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).

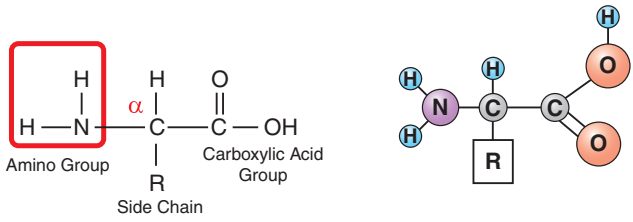


Fig. 1.1. Basic amino acid formula

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.

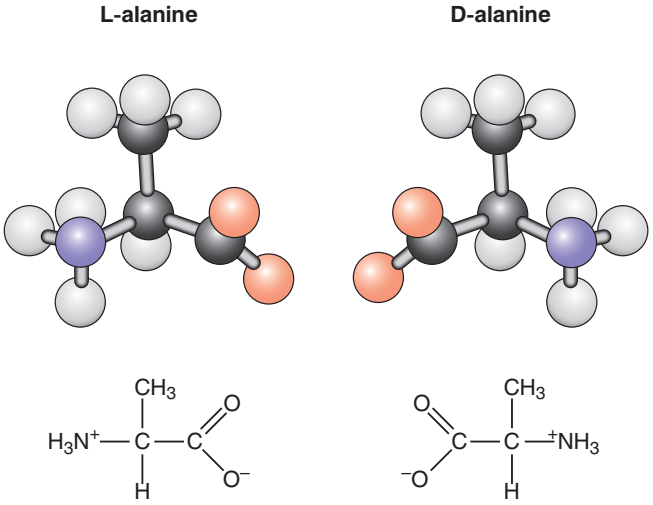


Fig. 1.2. Optical isomers of amino acids

- **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.

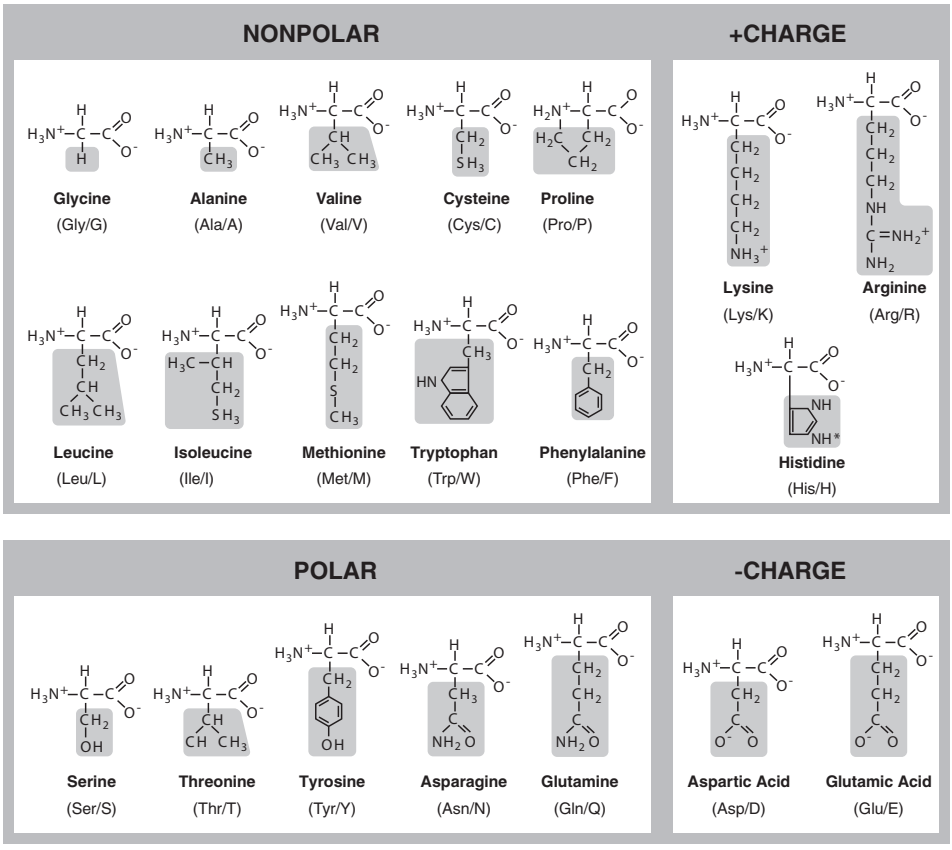
To designate amino acids in proteins, their trivial names are usually used. In addition, for the convenience of recording the amino acid sequence of peptides and proteins, their three-letter and one-letter designations are used (Table 1.1).

