Сеченовский Университет

Федеральное государственное автономное образовательное учреждение высшего образования Первый Московский государственный медицинский университет имени И.М. Сеченова Министерства здравоохранения Российской Федерации (Сеченовский Университет)

BIOCHEMISTRY WITH EXERCISES AND TASKS

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Министерство науки и высшего образования РФ

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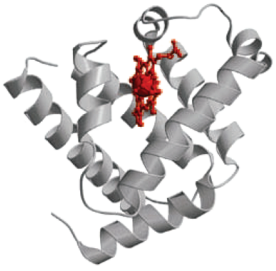


STRUCTURE, PROPERTIES AND FUNCTIONS OF PROTEINS

Main subjects:

1.1. Structure of amino acids. Peptide bond
1.2. Structural organization of proteins
1.3. Physicochemical properties of proteins
1.4. Structure and functioning of hemoglobin
1.5. Structure and functions of immunoglobulins
1.6. Main techniques of separation and purification of proteins

Proteins are macromolecular nitrogen-containing organic compounds whose structure has been studied since the mid 18th century. Dutch scientist Gerrit Jan Mulder at the beginning of 19th century discovered that the proteins are polymers built up of α-amino acids and proposed the term «proteins» (from the Greek *protos,* for the first) stressing the major role of these compounds in nature. The term «proteins» was used in Russia for the compounds secreted from the organism and resembled hen's egg white.

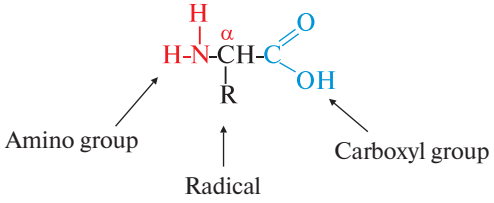
In human body proteins make up almost ¼ of body mass (about 15 kg). Proteins provide the basis of the cell's structure and are vital for all living cells. Phenotypic features and a variety of functions of each organism are conditioned by diversity in protein composition.

More than 50 000 proteins of human body are known to perform the following **functions**:

- **catalytic**; the acceleration of chemical reactions is realized by enzymes making up >50% of all proteins;
- **protective**; immunoglobulins (antibodies), complement system, lysozyme, lactoferrin etc., protection of the body against bacterial toxins, foreign proteins and other macromolecules;
- **transport**; the transport of different substances by blood occurs with the help of proteins, for example serum albumin (Na^+ ions, fatty acids etc.), transferrin (iron ions), hemoglobin (oxygen);
- **regulatory**; protein hormones control the cellular metabolism (insulin, glucagon, vasopressin etc.);
- **retractive**; muscles work due to proteins actin and myosin;
- **structural**; this function is performed by proteins of nucleosomes (histones), connective tissue proteins (collagen and elastin), fibrin of thrombus, keratin of hair and nails;
- **reserve**; the form of amino acids reserve (muscular proteins, blood plasma albumin).

1.1. STRUCTURE OF AMINO ACIDS. PEPTIDE BOND

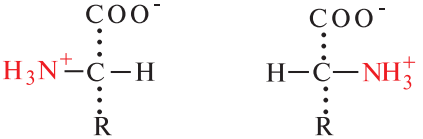
Proteins are macromolecular compounds composed of α-amino acids connected to each other by peptide bonds.



More than 300 different amino acids are known in nature, but only 20 are found in human and animal body, and other highest organisms. Each amino acid has a **carboxylic group and** an **amino group** at α-carbon (2), and a **radical** (side chain) which is distinctive in different amino acids.

At physiological pH (~7.4) the carboxylic group usually dissociates, and the amino group is protonated.

All amino acids (except glycine) include asymmetric carbon, and hence, can exist as L- and D-stereoisomers:



Only L-amino acids are used for synthesis of human body proteins. In proteins with long life-time L-isomers can slowly acquire D-configuration, this process occurring with the rate definite and characteristic for each amino acid. So, the dentin proteins include L-aspartate which is transformed into D-form at body temperature at the rate of 0.01% per year. Since enamel dentin is practically not metabolized and synthesized in adults in the absence of trauma, it is possible to determine

a person's age by D-aspartate content, using this in clinical practice and forensic analysis.

Radicals (side chains) of amino acids is a variable part of peptide and may include different functional groups:

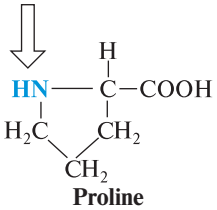
polar (hydrophilic):
- hydroxyl –OH;
- carboxyl –COOH;
- amino group $-NH_2$;
- imino group =NH;
- amido group $-CO-NH_2$;
- thiol –SH;

nonpolar(hydrophobic):
- methyl $-CH_3$
- phenyl  .

The chemical groups specify the properties of peptides which contain these radicals. Thus, the presence of nonpolar groups $-CH_3$,  enhances hydrophobicity, but the abundance of polar groups –OH, –SH, –COOH, $-NH_2$ make peptides hydrophilic. All amino acids which are present in proteins may be divided into four groups depending on the structure and polarity of radicals (Table 1.1).

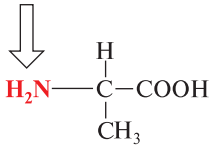
Human proteins include 19 amino acids and 1 cyclic imino acid proline which has imino group –NH-. The saturated aliphatic three-carbon chain forming 5-member cycle between α-carbon and imino-group plays the role of the hydrophobic radical in this molecule:

Imino group



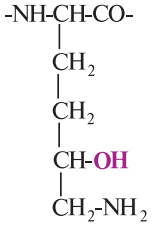
Proline

Amino group

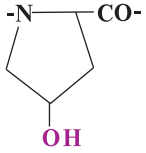


Alanine

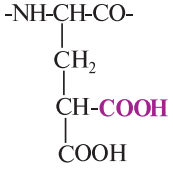
Some proteins involve amino acids with modified radicals not found in other proteins. Thus, collagen polypeptide chain includes hydroxylysine, collagen and elastin include hydroxyproline. Blood clotting factors prothrombin, proconvertin, and bone tissue proteins osteocalcin, sialoprotein contain γ-carboxyglutamic acid:



Hydroxylysine



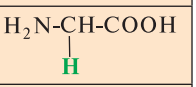
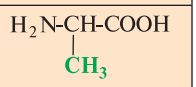
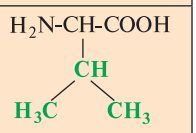
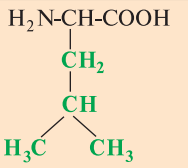
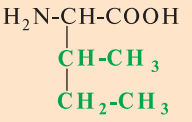
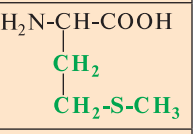
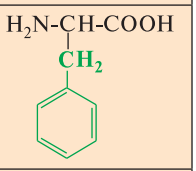
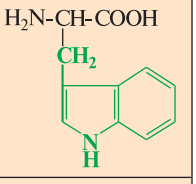
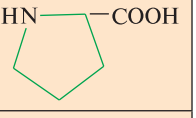
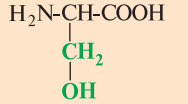
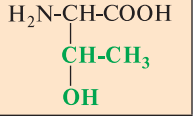
Hydroxyproline



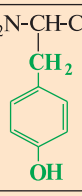
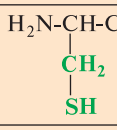
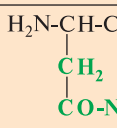
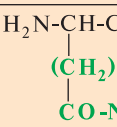
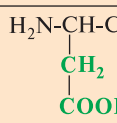
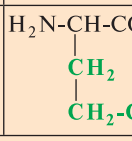
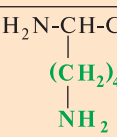
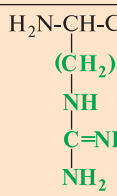
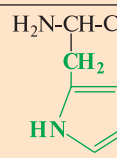
γ-Carboxyglutamic acid

Table 1.1

Classification of amino acids by radical polarity

№	Name		Formula for the amino acid
	full	abbreviations	
I. Amino acids with nonpolar radicals			
1	Glycine	Gly	
2	Alanine	Ala	
3	Valine	Val	
4	Leucine	Leu	
5	Isoleucine	Ile	
6	Methionine	Met	
7	Phenylalanine	Phe	
8	Tryptophan	Trp	
9	Proline	Pro	
II. Amino acids with polar uncharged radicals			
10	Serine	Ser	
11	Treonine	Thr	

Окончание таблицы 1.1

№	Name		Formula for the amino acid
	full	abbreviations	
12	Tyrosine	Tyr	
13	Cysteine	Cys	
14	Asparagine	Asn	
15	Glutamine	Gln	
III. Amino acids with polar anionic radicals			
16.	Aspartate	Asp	
17.	Glutamate	Glu	
IV. Amino acids with polar cationic radicals			
18.	Lysine	Lys	
19.	Arginine	Arg	
20.	Histidine	His	

The modification of these amino acid radicals usually occurs after their insertion into the polypeptide chain.

The content of certain amino acids in proteins can vary within wide ranges, the most common is glycine, and the most rarely encountered is tryptophan. Alanine, valine, leucine, serine as well as glutamic acid and glutamine are found very often in protein composition. The majority of proteins don't differ greatly from each other, but there are proteins with a unique and very characteristic amino acid composition. For example, the connective tissue protein collagen is built up of 30% from glycine and 20% from proline; chromosomal proteins histones include 30% of cationogenic arginine and lysine; plasma albumin is composed of 30% from anionogenic aspartic and glutamic acids. Unique amino acid composition is found in specific proteins of saliva: proline-rich proteins (PRP) (proline content reaches 20–40%), statherin (rich in tyrosine), histatins (histidine and tyrosine are predominant), as well as cystatins (rich in cysteine).

PEPTIDE BOND

Amino acids in polypeptide chain are bound by amide bond which is formed between α-carboxylic group of the first amino acid and α-amino group of the second one (Fig. 1.1). The covalent bond formed between amino acids is referred to as **peptide bond**, with oxygen and hydrogen atoms being in transposition.

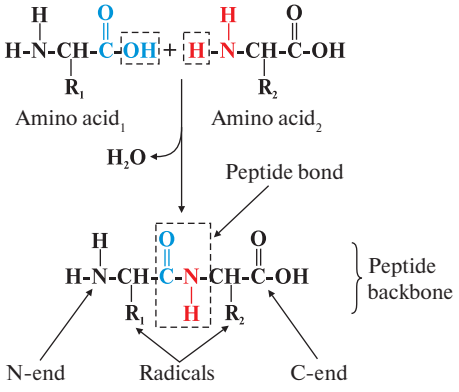


Fig. 1.1. The formation of the peptide bond

Each protein or peptide can be distinguished by:
N-terminal of a protein or peptide with a free α-amino group (**–NH_2**);
C-terminal with a free carboxyl group (**–COOH**);
peptide backbone of a protein composed of repeated fragments **–NH–CH–CO–**;
amino acid radicals (side chains) (**R_1** and **R_2**) — variable groups.

