), which play an important role in the functioning of the protein. They are located on different sides of the heme plane and are part of the F and E helices, between which the heme is located. The iron atom in the heme can form 6 coordination bonds, 4 of which hold Fe^{2+} in the center of protoporphyrin (connecting it with the nitrogen atoms of the pyrrole rings), and the 5th bond arises between $Fe2^{+}$ and the nitrogen atom of the imidazole ring His F8 (Fig. 1.19).

His E7, although not associated with heme, is necessary for the proper orientation and attachment of another ligand O_2 to myoglobin. The amino acid environment of the heme creates the conditions for a rather strong, but reversible binding of O_2 to Fe^{2+} myoglobin. The hydrophobic amino acid residues surrounding heme prevent water from entering the myoglobin binding site and the oxidation of Fe^{2+} to Fe^{3+}. Trivalent iron in the heme is not able to attach oxygen.

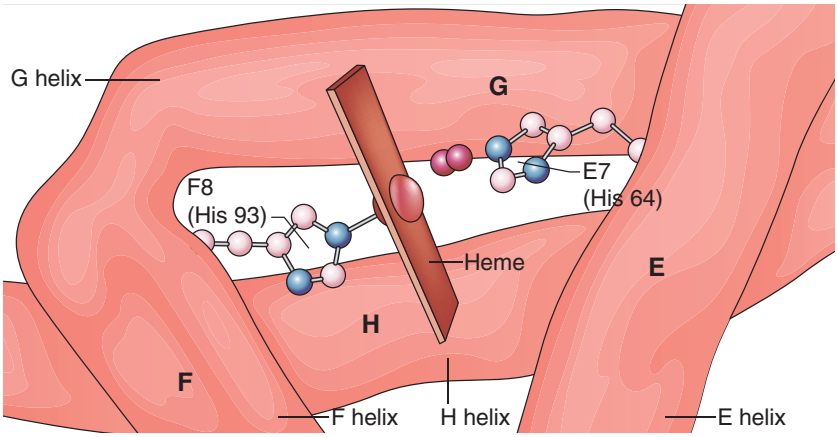


Fig. 1.19. The location of the heme in the active center of apomioglobin and hemoglobin protomers

Hemoglobin

Hemoglobins are related proteins found in human and vertebrate erythrocytes. They are also classified as heme-containing proteins. These proteins perform 2 important functions:

- transfer of oxygen from the lungs to the peripheral tissues;
- participation in the transfer of CO_2 and protons from peripheral tissues to the lungs for subsequent excretion from the body.

The most important characteristic of hemoglobin is its ability to regulate the affinity for O_2 depending on tissue conditions. Hemoglobins are similar in structure to myoglobin, but they have a quaternary structure (they consist of 4 polypeptide chains), which makes it possible to regulate their functions. In adult red blood cells, hemoglobin accounts for 90% of all proteins in a given cell.

Adult Hemoglobins:

- **hemoglobin A** (HbA) the main adult hemoglobin, accounts for about 98% of the total hemoglobin, tetramer, consists of 2 polypeptide chains α and 2β (2α2β);
- **hemoglobin A_2** is in the adult body in a lower concentration, it accounts for about 2% of total hemoglobin. It consists of 2α and 2δ chains (2α2δ);
- **hemoglobin A1c** is hemoglobin A modified by the covalent addition of glucose to it (the so-called glycosylated hemoglobin).

Hemoglobins synthesized during fetal development:

- **embryonic hemoglobin** (HbE) is synthesized in the fetal yolk sac a few weeks after fertilization. It is a tetramer 2ξ2ε. After 2 weeks of formation of the fetal

liver, hemoglobin F begins to be synthesized in it, which at 6 months replaces embryonic hemoglobin;

- **hemoglobin F** (HbF) is a fetal hemoglobin that is synthesized in the liver and bone marrow of the fetus before its birth. It has a tetrameric structure consisting of 2α and 2γ chains. After the birth of a child, it is gradually replaced by hemoglobin A.

The conformation of individual hemoglobin protomers is very similar to the conformation of myoglobin, despite the fact that only 24 amino acid residues are identical in the primary structure of their polypeptide chains. The hemoglobin protomers, like apomioglobin, consist of 8 helices folded into a dense globular structure containing an internal hydrophobic core and a «pocket» for heme binding. The heme compound with globin (protein part) is similar to that of myoglobin — the hydrophobic environment of the heme, with the exception of 2 residues His 7 and His F8 (Fig. 1.18, 1.19). However, the tetrameric structure of hemoglobin is a more complex structural and functional complex than myoglobin.

The role of histidine E7 in functioning of myoglobin and hemoglobin

Heme has a high affinity for carbon monoxide (CO). In the aquatic environment, the protein-free heme binds to CO 25,000 times stronger than O_2. The high degree of heme affinity for CO compared to O_2 is explained by the different spatial arrangements of the Fe^{2+} heme complexes with CO and O_2. In the Fe^{2+} heme complex with CO, the Fe^{2+}, carbon, and oxygen atoms are located on one straight line, and in the Fe^{2+} heme complex with O_2, the iron and oxygen atoms are located at an angle, which reflects their optimal spatial arrangement.

In myoglobin and hemoglobin above Fe^{2+} in the region of attachment O_2 His E7 is located, which disrupts the optimal arrangement of CO in the center of protein binding and weakens its interaction with heme. On the contrary, the same His E7 creates optimal conditions for the binding of O_2 (Fig. 1.19). As a result, the heme affinity to CO in proteins is only 200 times higher than its affinity for O_2. Decreased affinity for heme-containing proteins to CO is of great biological importance. CO is produced in small amounts during the catabolism of certain substances, in particular heme. This endogenously formed CO blocks about 1% of heme-containing proteins. If the heme affinity for CO did not decrease under the influence of the protein environment, then endogenous carbon monoxide could cause serious poisoning.

Quaternary hemoglobin structure

Four hemoglobin polypeptide chains connected together form an almost regular ball shape, where each α-chain is in contact with two β-chains (Fig. 1.20.). There are many hydrophobic radicals in the contact region between α1 and β1, and also between α2- and β2-chains. Therefore, a strong compound is formed between these polypeptide chains for hydrophobic as well as ionic and hydrogen bonds. As a result, dimers α1β1 and α2β2 are formed.

Polar (ionic and hydrogen) bonds arise between these dimers in the tetrameric hemoglobin molecule; therefore, when the pH of the medium changes to the acidic

or alkaline side, the bonds between the dimers are first destroyed. In addition, dimers are able to easily move relative to each other. Since the surface of the protomers is uneven, the polypeptide chains in the central region cannot adhere closely to each other; as a result, a «central cavity» is formed in the center, which passes through the entire hemoglobin molecule. The weaker interactions between dimers result in the two dimers occupying different relative positions in deoxyhemoglobin (T form) as compared with oxyhemoglobin (R form) (Fig. 1.21).