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**.

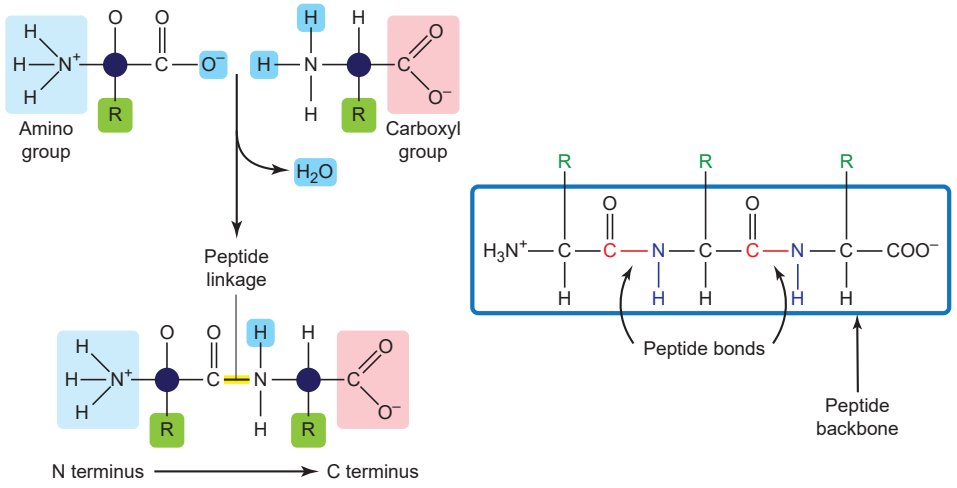


Fig. 1.3. Peptide bond formation

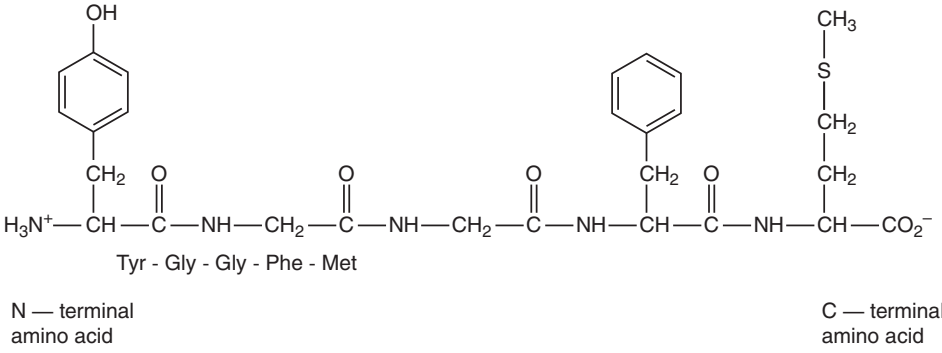


Fig. 1.4. Formula of pentapeptide Tyr-Gly-Gly-Phe-Met

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).

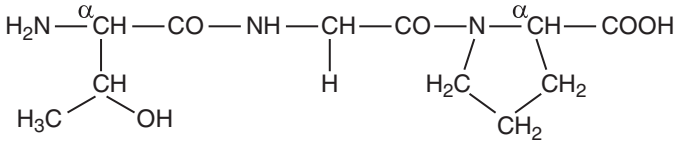


Fig. 1.5. Formation of a peptide bond between Threonine and Proline

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).