Abbreviated records of polypeptide chain, as well as the synthesis of protein in the cell necessarily begin with N-terminal and finish with C-terminal:

H_2N–Thr-His-Pro–COOH

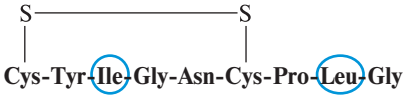
The name of amino acid included into the peptide and forming peptide bond has the ending **«-yl».** For example, tripeptide is referred to as **threonyl-histidyl-proline**.

The only variable part that distinguishes a protein from all others is the combination of radicals (side chains) of amino acids. Therefore the individual properties and functions of proteins are determined by the structure and order of the sequence of amino acids in the polypeptide chain.

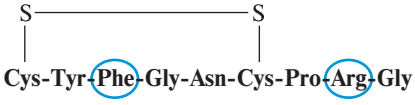
Polypeptide chains of different proteins of the body can include from several amino acids to hundreds and thousands of amino acid residues. Also, their molecular mass varies widely.

Thus, hormone vasopressin is composed from 9 amino acids, molecular mass 1070 kDa; insulin includes 51 amino acids (two chains), molecular mass is 5733 kDa; lysozyme is composed from 129 amino acids (one chain), molecular mass is 13930 kDa; hemoglobin involves 574 amino acids (4 chains), molecular mass is 64,500 kDa; collagen (tropocollagen) includes about 1000 amino acids (3 chains), molecular mass is 130,000 kDa.

Properties and functions of proteins are determined by the structure and the order of the sequence of amino acids in the polypeptide chain, and alteration of amino acid composition may considerably change them. Thus, two hormones of posterior pituitary gland: oxytocin and vasopressin are nanopeptides and differ in two amino acids among the nine (position 3 and 8):



Oxytocin



Vasopressin

The main biological effect of oxytocin is the stimulation of smooth muscles of uterus in labor, and vasopressin induces water reabsorption in kidney tubules (antidiuretic hormone) and possesses vasoconstrictive properties. Therefore, despite their structural similarity, there is a difference between physiological activity of these peptides andthe target tissues affected by them, i.e.the replacement of only 2 from 9 amino acids induces the substantial alterationof functions of this peptide.

Sometimes a minor alteration in the structure of the large protein results in the repression of its activity. Thus the enzyme alcohol dehydrogenase splitting ethanol in the human liver is built up of 500 amino acids (in four polypeptide chains). The activity of this enzyme in people of Asian region (Japan, China etc.) is much lower thanin Europeans. This occurs due to the replacement of glutamic acid to lysine at position 487 in polypeptide chain.

Each body protein functions during a particular period and then undergoes degradation and renovation. The lifetime of different proteins differs considerably. For example, the liver soluble proteins belong to short-living ones and are regenerated every 20–30 min., the enzyme RNA-polymerase is renewed every 2.5 hours, glucokinase — every 12 hours. Proteins of the blood clotting system are short-lived and their lifetime is several minutes or hours, proteins of oral cavity epithelium are renovated every 6–12 days, the life span of hemoglobin is 120 days, and connective tissue elastin is regenerated only by half during 75 years.

1.2. STRUCTURAL ORGANIZATION OF PROTEINS

Protein molecules are three-dimensional structures and have several levels of structural organization.

The **primary structure** is the order (sequence) of amino acids in polypeptide chain, it is formed by **peptide bonds** and is specific for different proteins (Fig. 1.2).

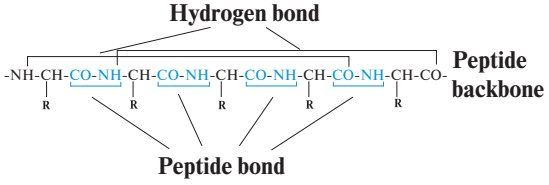


Fig. 1.2. Primary structure of a protein

Peptide bonds stabilize the primary linear structure (shown in blue);
Hydrogen bonds make up the three-dimensional secondary structure of proteins and are formed between the groups >C=O and >NH of peptide backbone.

The **secondary structure** of protein is a spatial structure formed by **hydrogen bonds** between carbonyl (>C=O) and imino (>N–N) groups of peptide backbone (Fig. 1.2).

Hydrogen bonds are weak electrostatic interactions between one electronegative atom (oxygen, nitrogen, etc.) and hydrogen atom covalently linked with another electronegative atom (Fig. 1.3). Hydrogen bonds are characterized by low strength, but when formed in large

amounts in the protein molecule they facilitate its compaction.

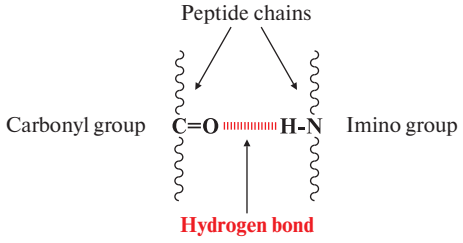


Fig. 1.3. Formation of hydrogen bonds between the groups of peptide backbone

There are 3 types of secondary structure.

In **α-helixall** >C=O and –NH–groups of polypeptide backbone are involved in the formation of hydrogen bonds. H-bonds are arranged very orderly forming the helical structure and orientated along its axis (Fig. 1.4). A single coil of helix includes 3,6 amino acid residues, i. e. the hydrogen bonds are formed between 1-st and the 4-th amino acids, mostly with short side chains. Proline and amino acids with long radicals damage helical packing. The α-helix is the strongest type of secondary structure prevailing in many proteins.

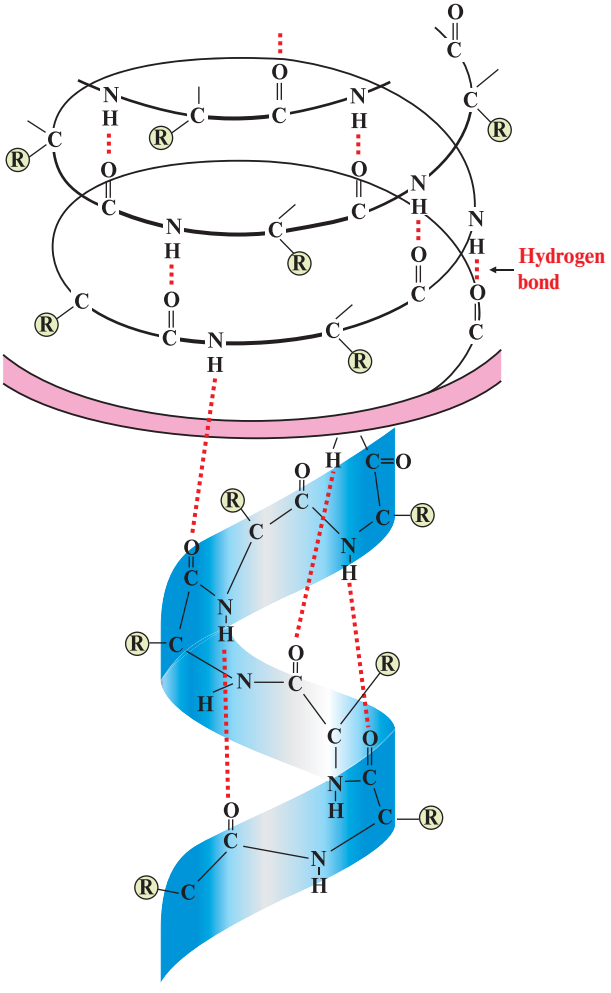


Fig. 1.4. The α-helix

β-structure (β-sheet) is of folding (pleated) type, hydrogen bonds occur less systemically forming pleated structure from polypeptide chain (Fig. 1.5, A), whereas β-structural polypeptide chain sections run either in one (Fig. 1.5, B) or opposite direction (Fig. 1.5, C). β-structure occurs more rarely than α-helix. Schematically β-structure is depicted as wide flat arrow denoting the direction from N- to C-terminal of the chain.

Nonrepetitive (irregular) structure does not have regular pattern, hydrogen bonds are disordered. The sections of polypeptide chain that form this type of secondary structure are usually small ones, and in this part of the molecule the chain can easily bend changing its direction.

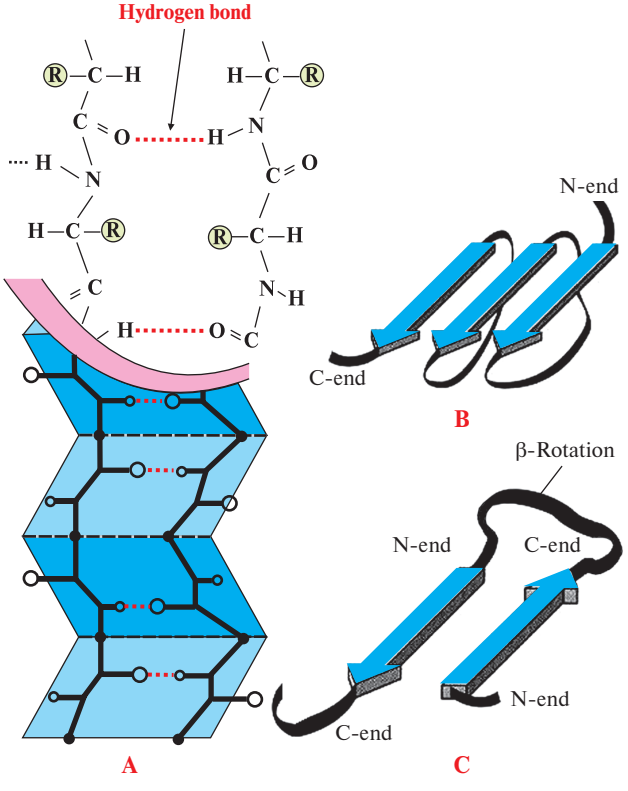


Fig. 1.5. The β-structure

A — formation of hydrogen bonds of β-structure;
B — parallel β-pleated sheet;
C — antiparallel β-pleated sheet.

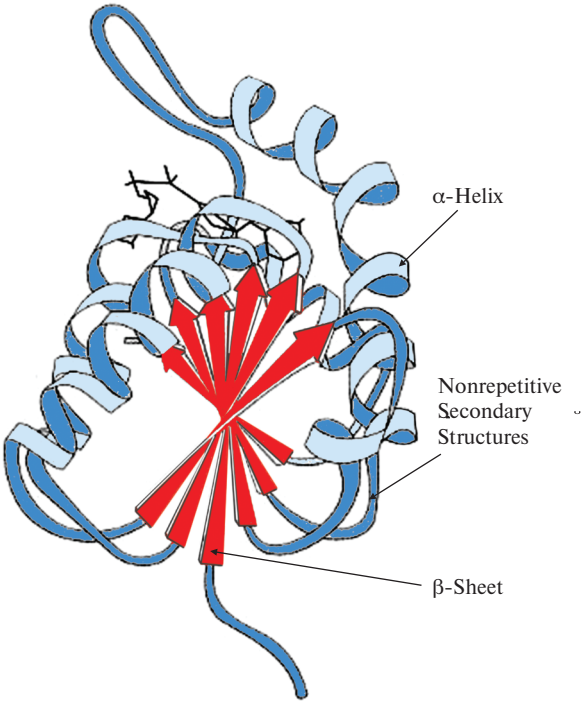


Fig. 1.6. Spatial structure of LDH domain. Combination of different types of the secondary structure

The regions with different types of secondary structure may considerably differ in proteins which is conditioned by primary structure of polypeptide chain. There are proteins with obviously predominant α-helix (hemoglobin and myoglobin, hormone insulin); other proteins involve more β-structures (chymotrypsin, immunoglobulins). More often proteins include all three types of secondary structures (enzyme lactate dehydrogenase, LDH) (Fig. 1.6).