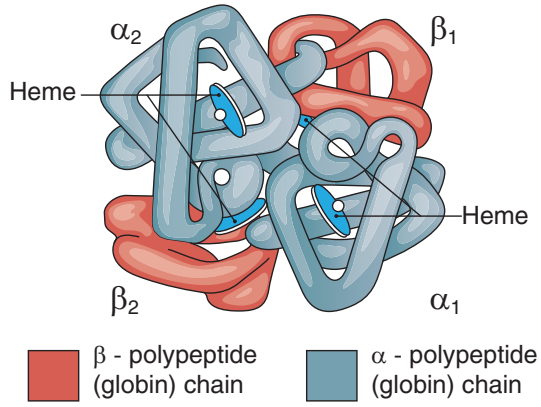


Fig. 1.20. Quaternary structure of hemoglobin A

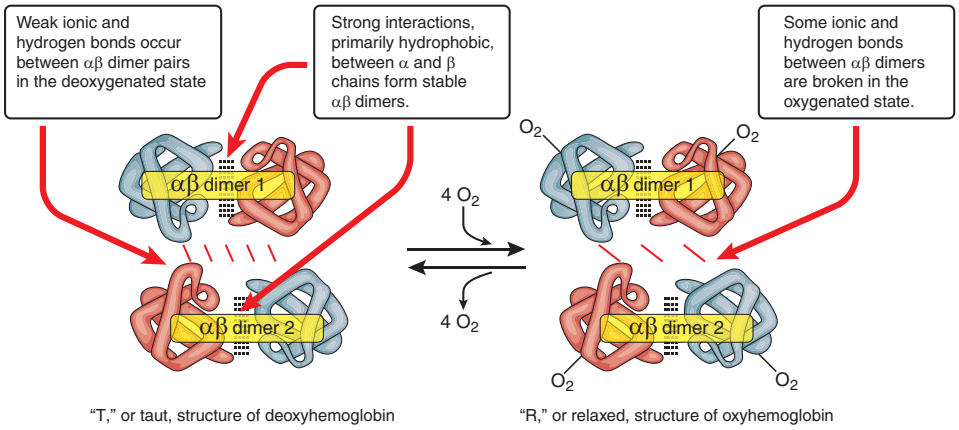


Fig. 1.21. Interaction between hemoglobin subunits

O_2 binds to hemoglobin protomers via Fe^{2+}, which is connected to four nitrogen atoms of the heme pyrrole rings and His F8 nitrogen atom. Oxygen is bound to the remaining free coordination bond Fe^{2+} on the other side of the heme plane in the His E7 region. In deoxyhemoglobin, the Fe^{2+} atom, due to the covalent bond with the

protein part, protrudes from the heme plane in the direction of His F8. The addition of O_2 to the Fe^{2+} ion of one protomer causes it to move to the heme plane; the His F8 residue and the polypeptide chain of which it enters also move behind it. Since proteins have conformational lability, the conformation of the whole protein changes. Conformational changes that occurred in other protomers facilitate the attachment of the next O_2 molecule, which causes new conformational changes in the protein and accelerates the binding of the next O_2 molecule. The fourth O_2 molecule attaches to hemoglobin 300 times easier than the first molecule (Fig. 1.22).

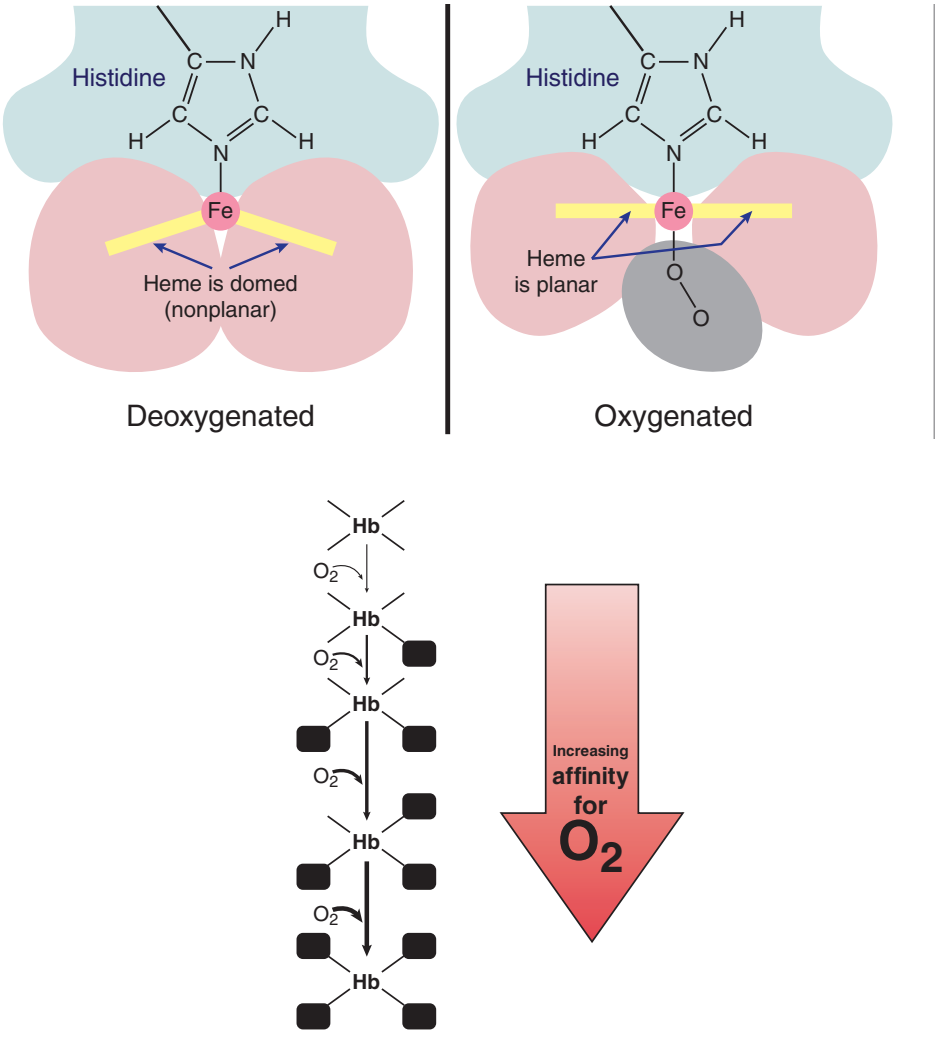


Fig. 1.22. Cooperative changes in the conformation of hemoglobin protomers upon addition of O_2

A change in the conformation and, as a consequence, the functional properties of all protomers of the oligomeric protein upon ligand attachment to only one of them is called **cooperative changes** in the conformation of protomers. Similarly, in tissues the dissociation of each O_2 molecule changes the conformation of all protomers and facilitates the cleavage of the subsequent O_2 molecules.

The cooperative effect of hemoglobin protomers can also be observed on the O_2 dissociation curves for myoglobin and hemoglobin (Fig. 1.23). The ratio of O_2 occupied protein binding sites to the total number of such binding sites is called the degree of oxygen saturation of these proteins. Dissociation curves show how saturated these O_2 proteins are for various values of the partial oxygen pressure.

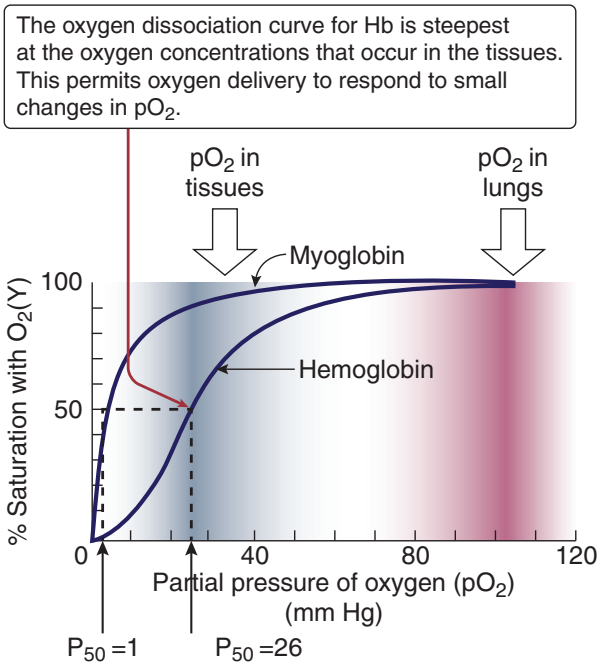


Fig. 1.23. Oxygen dissociation curves for myoglobin and hemoglobin depending on the partial pressure of oxygen

Myoglobin binds oxygen, which releases hemoglobin in the tissue capillaries, and myoglobin itself can release O_2 in response to increased demand for muscle tissue and intensive use of O_2 during exercise. Myoglobin has a very high affinity for O_2. Even at a partial pressure of O_2, equal to 1–2 mm Hg myoglobin remains bound to O_2 by 50%. Hemoglobin has a significantly lower affinity for O_2; half-saturation of hemoglobin occurs at a higher pressure of O_2 (about 26 mm Hg). The hemoglobin dissociation curve has a sigmoid shape (S-shaped). This indicates that the hemoglobin protomers work cooperatively: the more O_2 is released by the protomers, the easier is the cleavage of subsequent O_2 molecules.