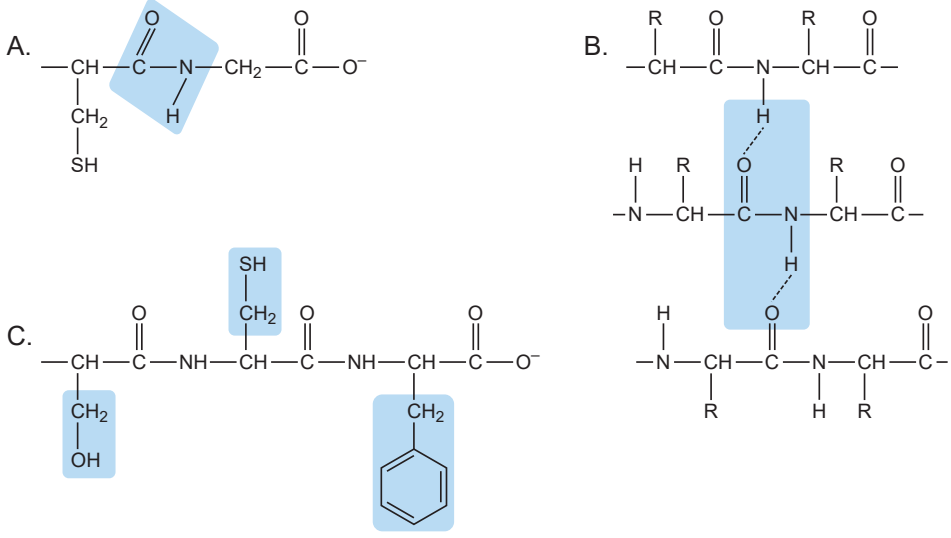


Fig. 1.6. Properties of the peptide bond

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.

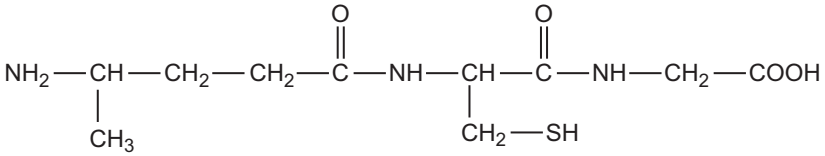
The biological role of amino acids and peptides

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.).

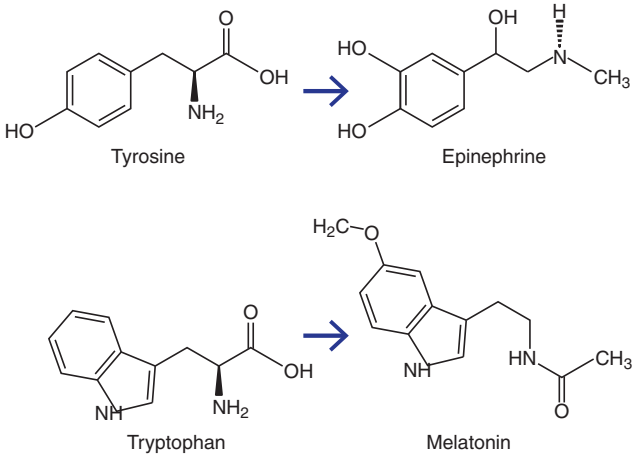
A number of hereditary and acquired diseases, accompanied by serious problems in the development of the organism, such as cystinosis, homocysteinemia, leucinosis, tyrosinemia, etc., are associated with metabolic disorders of amino acids. Phenylketonuria is the most famous example of metabolic disorders 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.