The **tertiary structure** of protein is the entire three-dimensional pattern resulted from interactions between amino acid radicals. Tertiary structure is formed by 4 types of chemical bonds: hydrophobic, hydrogen, ionic, and disulfide.

Hydrophobic bonds occur between nonpolar hydrophobic radicals (Fig. 1.7). They play the leading role in the formation of tertiary structure of protein molecule.

Nonpolar radicals are repelled by water molecules and hidden in the internal part of protein molecule (Fig. 1.8, A). The polar groups tend to move towards the external aqueous phase and form hydrophilic surface. That is why the majority of globular proteins are well-soluble in water in spite of large molecular mass.

On the contrary, in the integral membrane proteins located inside the lipid hydrophobic layer nonpolar radicals tend to move to the hydrophobic medium of the surface of molecule, while hydrophilic (polar) ones pass into

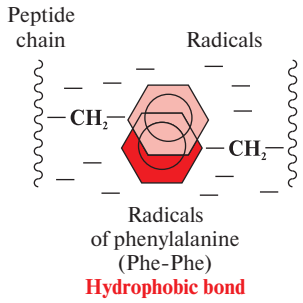


Fig. 1.7. Hydrophobic interactions between radicals

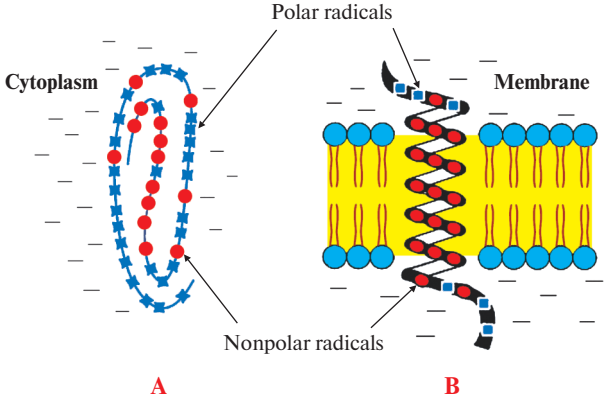


Fig. 1.8. Localization of hydrophobic and hydrophilic radicals in a protein molecule

A — hydrophilic cytoplasmic protein;
B — hydrophobic membrane protein.
■ — polar (hydrophilic) radicals;
● — nonpolar (hydrophobic) radicals.

the internal part of protein molecule (Fig. 1.8, B). It's as if the protein is turned inside out compared to the cytoplasmic hydrophilic proteins.

Hydrophilic radicals within the internal part of the protein globular can also interact with each other-forming weak hydrogen or ionic bonds.

The hydrogen bonds are formed between the polar (hydrophilic) uncharged radicals having «mobile» hydrogen atom and groups with electronegative atom (-O- or -N-) (Fig. 1.9).

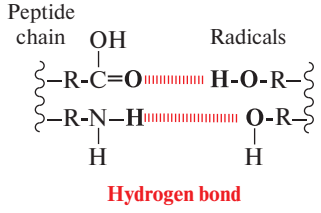


Fig. 1.9. Hydrogen bonds between radicals of amino acids

The ionic bonds are formed between polar (hydrophilic) ionogenic radicals with oppositely charged groups (Fig. 1.10).

The disulfide bond is covalent formed between two ionogenic (thiol) radicals of the cysteine residing in the different parts of polypeptide chain (Fig. 1.11). This bond is encountered in such proteins as insulin, insulin receptor, immunoglobulins, salivary protein mucin etc.

Disulfide bridges stabilize the spatial structure of the single polypeptide chain or link together 2 chains (for example, A and B chains of insulin) (Fig. 1.12).

Polypeptide chain of each protein necessarily has 3 levels of structural organization.

In the human body proteins perform important and varied functions which are realizedby interacting with other substances. The compound bound to the protein is referred to as **ligand**. Ligand may be a low-molecular, as well as high-molecular compound including another protein. Ligands are substrates of enzymes, cofactors, inhibitors and activators of enzymes, subunits in oligomeric proteins, etc. Ligand binds to a particular site on the surface of protein molecule named the binding site (active site).

The binding site (active site) is the part of protein molecule built up of amino acid residues which are drawn together during formation of the tertiary structure and responsible for specific interaction with ligand; binding site often resides in the hydrophobic cleft on the surface of protein molecule (Fig. 1.13).

The interaction of the ligand with the binding site occurs due to the principle of complementarity («lock-and-key model»). **Complementarity** is the geometrical (spatial) and chemical conformity of ligand and protein

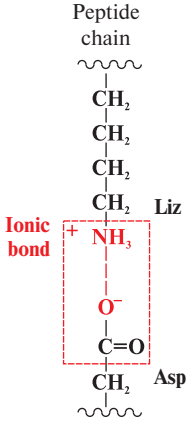


Fig. 1.10. Ionic bond between radicals of lysine and asparagine acid

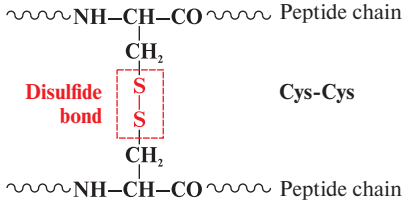


Fig. 1.11. Formation of disulphide bond

Cysteine as part of proteins plays a major role in folding since its thiol groups are able to form strong disulphide bond making up the tertiary structure of protein molecules. **Folding is the process of spatial arrangement of the synthesized polypeptide chain, formation of the single unique native structure of protein.** It is realized with the help of proteins-**chaperons** (heat shock proteins).

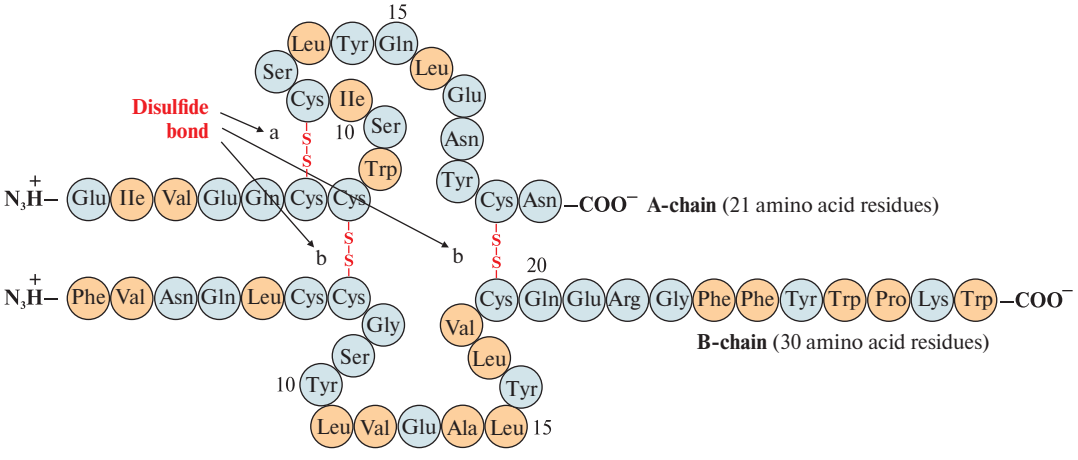


Fig. 1.12. Disulphide bonds in a molecule of insulin

Disulphide bonds: between the cysteine residues of one chain A (a), between the chains A and B (b).
Numbers identify the position of amino acids in polypeptide chains.

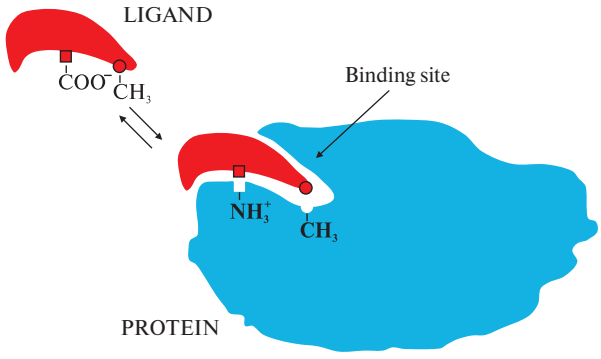


Fig. 1.13. The interaction of ligand and protein binding site

binding site. In their interaction noncovalent bonds are mostly formed: ionic, hydrogen, and hydrophobic.

Proteins with long polypeptide chains (more than 200 amino acid residues) often build up domain structures. **Domain** is the section of polypeptide chain forming the structure similar to globular which may be linked with other domains (globules) of the same chain. A single chain can make up several domains (for example, muscular protein actin), whereas domains may differ in the structure and functions (Fig. 1.14).

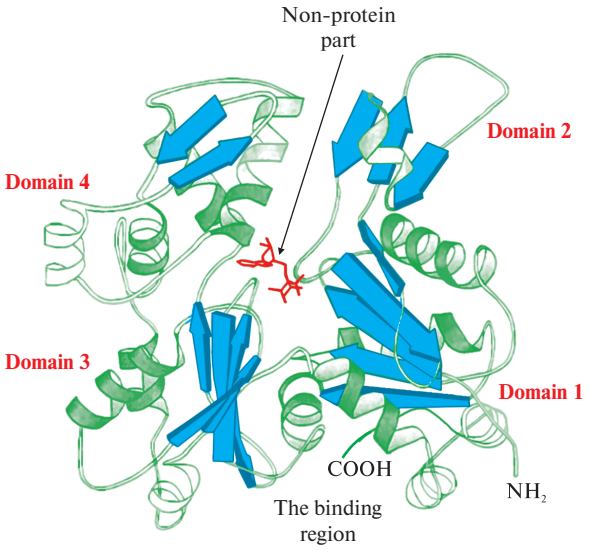


Fig. 1.14. The domain structure of protein actin

Quaternary structure of protein

There are proteins in the body composed of several identical or different polypeptide chains. Each of these chains has 3 levels of structural organization and is referred to as a protomer (subunit). **Quaternary structure** of protein is the spatial arrangement of several polypeptide chains (protomers or subunits). The protein that has quaternary structure is referred to as **oligomeric** (Fig. 1.15). The number of subunits in the oligomer depends on its function, and may range from two (the enzyme hexokinase) to dozens, and even hundreds (pyruvate dehydrogenase complex) of subunits.

The interaction of contact surfaces of subunits in oligomeric proteins occurs in strict accordance to the **principle of complementarity**, with only weak noncovalent bonds (hydrophobic, hydrogen, and ionic) being formed between two subunits. Oligomeric proteins can dissociate under certain conditions.