Due to the unique structure, each of the considered proteins is adapted to perform its function: myoglobin — attach O_2 released by hemoglobin, accumulate it in the cell and give it up, if necessary. Hemoglobin is an additive in the lungs to O_2, where its saturation reaches 100%, and release O_2 in the capillaries of tissues, depending on the change in O_2 pressure in them.

Transfer of H^+ and CO_2 from tissues to the lungs by hemoglobin. Bohr effect

In the mitochondria of cells, organic substances are oxidized with oxygen to produce energy. In this case, the final decomposition products CO_2 and H_2O are formed, the amount of which is proportional to the intensity of oxidation processes. CO_2 formed in the tissues is transported to red blood cells. There, under the action of the enzyme carbonic anhydrase, which turns it into carbonic acid, an increase in the rate of its formation occurs. Weak carbonic acid H_2CO_3 can dissociate into H^+ and HCO_3^-. In erythrocytes located in the tissue capillaries, the reaction equilibrium shifts to the right, since protons formed as a result of dissociation of carbonic acid can attach to specific parts of the hemoglobin molecule: His46 radicals of two β-chains, His122 radicals and terminal α-amino groups of two α-chains. All these 6 sites, upon the transition of hemoglobin from oxy to deoxy form, acquire a greater affinity for H^+ as a result of a local change in the amino acid environment around these sites (the approach of negatively charged carboxyl groups of amino acids). The addition of 3 pairs of protons to hemoglobin reduces its affinity for O_2 and enhances the transport of O_2 to tissues that need it (Fig. 1.24). The increase in the release of O_2 hemoglobin depending on the concentration of H^+ is called the Bohr effect, after the Danish physiologist Christian Bohr, who first discovered this effect.

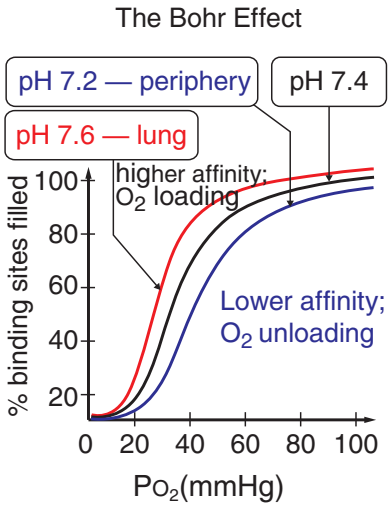


Fig. 1.24. Hemoglobin-oxygen dissociation curve and Bohr effect

In the lung capillaries, the high partial pressure of O_2 leads to oxygenation of the hemoglobin and removal of 6 protons. The reaction $CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$ is shifted to the left and the formed CO_2 is released into the alveolar space and is removed with exhaled air (Fig. 1.25). During evolution, the hemoglobin molecule acquired the ability to perceive and respond to environmental changes. An increase in the concentration of protons in the medium decreases the affinity of O_2 for hemoglobin and enhances its transport into tissues.

Most CO_2 is transported by blood in the form of HCO_3^- bicarbonate. A small amount of CO_2 (about 15–20%) can be transferred to the lungs, reversibly attaching to non-ionized terminal α-amino groups. As a result, carboxyhemoglobin is formed. The addition of CO_2 to hemoglobin also reduces its affinity for O_2.

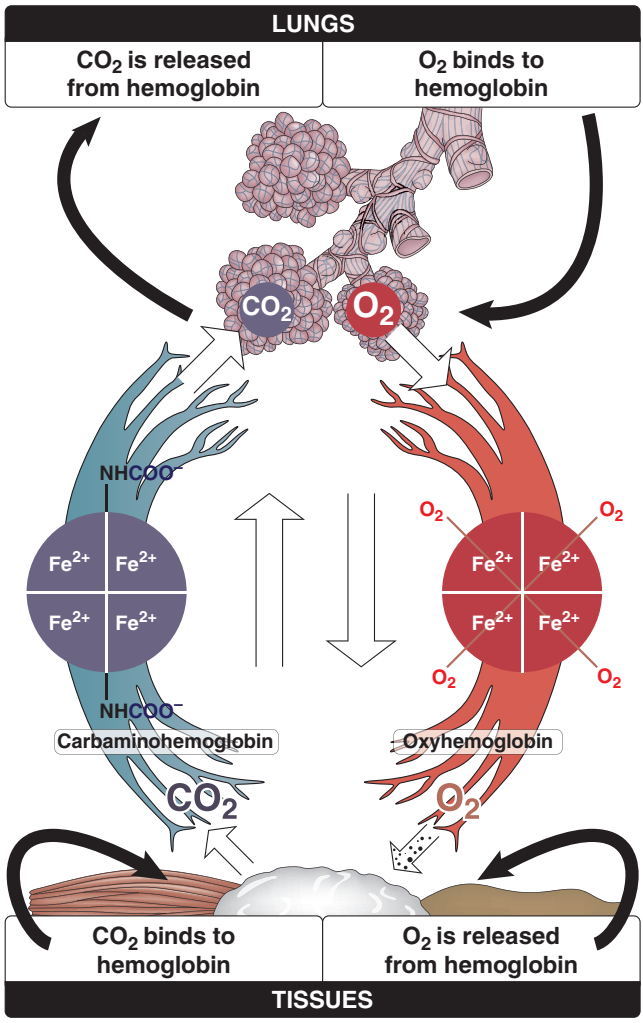


Fig. 1.25. Transfer of O_2 and CO_2 with blood

2,3-bisphosphoglycerate is an allosteric regulator of hemoglobin affinity for O_2

2,3-bisphosphoglycerate (2,3-BPG) is a substance synthesized in red blood cells from an intermediate product of 1,3-bisphosphoglycerate as a result of glucose oxidation. Under normal conditions, 2,3-bisphosphoglycerate is present in red blood cells at approximately the same concentration as hemoglobin. 2,3-BPG, when combined with hemoglobin, can also change its affinity for O_2.

At the center of the tetrameric hemoglobin molecule, there is a cavity formed by amino acid residues of all four protomers. The central cavity is the site of attachment of 2,3-BPG. The dimensions of the central cavity can vary; the removal of O_2 from oxyhemoglobin causes its conformational changes, which contribute to the formation of additional ionic bonds between dimers α1β1 and α2β2. As a result, the spatial structure of deoxyhemoglobin becomes more tense and the central cavity expands (Fig. 1.26).

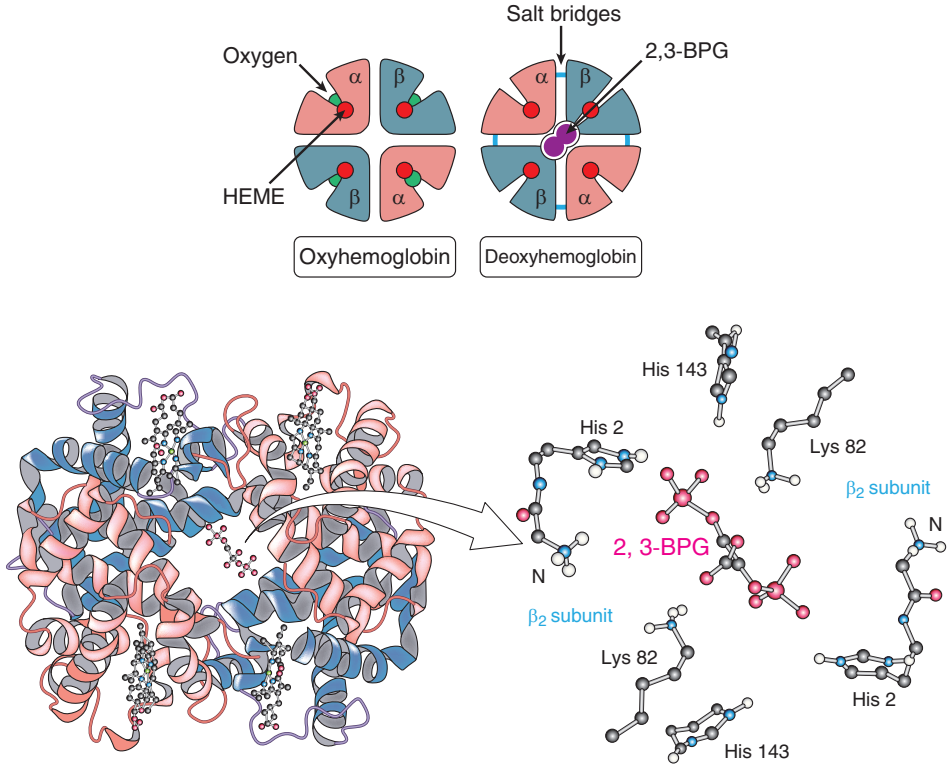


Fig. 1.26. Interaction of 2,3-bisphosphoglycerate with hemoglobin

In the central cavity, there are positively charged radicals of amino acid residues and positively charged α-amino groups of the N-terminal part of β-chains. 2,3-BPG, which has a strong negative charge, is attached to the expanded deoxyhemoglobin