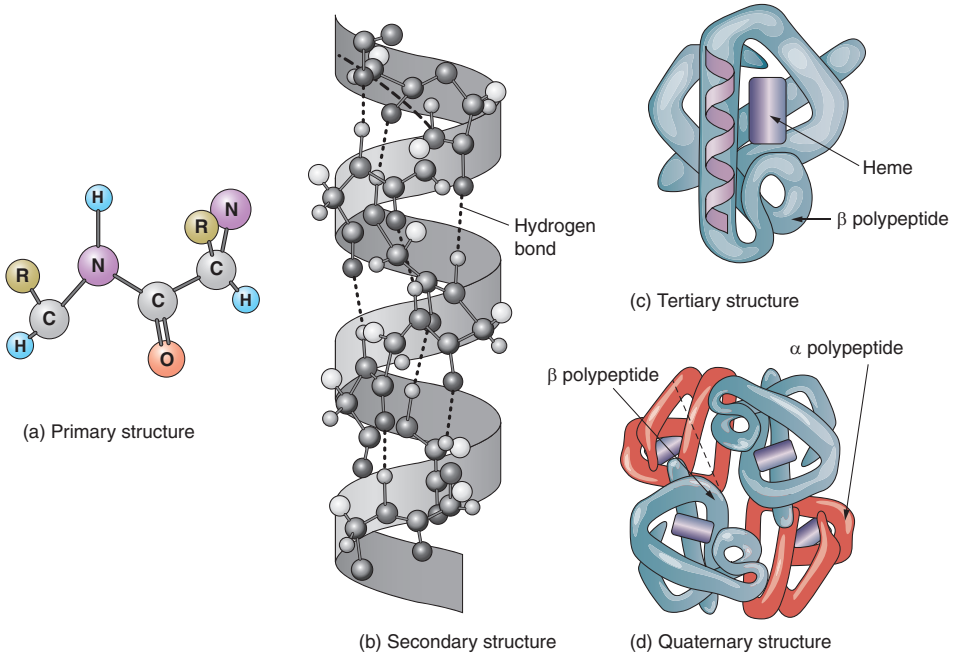


Fig. 1.7. Protein organization levels

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).

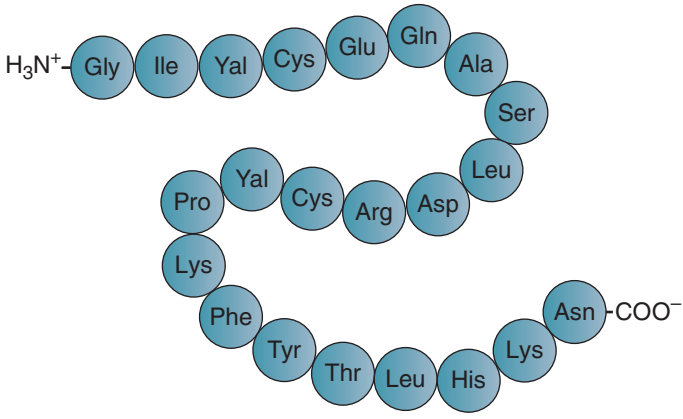


Fig. 1.8. Primary structure of the protein

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).

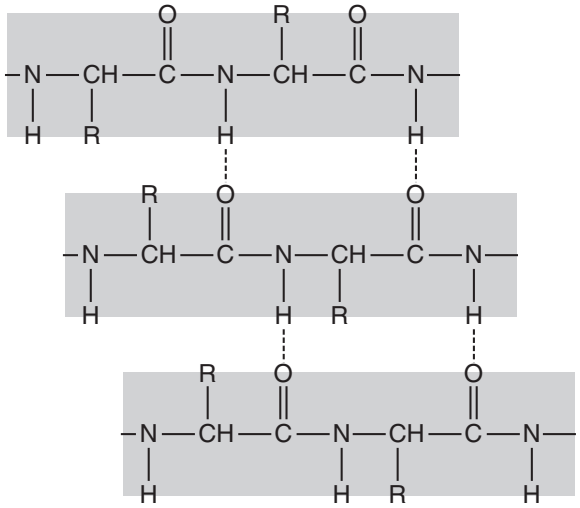


Fig. 1.9. Hydrogen bonds between peptide backbone groups form a secondary protein structure

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.