All molecules of the same native protein have the identical spatial structure because the information about all levels of structural folding is encoded in the primary structure of the protein molecule.

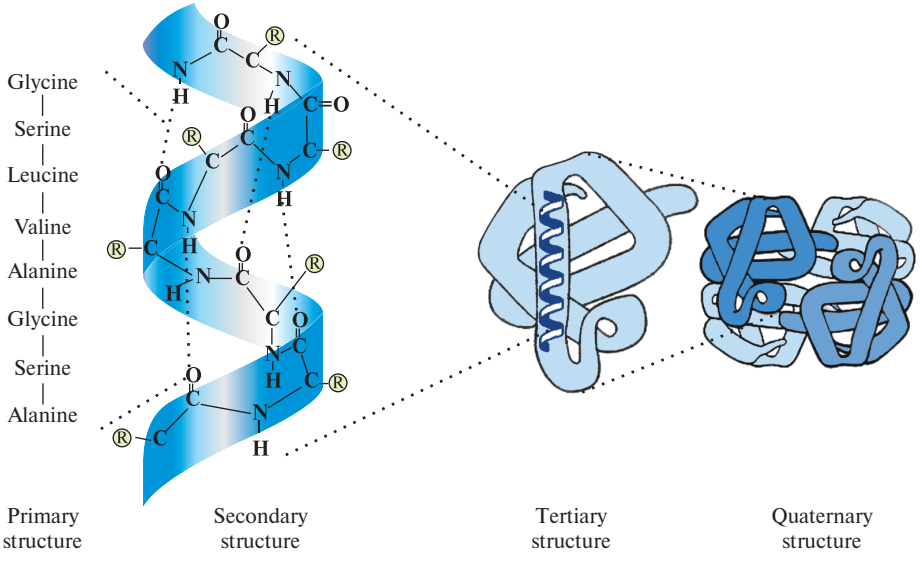


Fig. 1.15. The structural levels of protein molecule

The whole of all levels of structural organization (secondary, tertiary and quaternary structures) is referred to as **conformation** of the protein. The weak chemical bonds stabilizing the spatial structure are very sensitive to the changes of temperature, pH of medium, or the influence of specific ligands. Their rupture induces alteration of the protein conformation that is usually reversible. The ability of the protein to reversible conformational change is named the **conformational lability**.

1.3. PHYSICOCHEMICAL PROPETIES OF PROTEINS

Physicochemical properties of proteins and peptides (charge, mass, and solubility) are determined by its amino acid composition.

The net charge of the peptide is summed up from the charges of radicals of ionogenic amino acids forming part of it because they can ionize in the solution (Fig. 1.16):

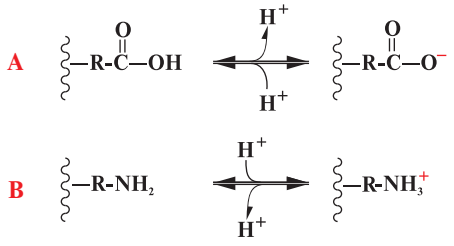
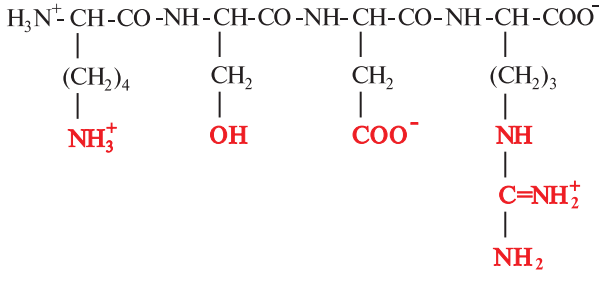


Fig. 1.16. The ionization of polar charged groups of the radicals of amino acids in the solution

A — carboxyl group in the solutions at pH of 7.0 is deprotonated and acquires negative charge; at pH <7.0 the uncharged form is formed;
B — amino group and other cationic groups in the solution at pH of 7.0 are protonated and acquire positive charge; at pH >7.0 they become unionized.

The charges of the terminal free amino- and carboxyl groups in the chain usually compensate each other and do not affect the net charge of the protein. For example, the net charge of the tetrapeptide **Lys–Ser–Asp–Arg** at pH 7.0 is equal to +1, because it is summarized from the charges of two cationogenic (Lys and Arg) and one anionogenic (Asp) amino acids in its structure:



Proteins are macromolecular compounds; their solutions have high viscosity, capacity to swell, and show mobility in the electric field. In the solution water dipoles form the hydrate layer surrounding the charged protein molecule. If a protein loses its charge water dipoles leave the molecule, hydrate coating is destroyed, and protein aggregates form the sediment. Thus the **solubility of the protein** depends on the charge and the mass of its molecule.

The charge of the protein molecule changes depending on pH of the medium since the level of ionization of radicals also changes. For example, serum protein albumin has high net negative charge as it is composed of many dicarboxylic acids. When the pH of the albumin solution changes from alkaline (pH >7.0) to strong acid (pH <<7.0), the ionization of carboxyl groups of aspartic and glutamic acid radicals will be changed affecting not only the charge but the solubility of the protein (Fig. 1.17).

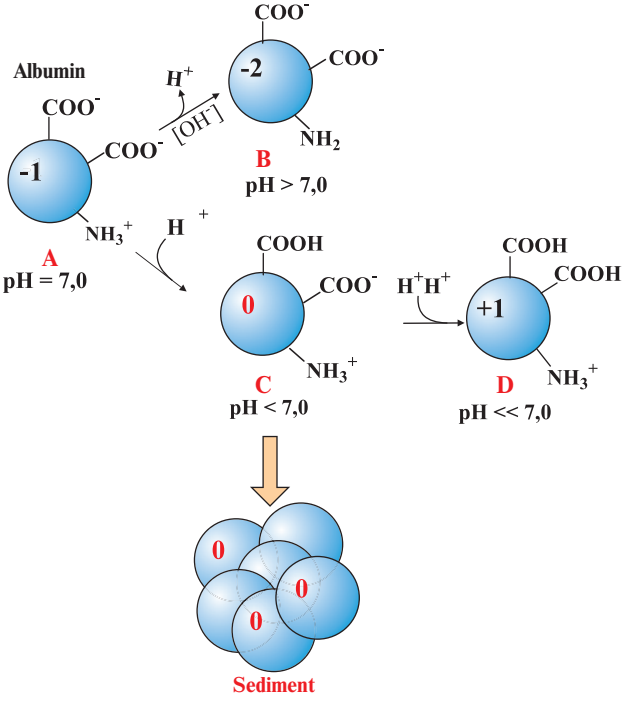


Fig. 1.17. Charge and solubility of albumin at different pH values of the environment

A — in the neutral environment the charge is –1;
B — in the alkaline environment the charge is –2;
C — in the weak acidic environment the charge is 0 (isoelectrical state), albumin precipitates;
D — in the strong acidic environment the charge is +1, the sediment dissolves, albumin passes into the solution.

At certain pH value the charge of protein molecule is compensated by ions of the medium and protein molecule becomes electroneutral; this state of protein molecule is referred to as **isoelectric state**. The protein loses the hydrate coating and precipitates out of solution. **Isoelectric point (pI)** is the pH of the medium at which a protein molecule is found in its isoelectric state (the charge of molecule is zero). The isoelectric point for serum albumin is encountered at pH <7.0 (pI=5.6).

The pI of the majority of proteinslies in weak acid medium, i. e. the anionogenic amino acids predominate in their structure (pepsin, albumin), and there are basic proteins as well (cytochrome C, lysozyme, and histones).

The solubility of polypeptide also depends on the shape of the molecule. The majority of proteins have oval, globular (or similar to it) shape (Fig. 1.18, A) and they are usually very soluble. **Globular proteins** predominate in the body; they include enzymes, hemoglobin, myoglobin, etc. **Fibrous proteins** with extended, threaded shape are more rarely encountered (Fig. 1.18, B). For example, collagen is the main component of connective tissue performing structural function; keratins are found in epidermis, hair, and nails. There are less fibrous proteins in the body and their hydrophobic properties are quite pronounced.

Weak chemical bonds stabilizing the spatial protein structure can be disrupted by the action of different factors and compounds (high temperature, X-ray radiation, concentrated acids and bases, urea, alcohols, phenol, formaldehyde, heavy metal salts etc.). This phenomenon is named **denaturation of the protein**. The primary structure is unaffected because it is stabilized by strong covalent bonds. Protein molecules of denatured protein acquire random conformation (Fig. 1.19). During this process the biological activity is completely lost since the structural alterations (II and III levels) result in the destruction of protein active site.

It is known that protein ribonuclease restores its conformation *in vitro* after denaturation by urea and the subsequent removal of denaturizing agent, that is, it is renatured (Fig. 1.19). This fact confirms the major role of primary structure of polypeptide chain as the source of information for spatial folding of protein molecule.

Within cells (in vivo) the complete denaturation of the protein is prevented by **chaperones (heat shock proteins)** whose synthesis is increased at elevated temperatures. Besides renaturation, chaperones facilitate the **folding** (formation of spatial structure) of newly synthesized proteins (see section 3).

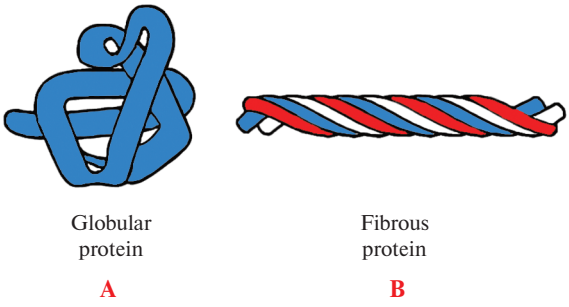


Fig. 1.18. Globular (A) and fibrous (B) proteins

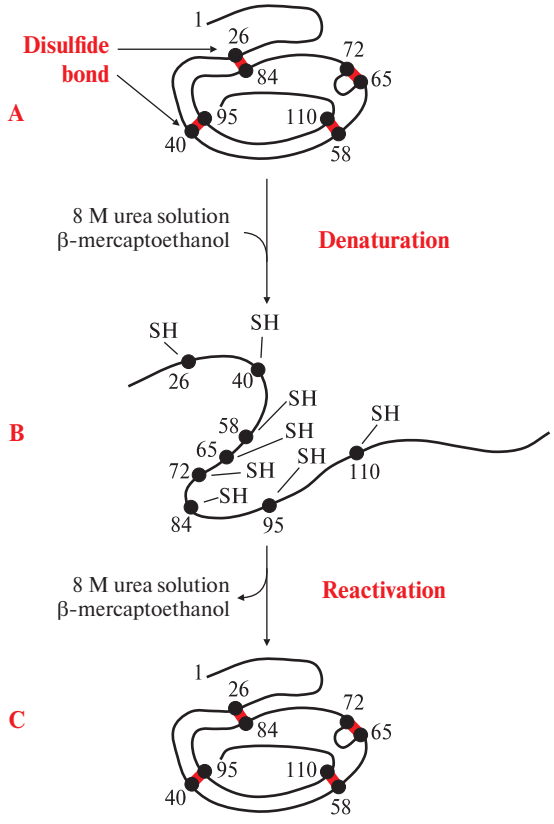


Fig. 1.19. Denaturation and renaturation of ribonuclease

A — native conformation of a protein with high activity;
B — denatured enzyme with low activity;
C — regenerated native conformation and high activity.