cavity by means of ionic bonds formed with positively charged functional groups. The addition of 2,3-BPG even more stabilizes the taut form structure of deoxyhemoglobin and reduces its affinity for O_2. The addition of 2,3-BPG to deoxyhemoglobin occurs in a different site, compared with the active site where O_2 binding occurs. Such a ligand is called **allosteric** and the center where the allosteric ligand is bonded is the **allosteric center** («allos» — is another, different, «steros» — is spatial). In the lungs, a high partial pressure of O_2 leads to hemoglobin oxygenation. The rupture of ionic bonds between dimers $\alpha 1\beta 1$ and $\alpha 2\beta 2$ leads to relaxation of the protein molecule, a decrease in the central cavity and the displacement of 2,3-BPG.

The concentration of 2,3-BPG in the erythrocytes of people living in certain climatic conditions is a constant value. However, in the period of adaptation to high mountains, when a person rises to an altitude of more than 4000 m above sea level, the concentration of 2,3-BPG in almost 2 days increases almost twice (from 4.5 to 7.0 mM). This reduces the affinity of hemoglobin for O_2 and increases the amount of O_2 transported to the tissue (Fig. 1.27) Changing the concentration of 2,3-BPG works as a mechanism for adapting the body to hypoxia. The same adaptation is observed in patients with lung diseases, in which general tissue hypoxia develops.

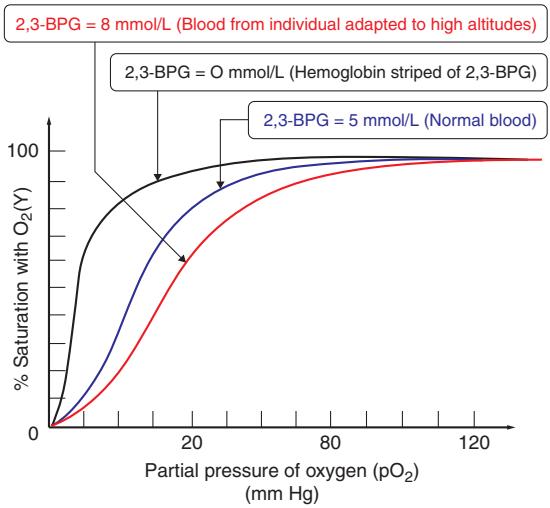


Fig. 1.27. Allosteric effect of 2,3-BPG on the oxygen affinity of hemoglobin

Fetal hemoglobin (HbF) replaces embryonic hemoglobin (HbE) after 2 weeks formation of the fetus liver. From 6 months of fetal development until birth, it is the main hemoglobin of red blood cells. After the birth of a child, it begins to be intensively replaced by hemoglobin A (HbA). Under physiological conditions, HbF has a higher affinity for O_2 than HbA, which creates optimal conditions for the transport of O_2 from maternal blood to fetal blood. This property of HbF is due to the fact that it is weaker than HbA binds to 2,3-BPG. The physiological features of HbF are related to the peculiarities of its structure: instead of β-globin chains in HbA, it contains two

γ-chains. Some of the positively charged amino acid radicals are absent in the primary structure of the γ chains. In a medium lacking 2,3-BPG, HbA and HbF exhibit the same high affinity for O_2.

Hereditary hemoglobinopathy

The importance of the primary structure of proteins for the formation of their conformation and function can be traced to the examples of hereditary diseases associated with a change in the primary structure of hemoglobin. At present, about 300 HbA variants are known that have only small changes in the primary structure of the α or β chains. Some of them barely affect protein function and human health, others reduce protein function, and especially in extreme situations, reduce the possibility of human adaptation, others cause significant impairment of HbA functions and the development of anemia, which leads to serious clinical consequences.

In 1904, a Chicago doctor, J. Herrick, described severe anemia in a student with the discovery of many elongated, sickle-like red blood cells in his blood. The disease was called **sickle cell anemia**, and only in 1949 did L. Pauling and his colleagues prove that it was caused by a change in the primary structure of HbA. In the hemoglobin S (HbS) molecule (the so-called abnormal hemoglobin), 2 β-chains are mutant, in which glutamate, the highly polar negatively charged amino acid at 6th position, was replaced by a valine containing a hydrophobic radical (Fig. 1.28).

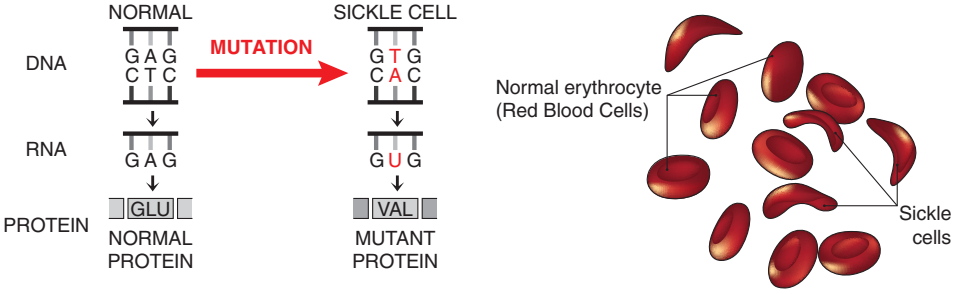


Fig. 1.28. Hemoglobin S (HbS) is a mutant form of hemoglobin

In deoxyhemoglobin S, there is a site complementary to another site of the same molecules containing an altered amino acid. As a result, deoxyhemoglobin molecules begin to stick together, forming elongated fibrillar aggregates that deform red blood cells and lead to the formation of abnormal red blood cells in the form of a sickle. In oxyhemoglobin S, the complementary region is «masked» as a result of changes in protein conformation. The inaccessibility of the site prevents the connection of oxyhemoglobin S molecules with each other. Consequently, the formation of HbS aggregates is facilitated by conditions that increase the concentration of deoxyhemoglobin in cells (physical work, hypoxia, decrease in pH, high altitude conditions, etc). Since sickle-shaped erythrocytes do not pass well through tissue capillaries, they often clog vessels and thereby create local hypoxia. Disruption of O_2

delivery to tissues causes pain and even cell necrosis in this area. Sickle cell anemia is a homozygous recessive disease; it only manifests itself in the case when 2 mutant genes β-chains of globin are followed from both parents (Fig. 1.29).