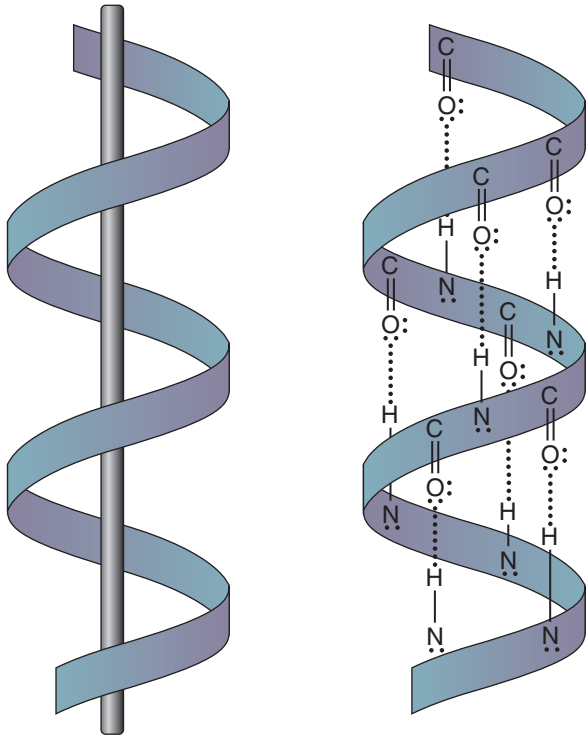


Fig. 1.10. The secondary structure of proteins in the form of α-helix

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.