CONJUGATED PROTEINS

All proteins can be classified as simple and conjugated. Simple proteins are composed only from amino acids and named **apoproteins**. Conjugated proteins — **holoproteins** — are built up of protein part (**apoprotein**) and nonprotein part (**prosthetic group**). When deprived of prosthetic group, holoprotein loses its activity.

Prosthetic groups may include metal ions, $H_2PO_4^-$ ions, heme, carbohydrates (mono- and oligosaccharides), nucleotides and nucleic acids, vitamins and their derivatives. Between apoprotein and prosthetic group covalent (strong bonds), as well as noncovalent ones are formed. The conjugated proteins that play an important role in the body are hemoglobin, myoglobin, immunoglobulins, enzymes and other proteins.

TESTS

1. Find a correspondence.

Characteristics of a radical:

A. Possesses hydrophobicity.
B. Involves sulfur.
C. At pH 7.0 has negative charge.
D. The six-membered cycle enters the structure.
E. Has NH-group which can bind H+.

Amino acid:

1. Ala.
2. Glu.
3. His.

2. Draw the tripeptide containing amino acids enumerated in the test 1.

a) specify N- and C-terminals of peptide, variable groups, peptide backbone;
b) determine the net charge; explain at which pH (7.0, <7.0, >7.0) the solubility of the peptide will decrease and form a sediment;
c) explain what is to be done to make the protein soluble again; present an appropriate scheme.

3. Draw the formula of C-terminal tripeptides of oxytocin and vasopressin.

a) point out the difference of these fragments;
b) characterize the polarity and the charge of radicals of these tripeptides;
c) explain, what protein — oxytocin or vasopressin — has higher value charge and why.

4. Draw the formulae of the peptide Glu–Met–Lys–Tyr:

a) determine the net charge of the peptide at pH 7.0; specify the region of its isoelectric point;
b) add 2 amino acids to this peptide so as net charge would be:
- Negative
- Neutral
- Positive;

c) take any of written peptides and show a single turn of the α-helix with dotted line, specify the stabilizing bond and groups forming this bond;
d) from written peptides choose the pairs of amino acids which are able to make bonds between radicals, specify the type of the bond, point out what level of structural organization they stabilize.

5. Find a correspondence.

The type of bond:

A. Ionic.
B. Disulfide.
C. Peptide.
D. Hydrogen.
E. Hydrophobic.

Interradical interaction:

1. Phe, Met.
2. Ser, Asn.
3. Arg, Asp.

6. Choose correct answers.

The binding site of the protein:

A. Is the part of polypeptide chain.
B. Is formed at the tertiary level of the structure.
C. Resides in the cleft of the protein surface.
D. Interacts with the ligand.
E. Is responsible for protein function.

7. Choose correct answers.

Oligomeric protein:

A. Includes several subunits linked by weak bonds.
B. Is stabilized by disulfide bridges.
C. May have only a single binding site.
D. Has 4 levels of structural organization.
E. Is formed by hydrogen bonds between groups of peptide backbone.

SITUATIONAL PROBLEMS

1. Amino acids are present in the proteins of bone tissue, their radicals contain one or even two carboxyl groups. Name these amino acids, draw their formulas and answer the following questions:

a) what radical is formed from an amino acid after incorporation of the latter into a polypeptide chain?
b) which of these amino acids can bind more calcium ions (Ca^{2+}) while forming the bone tissue?
c) what kind of bond is formed between the radicals of amino acid and Ca^{2+}?

2. Almost all hair waving products are designed on the basis of thio-organic compounds and their derivatives (thioglycol and thioglycolate). They act on the shape of a hair and change it even at normal temperature of human body. The action of these substances

is based on the effect of disruption of covalent bonds in the keratin molecules of hair. When oxidized by the fixatives these bonds are reconstructed, with hair shape changing.

Explain the principle of chemical hair curling, and for this purpose:

a) name the inter-radical covalent bonds in the keratin molecule;
b) draw the formula of the amino acid that forms these bonds;
c) indicate other inter-radical bonds that stabilize protein structure (give corresponding examples, draw formulas of amino acids taking part in their formation).

3. To disinfect root canals cotton wool soaked in formaldehyde is used. Explain the reasons for using formaldehyde if it is known that it penetrates the dentinal tubules and interacts with albumin. For this purpose:

a) explain what is protein denaturation and indicate what protein structural levels are respectively changed;
b) enumerate types of bonds that are broken down when denaturated, give examples of amino acids that form such bonds;
c) name the protein part which is responsible for its function, give the definition;
d) explain if biological activity of albumin changes or not after its interaction with formaldehyde, and why.

1.4. STRUCTURE AND FUNCTIONING OF HEMOGLOBIN

There are two proteins that perform the functions of delivery and storage of oxygen in the body, they are hemoglobin and myoglobin.

Hemoglobin (Hb) is a conjugated oligomeric protein built up of four subunits of two types (2α and 2β) including 574 amino acid residues. It is contained in erythrocytes and accounts for up to 90% of the protein mass in the cell. Hemoglobin provides the transport of oxygen from the lungs to the tissues and removal of carbon dioxide from the tissues.

Muscular tissue **myoglobin (Mb)** facilitates intracellular diffusion and short-time storage of oxygen. Mb is not oligomeric, it is a single-chain protein the conformation of which very similar to spatial structure of β-chain of hemoglobin (Fig. 1.20). Eight α-helical parts make up the major portion of Mb molecule and Hb subunits. They form a globule with hydrophobic

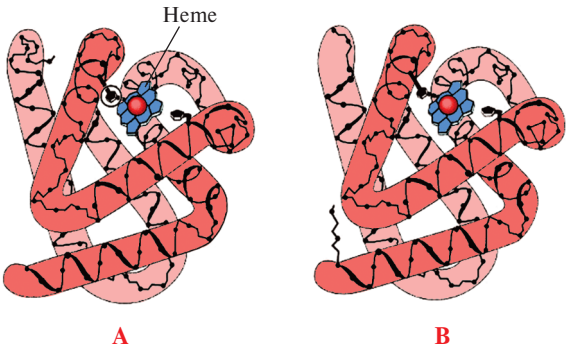


Fig. 1.20. Myoglobin structure and enaturation and β-chains of hemoglobin

A — myoglobin; **B** — β-chain of hemoglobin.

cleft (pocket) which involves the center for oxygen binding (**active site**), with polypeptide chains of myoglobin and hemoglobin subunits being only 20% identical.

Both proteins are holoproteins. The heme is prosthetic group which is found in the active site and takes part in the interaction with oxygen (Fig. 1.21). **Heme** (ferroprotoporphyrin) is the organic plane compound including 4 pyrrole rings and iron ion Fe^{2+}. It is a colored compound and gives a red color to hemoglobin, erythrocytes (red blood cells), and blood.

Heme binds to nonpolar radicals of active site by its pyrrole rings, as well as to histidine radical by Fe atom. Hemepyrrole rings lie in the single plane, but Fe^{2+} in nonoxygenated state of Hb protrudes above the plane at 0.6 Å. When binding oxygen, the iron ion is pulled into the planar structure of heme rings (Fig. 1.22). As a result, a part of polypeptide chain is shifted, weak bonds in hemoglobin are disturbed and the conformation of the whole globule is changed. Therefore, the oxygen binding results in the change of spatial structure of myoglobin molecule or hemoglobin subunit.

Myoglobin molecule can bind only one oxygen molecule to its active site:

$$Mb + O_2 \leftrightarrow MbO_2.$$

Hemoglobin is the oligomer and some features of its functioning are characteristic of all oligomeric proteins. Hemoglobin molecule is built up of 4 subunits and has 4 oxygen binding sites (active sites). Hemoglobin can exist in free (desoxyhemoglobin), as well as oxygenated state binding up to 4 oxygen molecules. Interaction of first subunit with oxygen induces the change of its conformation that leads to cooperative conformational changes of all other subunits (Fig. 1.23, A). The affinity to oxygen is increased and the binding of oxygen

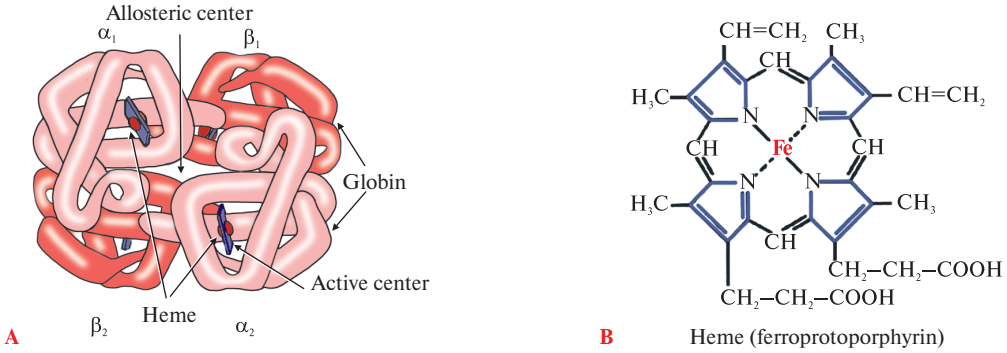


Fig. 1.21. Structure of hemoglobin and heme

A — **hemoglobin** — complex protein, oligomer, consists of 2α- and 2β-globin subunits, each of them has a binding site where heme, a non-protein part of the molecule, is located. It is involved in the binding of oxygen molecule. An allosteric site is formed between the protomers to bind the regulatory ligand of hemoglobin 2,3-bisphosphoglycerate;
B — **heme** — prosthetic group of hemoglobin, myoglobin and other hemoproteins. Binds to globin by hydrophobic bonds between pyrrol cycles and hydrophobic radicals of amino acids. There is an iron ion (Fe^{2+}) in the centre of the molecule, it forms 6 coordination bonds: 4 — with nitrogen atoms of hemepyrrol rings, 1 — with nitrogen of histidine radical in the globin chain, 1 — with oxygen molecule. One more histidine radical of the globin chain participates in the binding of O_2 to heme.