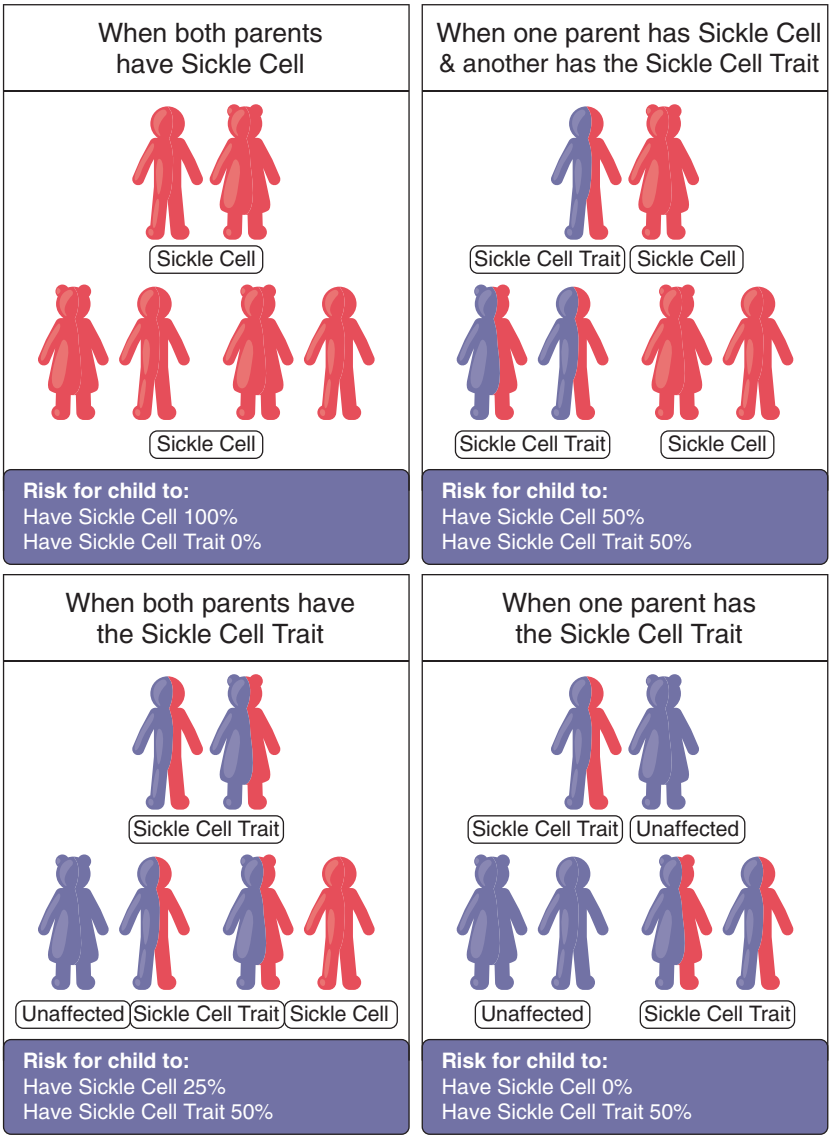


Fig. 1.29. Inheritance of sickle cell anemia and manifestation of the disease

After the birth of a baby, the disease does not appear until significant amounts of HbF are replaced by HbS. Patients reveal clinical symptoms characteristic for anemia: dizziness and headaches, shortness of breath, palpitations, pain in the limbs,

increased susceptibility to infectious diseases. Heterozygous individuals having one normal HLA gene and another HbS gene in blood have only traces of sickle cells and a normal lifespan; clinical symptoms of the disease usually do not appear**.**

Another example of hemoglobinopathy is **methemoglobin**. Hemoglobin M is a variant of hemoglobin A, where the presence of a mutation in the α- or β-chain gene, the His E7 or His F8 is replaced by tyrosine. As a result of such mutation, Fe^{2+} is oxidized to Fe^{3+} and stabilizes in this form. The hemoglobin containing Fe^{3+} in the heme is called methemoglobin (hemoglobin M). Instead of O_2, H_2O is attached to Fe^{3+}. Usually, changes affect either the α- or β-chains, so no more than two O_2 molecules can be carried by hemoglobin. In heterozygous people, cyanosis associated with impaired O_2 transport is noted, and homozygosity for this gene leads to death.

There are several hereditary hemoglobinopathies that most often lead to impaired oxygen transport as well as to hypoxia or cyanosis.

1.5. PHYSICAL CHEMICAL PROPERTIES OF PROTEINS

Individual proteins differ in their physicochemical properties: the shape of the molecules, molecular weight, total charge, solubility, etc.

Differences in the shape of molecules

As mentioned above the form of protein molecules is divided into globular and fibrillar. Globular proteins have a more compact structure, their hydrophobic radicals are mostly hidden in a hydrophobic core, and they are much better soluble in body fluids than fibrillar proteins (the exception is membrane proteins).

Molecular weight differences

Proteins are high-molecular compounds, but can vary greatly in molecular weight, which ranges from 6,000 to 1,000,000 D and higher. The molecular weight of the protein depends on the number of amino acid residues in the polypeptide chain, and for oligomeric proteins it depends on the number of protomers (or subunits) included in it.

Total charge of protein

Proteins have the property of amphotericity. That is, depending on the conditions, they exhibit both acidic and basic properties. In proteins, there are several types of chemical groups capable of ionization in an aqueous solution: carboxylic acid residues of the side chains of acidic amino acids (aspartic and glutamic acids) and nitrogen-containing groups of the side chains of basic amino acids (primarily the amino group of lysine and the amid residue CNH (NH_2) of arginine).

Protein charge depends on the ratio of acidic and basic amino acids. Therefore, like amino acids, proteins charge positively with decreasing pH and negatively with increasing it. If the pH of the solution corresponds to the isoelectric point of the protein, then the charge of the protein is 0. If acidic amino acids (glutamate and aspartate) predominate in the peptide or protein, then the protein is acidic, at neutral pH the protein charge is negative and the isoelectric point is in the acidic environment. For most natural proteins, the isoelectric point is in the pH range of

4.8–5.4, which indicates the predominance of glutamine and aspartic amino acids in their composition. If the protein is dominated by basic amino acids (lysine and arginine), then at neutral pH the protein charge is positive and it is due to these positively charged amino acids.

Amphotericity is important for proteins to perform certain functions. For example, the buffer properties of proteins, i.e. the ability to maintain unchanged blood pH, based on the ability to attach H^+ ions during acidification of the environment or give them when alkalizing. On the practical side, the presence of amphotericity makes it possible to separate proteins by charge (electrophoresis) or use a change in the pH of the solution to precipitate any known protein. The presence of both positive and negative charges in a protein determines their ability to salting out, which is convenient for isolating proteins in a native (living) conformation.

As the pH in the solution changes, the concentration of H^+ ions changes too. When the medium is acidified (with a decrease in pH) below the isoelectric point, H^+ ions attach to the negatively charged groups of glutamic and aspartic acids and neutralize them. The protein charge in this case becomes positive. With increasing pH in the solution above the isoelectric point, the concentration of H^+ ions decreases and positively charged protein groups (NH_3^+ groups of lysine and arginine) lose protons, their charge disappears. The total charge of the protein becomes negative (Fig. 1.30).

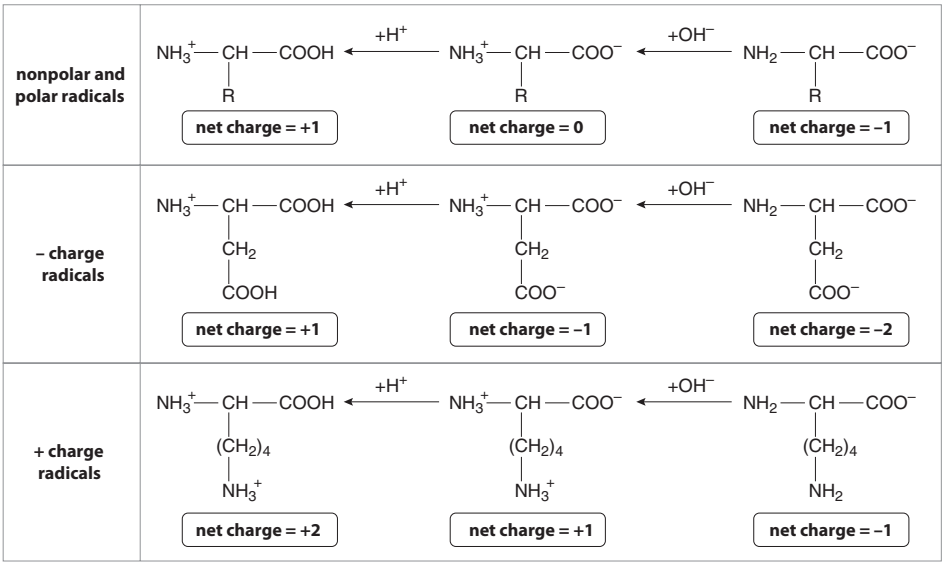


Fig. 1.30. Changes in the total charge of a protein with a change in pH

The pH value at which the protein acquires a total zero charge is called the isoelectric **point** and is denoted as **pI**. At the isoelectric point, the number of positively and negatively charged protein groups is the same, i.e. the protein is in an isoelectric state. Since most of the proteins in the cell contain more anionic groups (-COO-),

the isoelectric point of these proteins lies in a slightly acidic medium. The isoelectric point of the proteins, in which cationogenic groups predominate, is in an alkaline environment. The most striking example of such intracellular proteins containing a lot of arginine and lysine are histones, which are part of chromatin.