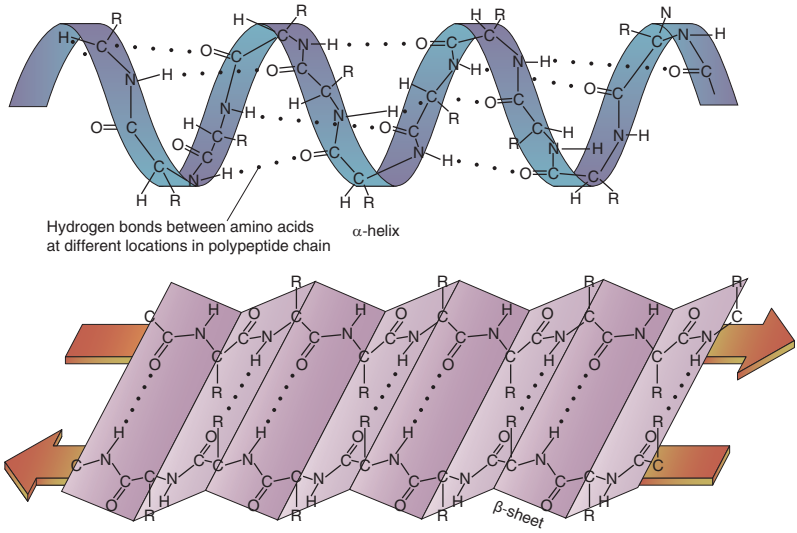


Fig. 1.11. The secondary structure of proteins in the form of β-sheet

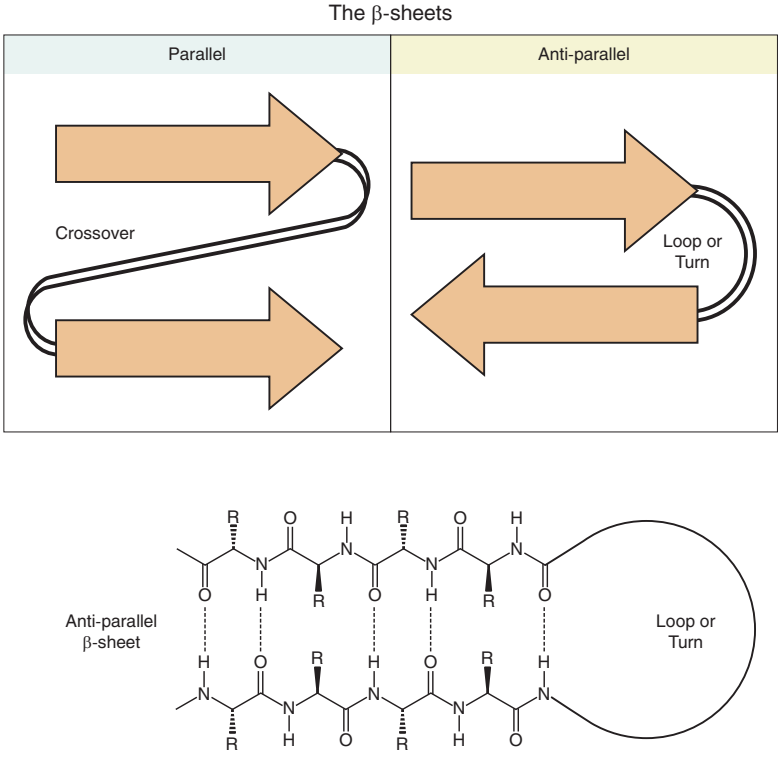


Fig. 1.12. Parallel and antiparallel β-sheets

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).

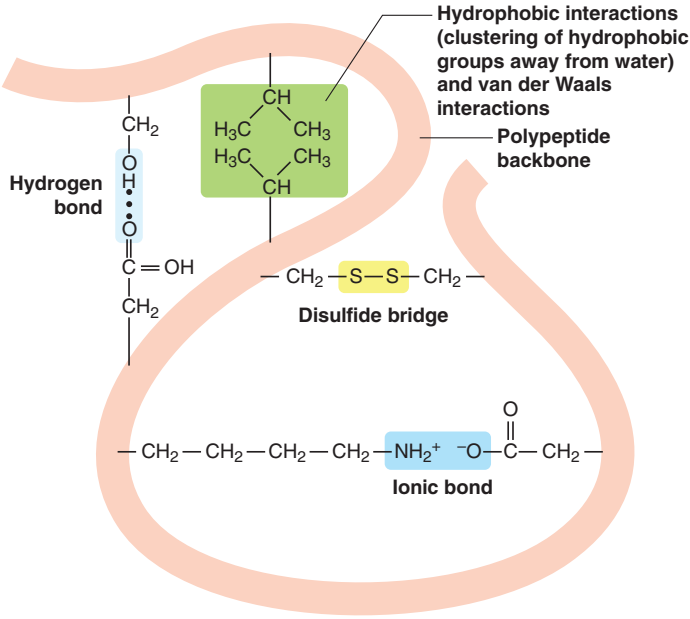
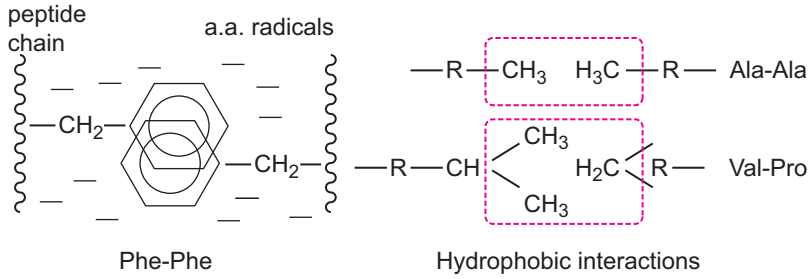