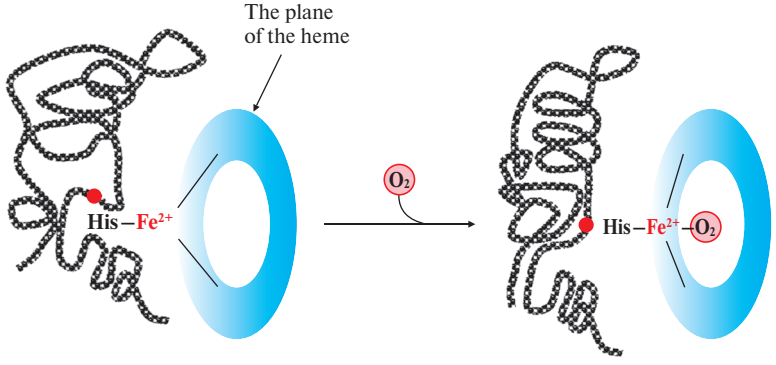


Fig. 1.22. Interaction of oxygen with heme in hemoglobin

molecule to the active site of the second subunit occurs easier producing further conformational reconstruction of the whole molecule. As a result, the structure of the remaining subunits and their active sites is altered and interaction with oxygen is facilitated even more. After this, the fourthoxygen molecule binds to Hb approximately 300 times easier than the first one (Fig. 1.23, B). It takes place in the lungs at high oxygen pressure. In tissues where oxygen content is lower, the cleavage of each O_2 molecule, on the contrary, facilitates the release of the subsequent molecules.

Therefore, the interaction of oligomeric protein hemoglobin with ligand (O_2) in one binding site trigger a conformational change of the whole molecule and others spatially distant centers in other subunits («domino effect»). Such interrelated changes of protein structure are referred to as **cooperative conformational changes**. They are characteristic of all oligomeric proteins and used for regulation of their activity.

Interaction of both proteins (Mb and Hb) with oxygen depends on its partial pressure in tissues. This dependency is of different character which is determined by specific characteristics of their structure and functioning (Fig. 1.24).

Hemoglobin has S-shaped curve of oxygen saturation which elucidates that subunits works cooperatively, the more oxygen they release the easier the oxygen molecules are liberated. The process depends on partial pressure of oxygen in tissues.

The graph of myoglobin saturation is a simple hyperbola, i.e. saturation of Mb by oxygen occurs rapidly and reflects its function — the reversible binding of oxygen being released by hemoglobin and O_2 release in intensive exercises.

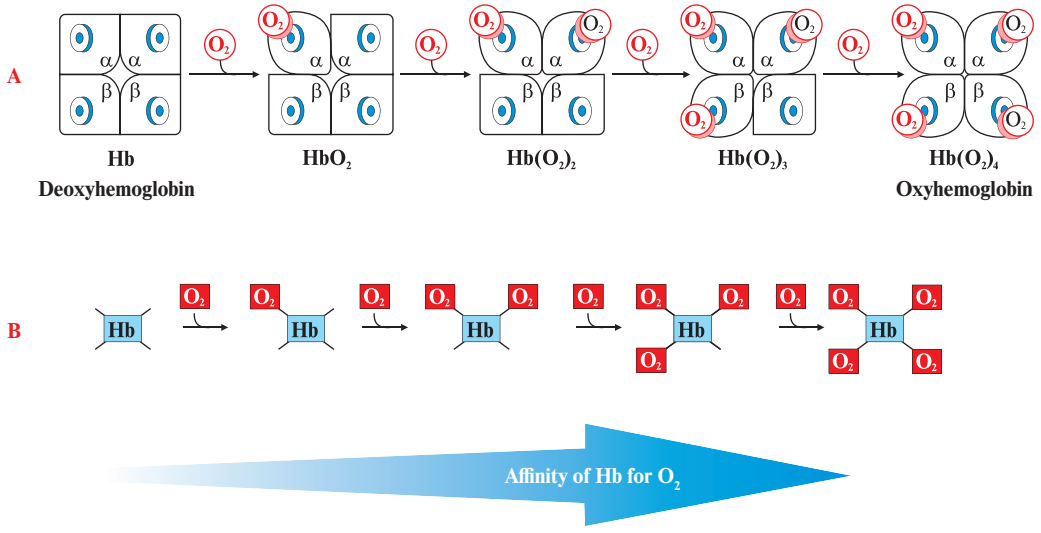


Fig. 1.23. Cooperative conformational changes in a hemoglobin molecule on interacting with oxygen

A — when deoxyhemoglobin Hb interacts with O_2 cooperative conformational changes occur, these changes accompany the binding of each subsequent oxygen molecule;
B — changing of active site conformation results in the increase of affinity of Hb for oxygen, the fourth oxygen molecule binds to oxygenated hemoglobin [$Hb(O_2)_3$] 300 times easier than the first one.

The change of hemoglobin's O_2 affinity ensures rapid saturation of blood by oxygen in lungs, as well as its release and delivery to tissues. Myoglobin possesses higher affinity to oxygen that is why it binds and transfers oxygen to cell mitochondria which is transported to muscles by hemoglobin.

Hemoglobin delivers up to 600 L (850 g) of oxygen per 24 hours and facilitates the removal of 500 L (1000 g) of CO_2 from tissues. The motive force of these flows is the gradient of O_2 concentration between alveolar air and intracellular fluid. Partial pressure of O_2 in the alveolar air is 100 mm Hg. Partial pressure of O_2 in tissues is much lower (~40 mm Hg) which is conditioned by influx and usage of oxygen by cell mitochondria where O_2 is converted to H_2O. In this way O_2 is consumed by the cells.

The exchange of O_2 and CO_2 takes place in capillaries: in the lungs O_2 passes from alveolar air to erythrocytes, while CO_2 moves in the opposite direction; in tissue capillaries O_2 moves from erythrocytes to tissue cells, and CO_2 in the opposite direction (Fig. 1.25).

Protons H^+ play the **major role** in the **regulation** of hemoglobin function.

- Hb enters the **tissues** predominantly as $Hb(O_2)_4$. At low partial pressure of O_2 some oxygen is cleaved off. The higher content of incompletely oxygenated Hb facilitates the release of O_2.

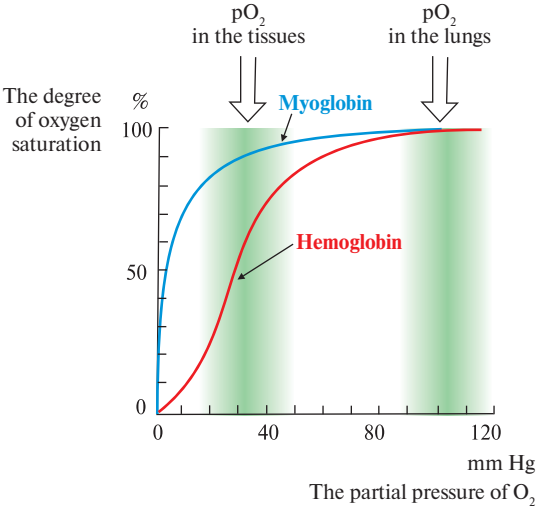


Fig. 1.24. Oxygen saturation curves for myoglobin and hemoglobin

Significant amount of CO_2 is formed in muscles, it enters the blood and then into erythrocytes where it is converted to carbonic acid H_2CO_3 by action of **carbonic anhydrase** (Zn^{2+}-dependent enzyme). H_2CO_3 dissociates to proton H^+ and bicarbonate ion:

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^-$$

The increase of H^+ concentration induces the protonation of ionogenic groups of hemoglobin resulting in the change of its charge and decrease of its affinity for O_2:

$$H^+ + HbO_2 \rightarrow H^+Hb + O_2$$

- Blood with high content of protonated deoxyhemoglobin enters the **lungs.** This form of Hb has lower affinity for O_2.

CO_2 diffuses from capillaries where it is formed as a result of the reaction:

$$H^+ + HCO_3^- \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O$$

This stimulates the deprotonation of hemoglobin:

$$nH^+Hb \rightarrow nH^+ + Hb$$

High partial oxygen pressure leads to Hb oxygenation.

Partial hemoglobin oxygenation increases its affinity for oxygen. All reactions written above occur in reverse order.

The dependence of hemoglobin affinity for oxygen on the concentration of protons H^+ is referred to as **Bohr effect** named after the Danish physiologist who studied hemoglobin function (Fig. 1.25).

Therefore, the volume of oxygen transported to tissues by hemoglobin is regulated and increased when the content of CO_2 and H^+ in blood is elevated (for example, during intensive physical work). If pH of blood is near alkaline (**alkalosis**) or acidic (**acidosis**) level, the delivery of oxygen to tissues decreases.

The alteration of protein functional activity when interacting with other ligands due to the conformational changes is named **allosteric regulation**, and regulatory compounds are **allosteric ligands**. The ability to allosteric regulation is characteristic, as a rule, ofoligomeric proteins, i.e.the interaction of subunits is requiredto reveal allosteric effect. During the interaction with allosteric ligands proteins change their conformation (including that of the active site) and function.

Hemoglobin molecule is able to bind with several ligands: O_2, H^+, CO, 2,3-bisphosphoglycerate (BPG). BPG is an allosteric regulator of hemoglobin activity and binds to the sites (allosteric sites) spatially distant from the active site.

The increase of concentration of the allosteric ligand decreases the affinity of hemoglobin for oxygen.

BPG formed from glucose in erythrocytes is one of regulators of hemoglobin function. Its molar concentration is close to molar Hb concentration. In the central part of hemoglobin molecule the polypeptide chains of 4 subunits form a cavity (allosteric site), and its size increases in deoxyhemoglobin and decreases in oxyhemoglobin. BPG enters the cavity of deoxyhemoglobin and binds to positively charged groups in β-subunits (Fig. 1.26), affinity of Hb for O_2 decreasing 26 times. As a result oxygen is liberated in tissue capillaries at low oxygen partial pressure.

In the lungs the partial pressure of O_2, on the contrary, leads to Hb oxygenation and the release of BPG.

$$Hb{\cdot}BPG \rightarrow Hb + BPG$$

The amount of BPG in human erythrocytes corresponds to that of hemoglobin and increases at low oxygen content in the air (hypoxia), or shortness of breath in lung disorders. Decrease in BPG concentration impairs the supply of tissues with oxygen. This is important when administering blood transfusion and it's necessary to maintain the required BPG concentration during the storage of the blood. The transfusion

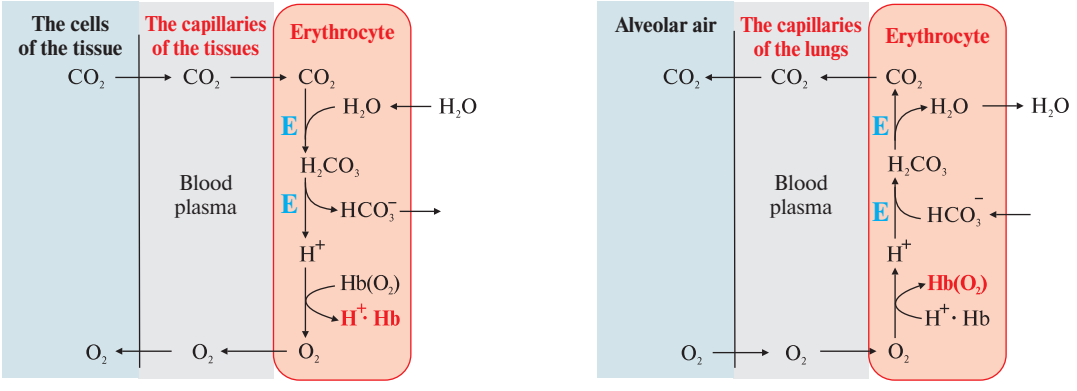


Fig. 1.25. Transport of oxygen by the erythrocyte. Bohr effect

E — carbonic anhydrase.

of donor's blood with low BPG concentration can lead to hypoxia and death of the patient.