Proteins having a total positive or negative charge are better soluble than proteins located at an isoelectric point. The total charge increases the number of water dipoles that can bind to a protein molecule, and prevents the contact of the same charged molecules, as a result, the solubility of proteins increases. Charged proteins can move in an electric field: anionic proteins having a negative charge will move to a positively charged anode (+), and cationic proteins to a negatively charged cathode (–). Proteins in an isoelectric state do not move in an electric field. At the isoelectric point, proteins are the least stable in a solution and easily precipitate. The isoelectric point of the protein is highly dependent on the presence of salt ions in the solution; at the same time, its value is not affected by protein concentration.

With a shift in the acid-base balance of the body towards an increase in acidity (decrease in pH), acidosis occurs. For example, with diabetes, there is an increase in the production of ketone bodies (ketoacidosis). This is a dangerous condition for the body, proteins lose their charge and their solubility decreases, while they can aggregate.

Protein solubility

Most proteins carry many charged groups on the surface; therefore, they are soluble in water. Solubility is due to the presence of a charge, as well as the repulsion of charged protein molecules. Besides, the presence of a hydration shell, i.e. environment of a protein molecule with water dipoles and their interaction with polar and charged groups on the surface of a protein globule.

Protein isolation and purification methods

Obtaining individual proteins from biological material (tissues, organs, cell cultures) requires sequential operations, including:

- crushing of biological material and destruction of cell membranes;
- fractionation of organelles containing certain proteins;
- protein extraction (translating them into a dissolved state);
- separation of a mixture of proteins into individual proteins.

Protein separation methods are based on the physicochemical properties of proteins. The table (Table 1.3) shows the main methods of protein purification.

Ion exchange chromatography

The method is based on the separation of proteins that differ in total charge at certain pH and ionic strength of the solution. When a protein solution is passed through a chromatographic column filled with solid porous charged material, some of the proteins are retained on it as a result of electrostatic interactions. As the stationary phase, ion exchangers are used — polymer organic substances containing charged functional groups. There are positively charged anion exchangers containing cationic groups and negatively charged cation exchangers containing anionic groups. The choice of an ion

Table 1.3. The main methods of protein purification

Characteristic	Procedure
1. Charge	1. Ion-exchange chromatography 2. Electrophoresis 3. Isoelectric focusing
2. Polarity	1. Adsorption chromatography 2. Paper chromatography 3. Reverse-phase chromatography 4. Hydrophobic chromatography
3. Size	1. Dialysis and ultrafiltration 2. Gel electrophoresis 3. Gel filtration chromatography 4. Ultracentrifigation
4. Specificity	1. Affinity chromatography

exchanger is determined by the charge of the protein released. So, to isolate a negatively charged protein, an anion exchanger is used. When a protein solution is passed through a column, the binding strength of the protein to the anion exchanger depends on the number of negatively charged carboxyl groups in the molecule. Proteins adsorbed on the anion exchanger can be washed off (eluted) with buffer solutions with different salt concentrations, most often NaCI, and different pH values. Chlorine ions bind to the positively charged functional groups of the anion exchanger and displace the carboxyl groups of the proteins. At low salt concentrations, proteins weakly bound to the anion exchanger elute. A gradual increase in salt concentration or a change in pH, which changes the charge of a protein molecule, leads to the release of protein fractions, one of which contains the desired protein (Fig. 1.31).

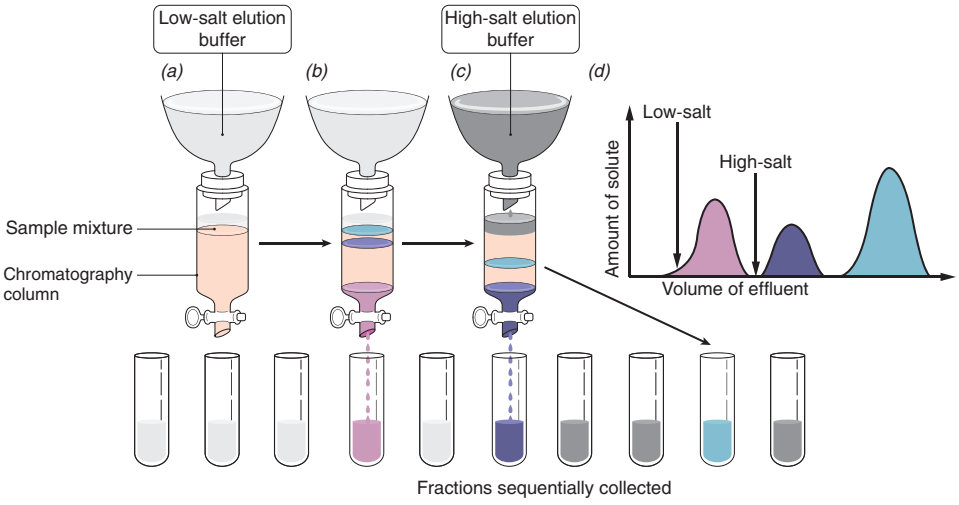


Fig. 1.31. The basic principles of ion exchange chromatography

Electrophoresis

The method is based on the property that, at a certain pH and ionic strength of a solution, proteins move in an electric field at speed proportional to their total charge. Proteins with a total negative charge move to the anode (+), and positively charged proteins move to the cathode (–). Electrophoresis is carried out on various media: paper, starch gel, polyacrylamide gel, etc. Unlike paper electrophoresis, where the speed of proteins is proportional only to their total charge, in polyacrylamide gel the speed of movement of proteins is proportional to their molecular weights (Fig. 1.32).