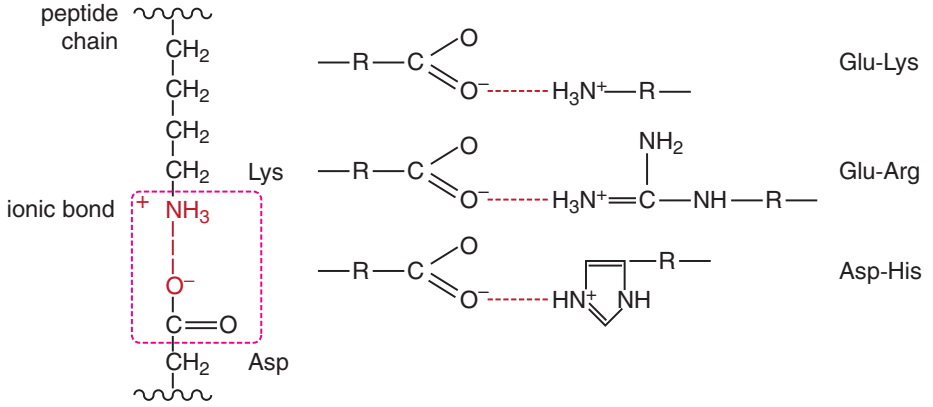
Fig. 1.13. Types of chemical bonds are involved in the formation of the tertiary structure

- **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

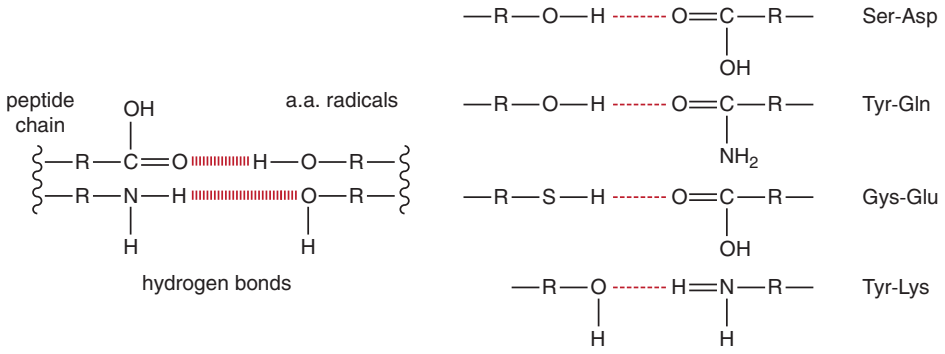
within the globular structure of water-soluble proteins. Between them, so-called hydrophobic interactions arise, as well as van der Waals forces between closely adjacent atoms. As a result, a hydrophobic core forms inside the protein globule.



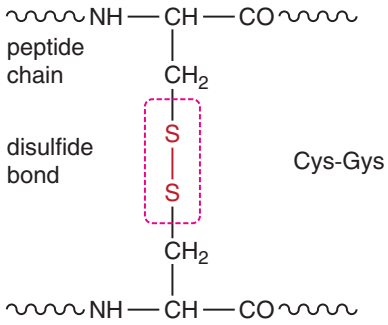
- **Ionic bonds:** Ionic bonding can occur between negatively charged (anionic) carboxyl groups of aspartic and glutamic acid radicals and positively charged (cationic) groups of lysine, arginine and histidine radicals.



- **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).

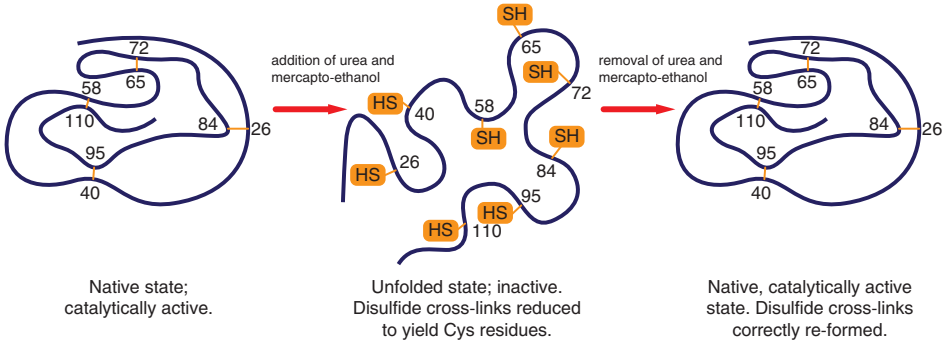


Fig. 1.14. Denaturation and renaturation of ribonuclease

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).