1.5. STRUCTURE AND FUNCTIONS OF IMMUNOGLOBULINS

There are homologous proteins in the body that have similar conformation, but differ in polypeptide chains structure, like, for example, hemoglobin and myoglobin. They arose in the evolution due to the replacement of the definite amino acid residues in the chain. Their conformation usually includes the equal number of α-helixes and β-structures; they have similar turns and bends of polypeptide chains. Homologous proteins are quite numerous and combined into families of proteins (immunoglobulins, serine proteases, hemoglobin family, albumin family etc.)

The most numerous superfamily is composed of immunoglobulins.

Immunoglobulins (antibodies) are proteins (Y-shaped glycoproteins) synthesized by B-lymphocytes in response to the entry of foreign structures (antigens), and providing their detoxification. **Antigens** are usually compounds that induce immune response, for example, foreign proteins or other macromolecules, bacteria, viruses, mushrooms etc. Human body produces more than 10^7 types of immunoglobulins; each of them is synthesized by its own clone of B-lymphocytes. Antibodies are localized on the surface of immune cells or present in a free form in the blood plasma. They react with antigens in the blood, lymph, intercellular fluid, saliva and other glandular secretions. Immunoglobulin function is realized in two stages: recognition and binding of the appropriate complementary antigen with the help of antigen-binding sites; and triggering the process that results in inactivation and degradation of the antigen (activation of the complement system).

Structural features of immunoglobulins

Immunoglobulin molecules have characteristic Y-shaped structure two ends of which bind the antigen (Fig. 1.27). Each molecule is built up of 4 polypeptide chains: 2 identical heavy (H-chains, molecular weight is 50,000 kD) and 2 light ones (L-chains, molecular weight is 25,000 kD). Chains are bound to each other by 4 disulfide bridges. Heavy chains are divided into 5 types specific to each class of immunoglobulins, and light ones — into 2 types present in all types of immunoglobulins.

Light chains are composed of two domains; variable (V_L) and constant (C_L). Heavy chains have 4 domains:

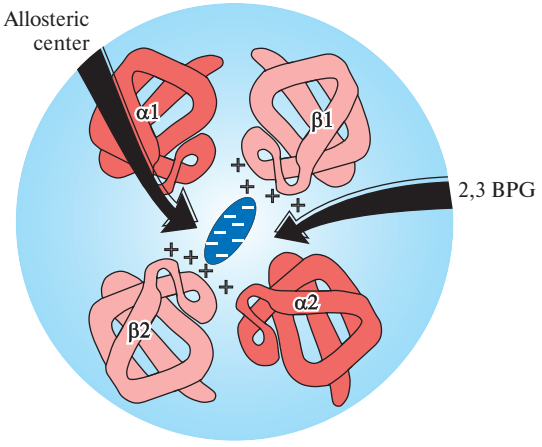


Fig. 1.26. BPG binding to deoxyhemoglobin

BPG binding site is found in a positively charged cavity between 4 protomers of hemoglobin. BPG interaction with the binding site changes the conformation of Hb α- and β-protomers and their active sites. Affinity of Hb for O_2 molecules decreases and oxygen is released into the tissues. In the lungs at high partial pressure of oxygen, hemoglobin active sites are saturated at the expense of conformational change and BPG is forced out of the allosteric site.

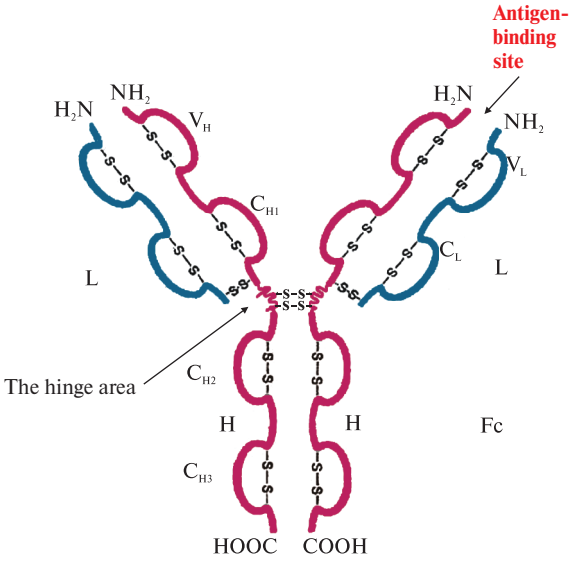


Fig. 1.27. Structure of immunoglobulin monomer

H — heavy chain;
L — light chain;
V_L, V_C — variable regions of heavy and light chains;
C_L, C_{H1}, C_{H2}, C_{H3} — constant regions of heavy and light chains;
F_C — fragment responsible for the binding with other components of immune system.

one is variable (V_H) and three constant (C_{H1}, C_{H2}, C_{H3}). All domains have β-folding structure and are stabilized by disulfide bonds between cysteine residues.

The heavy chain contains many proline residues between C_{H1} and C_{H2} domains preventing the formation of secondary structure and interaction of chains in this fragment. This part is referred to as «hinge region» because it gives to immunoglobulins the intramolecular mobility.

The binding site for antigen is formed by variable regions of H- and L-chains. The interaction with antigen occurs due to the noncovalent bonds. The unique possibility of each clone of antibodies to recognize and link the appropriate antigen is defined by hypervariableregion resided on variable domains of H- and L-chains and composed of 25–30 amino acids. There are 25^{20} possible combinations of this number of amino acids, i. e. due to the exchange of amino acids in hypervariableregions of heavy and light chains, practically an unlimited number of different immunoglobulins can exist in the human body.

Immunoglobulin classes, their structural and functional features

Human immunoglobulins are divided into 5 classes (A, D, E, G, M) differing in the structure of heavy chains (α, δ, ε, γ, μ). These differences determine the conformation characteristic of each class as well as physiological function (Fig. 1.28). The binding of antigen induces the change of conformation of immunoglobulin constant domains and this determines the subsequent course of its elimination. The light chains have two domains (κ and λ) found in all classes of immunoglobulins.

Immunoglobulins M (IgM) are secreted at early stages of the **primary immune response** and exist in two forms.

- Monomeric (early form) bound to B-lymphocyte surface is the receptor identifying antigen. It is built up of single monomer which has a hydrophobic part at C-terminal of heavy chains providing its incorporation into B-lymphocyte membrane.
- Secretory (latter form) is built up of 5 monomers linked by additional J-chain and has 10 sites for antigen binding (Fig. 1.28). This form is secreted into blood in the primary response and the most effective against viruses and bacteria. Repeated introduction of the same antigen leads to the massive secretion of IgG. This property of immune system is referred to as immune memory. When a child is born the level of IgM increases because a newborn is exposed to antigen stimulation.

Immunoglobulins G (IgG) are the main class of immunoglobulins secreted in the **secondary immune response** and comprise up to 75% of all immunoglobulins of the blood. IgG is a monomer form without hydrophobic region at C-term and are not incorporated into cell membranes. It is the main anti-infective immunoglobulin neutralizing bacterial toxins and increasing phagocytosis by binding to microorganisms. They are the only class of antibodies which can penetrate placenta barrier, they provide prenatal defense of fetus and passive immunity of newborns during the first weeks of life.

Immunoglobulins A (IgA) are the main class of antibodies found in **secretions** (saliva, respiratory, gastric, urogenital secretions, and milk) being the first row of defense. They are composed of 2, 3, or 4 monomers bound by J-chain. Microorganisms coated by IgA can't

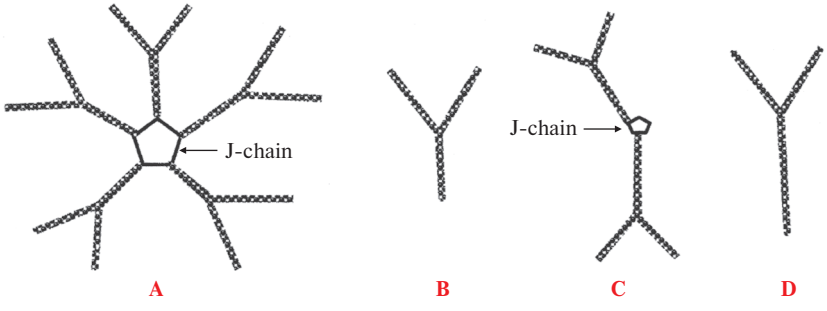


Fig. 1.28. Structure of immunoglobulins of different classes

A — IgM, secretory form (pentamer that has a linking J-chain);
B — IgG (monomer);
C — IgA (di- and tetramer that has a linking J-chain);
D — IgE (monomer).

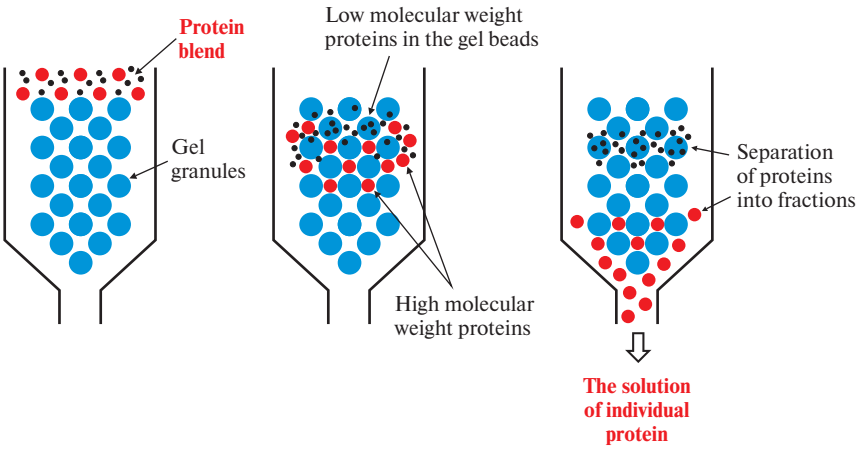


Fig. 1.29. Separation of proteins using gel-filtration method

bind to the surface of mucus membranes and are not involved in the infection. The saliva contains specific secretory form sIgA ensuring immune response of oral cavity.

Immunoglobulins E (IgE) are antibodies that bind to the receptors of **mast cells and eosinophils** after secretion. The interaction with the cell membrane occurs with the participation of hydrophobic terminal like in the monomeric IgM. The binding of antigens stimulate the cell to secret the histamine responsible for development of inflammatory reaction; in allergy, the histamine secretion is increased. The function of IgE in healthy individuals is to ensure antiparasitic immunity.