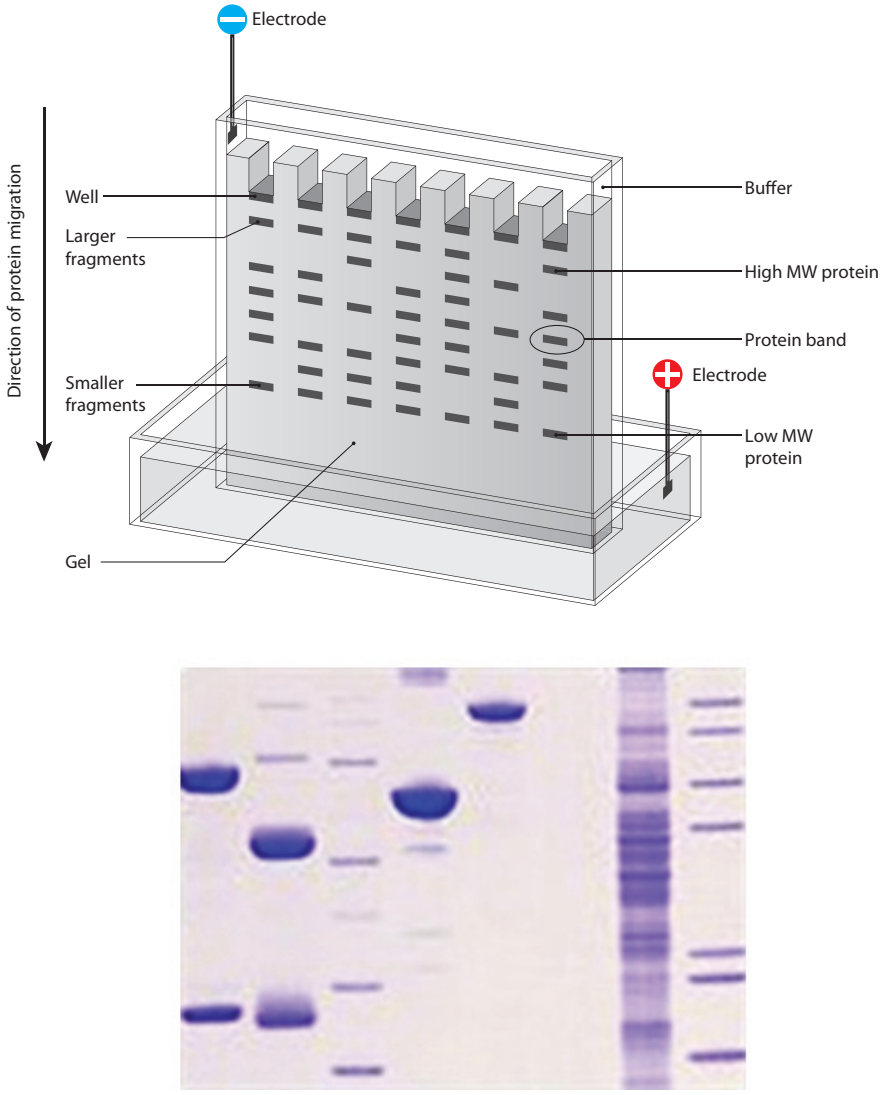


Fig. 1.32. The principle of separation of proteins using electrophoresis gel. Gel stained by Coomassie brilliant blue reagent

The resolution of polyacrylamide gel electrophoresis is higher than on paper. To detect protein fractions, strips of paper or polyacrylamide gel are dyed. The colored complex of proteins with a dye reveals the location of various fractions on the carrier.

Gel filtration chromatography

The method of protein separation using gel filtration chromatography is based on the fact that substances with different molecular weights are distributed differently between the stationary and mobile phases of the chromatographic column. It is filled with granules of a porous substance (Sephadex, agarose, etc.), cross-links are formed in the structure of the polysaccharide and granules with «pores» are formed, through which water and low molecular weight substances easily pass. Depending on the conditions, granules with different pore sizes can be formed. The stationary phase is the liquid inside the granules into which low molecular weight substances and proteins with a small molecular weight can penetrate. A mixture of proteins deposited on a chromatographic column is washed (eluted) by passing a solvent through the column. The largest molecules move along with the solvent front. Smaller molecules diffuse inside the Sephadex granules and fall into the stationary phase for some time, as a result of which their movement is delayed. The size of the pores determines the size of the molecules that can penetrate into the granules (Fig. 1.33).

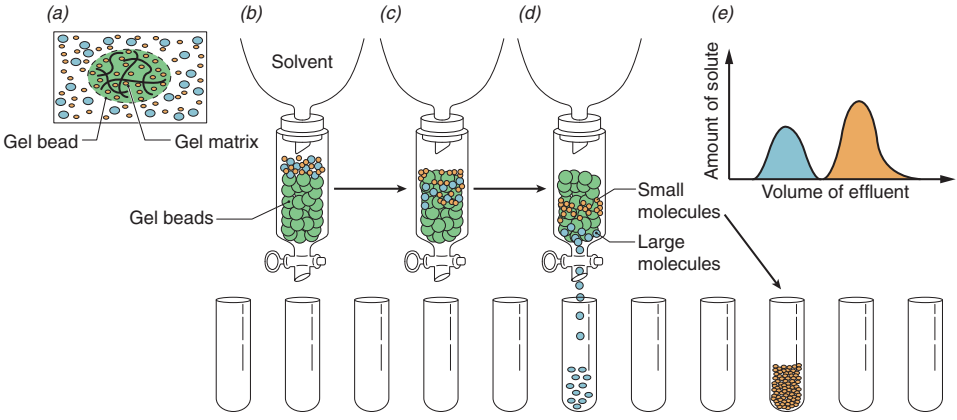


Fig. 1.33. The basic principles of gel filtration chromatography

Affinity chromatography

This is the most specific method for isolating individual proteins, based on the selective interaction of proteins with ligands attached (immobilized) to a solid carrier. As a ligand, a substrate or coenzyme can be used if any enzyme, antigens for the isolation of antibodies, etc. are isolated. A solution containing a protein mixture is passed through a column filled with an immobilized ligand. Only a protein that specifically interacts with it is attached to the ligand; all other proteins come out with the eluate. The protein adsorbed on the column can be removed by washing it with

a solution with a changed pH value or a changed ionic strength. In some cases, a detergent solution is used to break the hydrophobic bonds between the protein and the ligand. Affinity chromatography is highly selective and helps to purify the secreted protein thousands of times (Fig. 1.34).

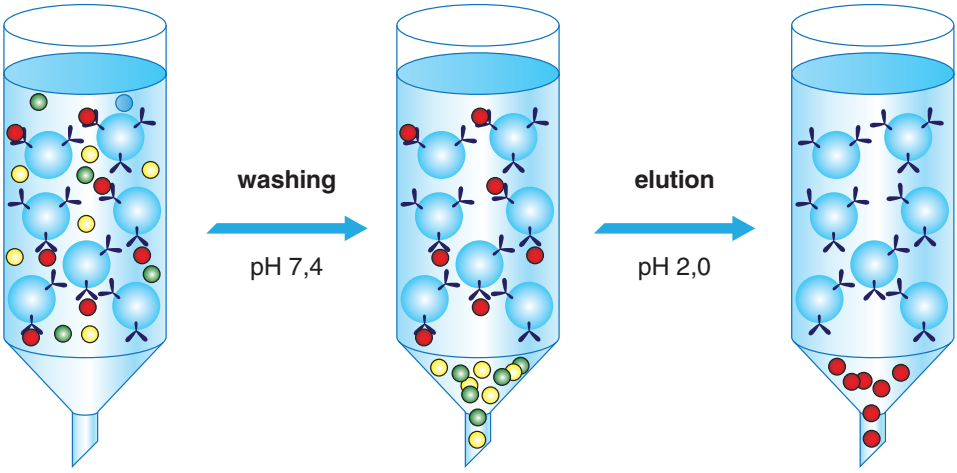


Fig. 1.34. The basic principles of affinity chromatography

1.6. DISEASES ASSOCIATED WITH STRUCTURE AND FUNCTION PROTEINS DISORDERS

The protein composition of an adult is more or less constant. However, some changes in the content of certain proteins are possible depending on physiological activity, food composition, etc. In diseases, the protein composition of tissues also changes. These manifestations of the disease are called proteinopathies.

In medicine, proteopathy refers to a class of diseases in which certain proteins become structurally abnormal, and thereby disrupt the function of cells, tissues and organs of the body. Often the proteins fail to fold into their normal configuration; in this misfolded state, the proteins can become toxic in some way (a gain of toxic function), or they can lose their normal function. The proteopathies (also known as protein conformational disorders or protein misfolding diseases) include such diseases as Alzheimer's disease, Parkinson's disease, amyloidosis, multiple system atrophy and a wide range of other disorders. Separate primary and secondary proteinopathy.

Primary proteinopathy

Hereditary proteinopathy develops as a result of a damage in the genetic apparatus of the individual. Any protein is not synthesized at all or is synthesized, but its primary

structure is changed. Examples of hereditary proteinopathy are hemoglobinopathies, that have been discussed above. Depending on the role of the defective protein in the vital activity of an organism, on the degree of disturbance of the conformation and function of proteins, on the homo-or heterozygosity of an individual for this protein, hereditary proteinopathies can cause diseases with varying degrees of severity, even death before birth or in the first months after birth.