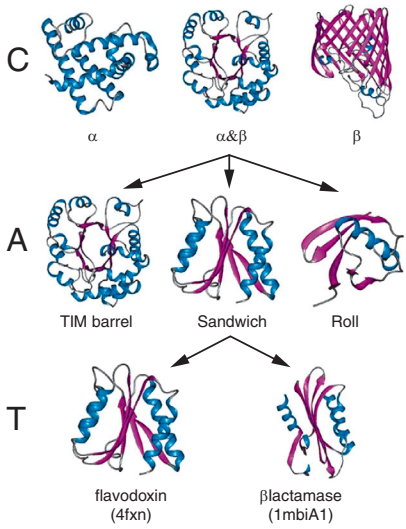


Fig. 1.15. Examples of protein domains classified by CATH (class, architecture, topology, homology)

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).

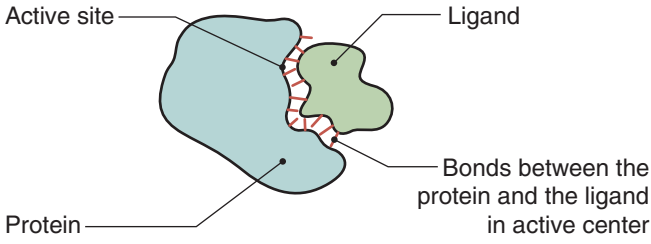


Fig. 1.16. The active site of the protein and its interaction with the ligand

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)

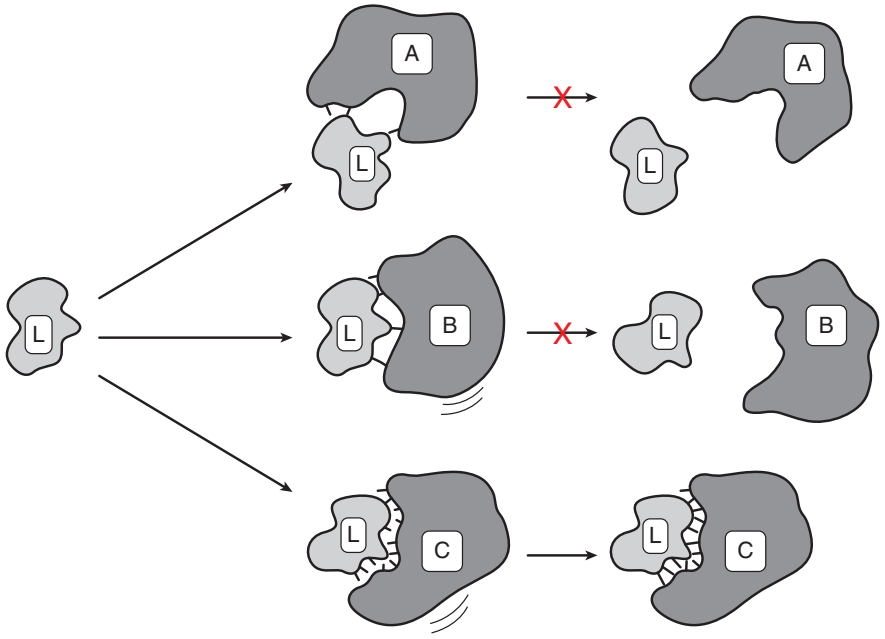


Fig. 1.17. The interaction of protein with a ligand in the active site. A and B are incomplete interaction. C is complementary interaction, L-ligand

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