Secondary proteinopathy

Any disease is accompanied by a change in the protein composition of the body, i.e. secondary proteinopathy develops. At the same time, the primary structure of proteins is not disturbed, and usually there is a quantitative change in proteins, especially in those organs and tissues in which the pathological process develops. For example, pancreatitis reduces the production of enzymes necessary for the digestion of nutrients in the gastrointestinal tract. In some cases, acquired proteinopathy develops as a result of changes in the conditions in which proteins function. So, when the pH of the medium changes to the alkaline side (alkalosis of different nature), the conformation of hemoglobin changes, its affinity for O_2 increases, and the delivery of O_2 to tissues (tissue hypoxia) decreases.

Sometimes as a result of the disease, the level of metabolites in the cells and blood serum increases, which leads to the modification of certain proteins and the disruption of their function. Thus, elevated blood glucose concentrations in diabetes mellitus lead to non-enzymatic attachment of glucose to proteins (glycosylation of proteins). An example is an increase in the level of glycated hemoglobin in red blood cells, which increases its affinity for O_2 and reduces the transport of O_2 into tissues. Glycosylation of proteins of the lens of the eye leads to its clouding and the development of cataracts.

In some cases, biochemical data on changes in the protein composition of the blood or urine can be leading in the diagnosis. For example, in myeloma (malignant degeneration of plasma cells that synthesize immunoglobulins), Bens-Jones proteins appear in the blood and urine, they are present in low concentrations in the blood of healthy people. These proteins are the light chains of immunoglobulin G, the synthesis of which is enhanced in malignantly reborn cells.

Conformational disorders

Some water-soluble proteins, when the conditions change, can acquire the conformation of poorly soluble molecules capable of aggregation, forming fibrillar deposits in cells called amyloid (from lat. amylum — starch). Like starch, amyloid deposits are detected by iodine staining of the tissue. This may occur:

- with the overproduction of certain proteins, as a result of which their concentration in the cell increases;
- when proteins enter the cells or form proteins in them that can affect the conformation of other protein molecules;
- upon activation of proteolysis of normal body proteins, with the formation of insoluble fragments prone to aggregation;
- as a result of point mutations in the protein structure.

As a result of the deposition of amyloid in the organs and tissues, the structure and function of cells are disrupted, their degenerative changes and the proliferation of connective tissue cells are observed. A disease called amyloidosis develops. Each type of amyloidosis is characterized by a specific type of amyloid. Currently, more than 15 such diseases are described.

Alzheimer's disease is the most frequently observed β-amyloidosis of the nervous system, usually affecting elderly people and characterized by progressive memory disorder and complete personality degradation. In the brain tissue, β-amyloid is deposited — a protein that forms insoluble fibrils that disrupt the structure and function of nerve cells. Protein β-amyloid is a product of a change in the conformation of a normal protein of the human body. It is formed from a larger precursor by partial proteolysis and is synthesized in many tissues. Protein β-amyloid, unlike its normal precursor, which contains many α-helical regions, has a secondary β-fold structure that can aggregate with the formation of insoluble fibrils, and is resistant to proteolytic enzymes.

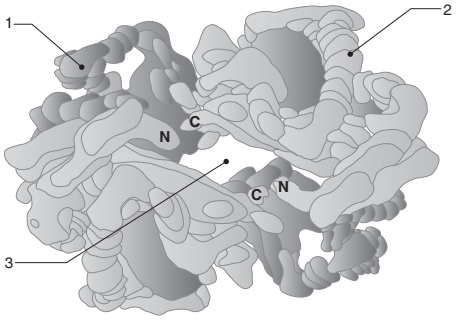
Parkinson's disease was first described in 1817 by the English physician James Parkinson (he called it «trembling paralysis») and is one of the most common neurodegenerative disorders. Most often, parkinsonism and other neurodegenerative disorders (such as Alzheimer's disease) are found in the elderly and along with oncological diseases occupy a leading position among the causes of death. But parkinsonism is not only a disease of the elderly: with the improvement of diagnostic methods, there is more and more evidence that the disease affects people under 40 years of age. As follows from the original name of the disease, its characteristic symptoms are motor disorders: trembling (tremors) of fingers, lower jaw and tongue, head and eyelids, slowness and impoverishment of the pattern of movements, stiffness of the body, difficulty in starting and stopping movement, impaired coordination, etc. Such disorders are caused by the flexibility of nerve cells, primarily the loss of pigment-containing neurons of the *substantia nigra*, which produce the dopamine neurotransmitter. Dopamine is the biochemical precursor of norepinephrine and adrenaline. In many patients who died from Parkinson's disease, protein clusters are found during an autopsy in the *substantia nigra* (they are called Levy bodies by the name of the German pathologist who discovered them in 1912). Parkinson's disease develops as a result of disturbances in the functioning of the chaperone and ubiquitin-proteasome systems. Apparently, the situation is as follows. Some damage in the neurons of the *substantia nigra* triggers a cascade of reactions leading to the appearance of a large number of improperly packaged proteins.

Review tests

1. Choose one correct answer. HbS in contrast to HbA:
A. Has a 6-position α-chain Val.
B. Contains 2α and 2γ chains.
C. Deoxyform is poorly soluble in water.
D. Has a high affinity for O_2.
E. In the active center there is an amino acid substitution.

2. Choose one correct answer. Isoelectric point of proteins:
A. The amount of protein charge.
B. The ratio of polar and nonpolar amino acid radicals.
C. The pH value at which the protein has a charge equal to zero.
D. The pH value at which the protein is best soluble in water.
E. The charge of the protein at which it is most actively moving in the electric field.

3. Match the figure with the letter. Hemoglobin molecule:
A. α chain Hb.
B. β chain Hb.
C. Active center.
D. Central cavity.
E. Domain.

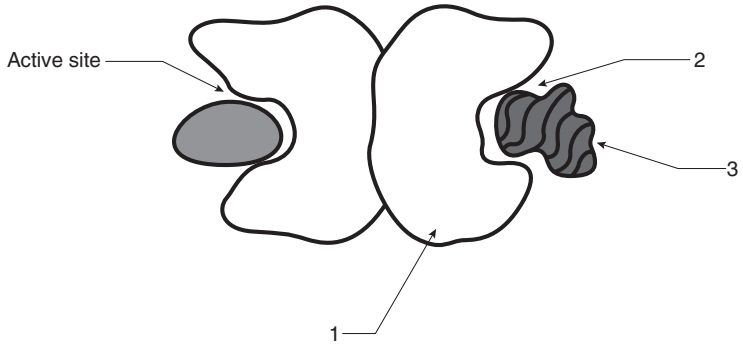


4. Match the figure with the letter. Protein separation methods:
1. Gel filtration.
2. Electrophoresis.
3. Affinity chromatography.

The principles of separation by difference:
A. Charges.
B. Solubility in water.
C. Molecular weight.
D. Sedimentation rate in solution.
E. Affinity for a specific ligand.

5. Choose all correct answers. The affinity of HB to oxygen decreases with:
A. An increase in the concentration of protons.
B. A decrease in the concentration of protons.
C. A decrease in the concentration of 2,3-BPH.
D. An increase in the concentration of 2,3-BPH.
E. Sequential cleavage of oxygen molecules.

6. Match the figure with the letter. Functional parts of the oligomeric protein:



A. Subunit.
B. An allosteric center.
C. Active center.
D. An oligomer.
E. An effector.

Situational problems

1. In high altitude conditions, climbers usually feel the clinical signs of hypoxia: headache, shortness of breath, nausea, increased heart rate. However, after 2 days of rest in the base camp, the symptoms disappear. How does the body adapt to high altitude conditions and increase oxygen delivery to tissues?
2. The fetal HbF has a higher affinity for oxygen than the mother HbA. What structural features of these proteins determine their difference in the ability to bind oxygen and what role does 2,3-bisphosphoglycerate play in this? What is the physiological meaning of the different affinities of these Hb forms for oxygen?
3. The patient went to the clinic with complaints of dizziness, shortness of breath, palpitations and pain in the limbs, which sharply worsened after a short rest in the mountains. A reduced number of red blood cells were found in the patient's blood, as well as immature crescent-shaped cells and red blood cells. What are the causes of this pathology? Why did the disease worsen in conditions of low partial pressure of oxygen?