Immunoglobulins D (IgD) found on very few B-lymphocytes (~1.5%) have monomeric structure and play the role of surface **receptor for antigen recognition.**

1.6. MAIN TECHNIQUES OF SEPARATION AND PURIFICATION OF PROTEINS

The comprehensive study of physical-chemical properties, chemical composition and structure is possible only in analyzing purified protein preparation. For extraction and fractionation of the individual proteins the following techniques are used: salting out, sedimentation by organic solvents, gel filtration, electrophoresis, ion-exchange chromatography, and affinity chromatography.

Salting out is based on the dependency of protein solubility on medium properties. In distilled water, proteins are dissolved worse than in weak salt solutions because low ion concentrations stabilize their hydrate coats. But at high salt concentrations protein molecules lose hydrate coats and aggregate forming the sediment. Once the salt removed, proteins are soluble again maintaining native properties and conformation.

The change of solubility at different salt concentrations and pH are used for the extraction of individual proteins. Ammonium sulfate solutions of different concentrations are more often used for salting out.

The sedimentation of proteins without denaturation is carried out by dehydrating agents such as organic solvents (ethanol, acetone).

Gel-filtration is based on the separation of proteins according to the size and shape of molecule. The separation is carried out in chromatographic columns filled with granules of porous gel (sephadex, agarose) in a buffer solution with certain pH value. Gel granules (beads) are permeable for proteins because of internal pores (cavities) with a definite medium diameter the size of which depends on the type of gel (sephadex G-25, G-200 etc.). The mixture of proteins is loaded into a column and then it is eluted by buffer solution with the definite pH. The large protein molecules can't penetrate into the bead pores and move quickly with the solvent through the column. Small molecules of low-molecular impurity (salt) or another protein are retained by gel beads and eluted with a lower rate (Fig. 1.29). When leaving the column the solution (eluate) is collected as a series of separate fractions.

Electrophoresis is based on the property of charged protein molecules to move in the electric field at a rate proportional to their net charge. At a given pH proteins with a net negative charge move to the anode, and those with a net positive charge move to the cathode. Electrophoresis is performed on different carriers: paper, starch gel, polyacrylamide gel etc. The rate of movement depends on charge, weight, and shape of protein molecule. At the end of the procedure the band is stained with special dyes (Fig. 1.30, A).

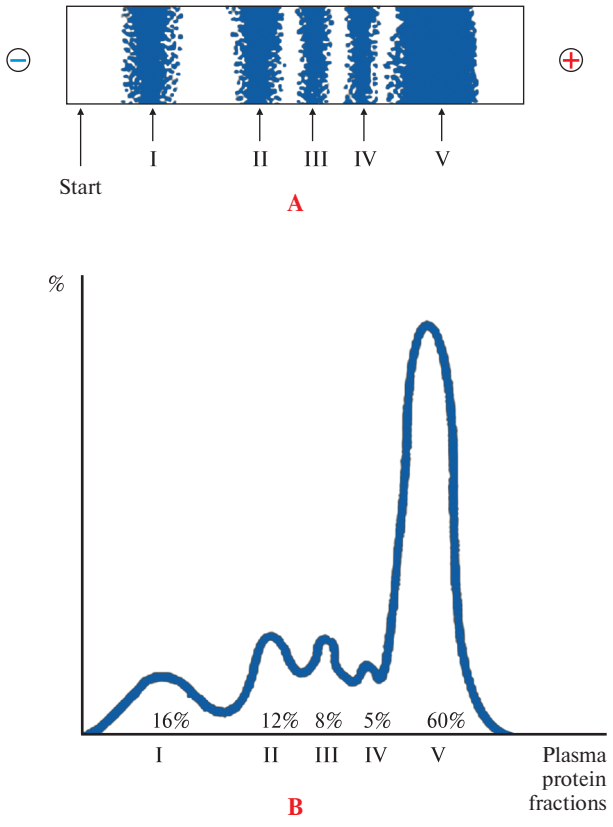


Fig. 1.30. Electrophoretogram of the serum proteins of a healthy person

A — electrophoretogram of serum proteins on paper;
B — number of plasma proteins of different fractions;
I — γ-globulins; II — β-globulins; III — α_2-globulins; IV — α_1-globulins; V — albumins.

The resolving power of electrophoresis in the gel is higher than on paper. When performing serum protein electrophoresis on the paper 5 fractions are separated (albumins, α_1- , α_2- , β- , γ-globulins), and in polyacrylamide gel till 18 fractions are obtained (Fig. 1.30, B).

Ion-exchange chromatography is based on the separation of proteins with different net charge. The solution of protein with certain pH passes through the chromatographic column filled with solid porous sorbent, and some proteins are retained due to electrostatic interaction. The ion-exchange substances are used as sorbents: anion exchangers (containing cation groups) for acidic protein separation; and cationexchangers (containing anionic groups) for alkaline protein separation.

When a protein flows through the column the strength of its binding to ion-exchange sorbent depends on the protein charge opposite to that of the sorbent. The proteins adsorbed on ion-exchange sorbent are eluted by buffer solutions with different salt concentration and pH producing different protein fractions.

Affinity chromatography is based on the specificity of protein binding to a ligand attached to a solid sorbent. Enzyme substrates, prosthetic group of holoproteins, antigens, among other compounds, are used as ligands. When a protein mixture migrates through the column, only the complementary protein binds to ligand (Fig. 1.31, A), whereas all the other substances are washed with the solution. Adsorbed protein is eluted by the solution with another pH value (Fig. 1.31, B). This technique is highly specific and permits to extract highly purified proteins.

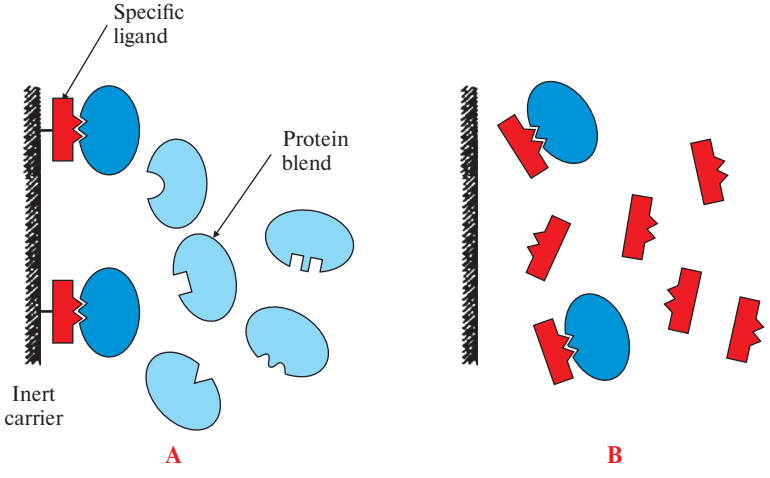


Fig. 1.31. Separation of proteins using affinity chromatography

A — binding of the separated protein to the specific ligand linked to neutral carrier;
B — production of the individual protein solution.

Separation and purification of a protein are usually realized in several stages using different methods. The sequence of stages is selected empirically and may differ for different proteins. High degree of purification of proteins is very important for their application as drugs (hormone insulin, etc.), and in diagnostics of different disorders based on the alteration of protein composition in blood, tissues, saliva, etc.

The set of proteins in the cells of different organs of an adult is individual and is maintained relatively stable during the life time. Specialized tissues can involve specific proteins, for example, hemoglobin in erythrocytes, actin and myoglobin in muscles, different collagen types in bones and connective tissues. The same proteins are found in many tissues but in different quantity. Some changes of protein composition in tissue and blood are possible and related, first of all, to diet, food components, and physical activity.

In disease, the protein composition of blood and tissues can be considerably changed, often the deficiency of a particular protein or decrease of its activity are developed and referred to as **proteinopathia.** Therefore, evaluation of the evident changes in protein composition in blood and tissues is used in clinical trials to diagnose various disorders.

TESTS

1. Choose the correct answer.

Heme:

A. Participates in the interaction with BPG.
B. Kept in the cavity between subunits of oligomeric protein.
C. Is peptide bynature.
D. Is the prosthetic group of hemoglobin.
E. Is the allosteric regulator of hemoglobin.

2. Choose the correct answer.

Hemoglobin and myoglobin:

A. Are choloproteins.
B. Are oligomeric proteins.
C. Are regulated by BPG.
D. Are found in erythrocytes.
E. Are subjected to cooperative changes when interacted with O_2.

3. Determine the correspondence.

A. IgM.
B. IgA.
C. IgD.
D. IgG.
E. IgE.

1. Is charged with primary immune response.
2. Is formed as dimer.
3. Is charged with secondary immune response.

4. Choose the correct answers.

Immunoglobulins:

A. Are oligomers.
B. Have 2 types of H-chains.
C. Are synthesized in B-lymphocytes.
D. Interact with macromolecules entering the body.
E. Have the single site for antigen binding.

5. Make the correspondence.

A. Salting out.
B. Electrophoresis.
C. Gel-filtration.
D. Affinity chromatography.
E. Ion-exchange chromatography.

1. Is used to remove low-molecular impurities.
2. Is based on the mobility of molecules in the electric field.
3. Is based on the interaction with specific ligand.

SITUATIONAL PROBLEMS

1. The content of bisphosphoglycerate (BPG) in blood increases from 4.5 to 7.0 mM/l while staying for two days at the height of 4.5 km above the sea level. When going down to the valley the BPG concentration returns to its initial level. Explain the meaning of such changes. For this purpose:

a) draw and describe the scheme of hemoglobin function;
b) name the center which binds BPG and explain at which level of the structural organization it is formed and show its localization on the figure;
c) indicate which form of hemoglobin BPG will interact (oxyhemoglobin or deoxyhemoglobin) and how it will affect the affinity of hemoglobin for oxygen.

2. As a result of mutation in the gene of hemoglobin β chain in the position 16 glutamate is replaced by asparagine. How will cumulative negative charge of the protein molecule change? To answer this question:

a) explain how many protomers are included in the hemoglobin molecule, what are the bonds that stabilize the quaternary structure of this protein, is hemoglobin a simple or a conjugated protein, and why;
b) draw the formulas of amino acids that determine the protein charge;
c) characterize the radicals of amino acids — glutamate and asparagine, and resolve the task.

3. Oxygen (O_2) is necessary for cells to oxidize substances and generate energy. Its deficiency or excess

is disastrous for tissues. How is the volume of O_2 delivered to tissues strictly in accordance with cells requirements regulated? To answer this question:

a) draw a schematic diagram explaining the ability of hemoglobin to bind O_2 in the lungs and transport it to tissues as well as transporting carbon dioxide (CO_2) from tissues to the lungs;
b) write the definition of the Bohr effect;
c) show the relation of this effect with metabolic tissue activity;
d) explain the change in volume of O_2 entering the tissues when a person intensively works.

4. Mutation resulting in substitution of the amino acid glutamate by valine in the position 6 of hemoglobin β-chains produces a severe hereditary disease — sickle cell anemia when oxygen delivery to the tissues is decreased. The erythrocytes in this disorder have the shape of a sickle. Explain the molecular mechanisms this disease. For this purpose:

a) give the definition the concept of «primary structure of a protein»;
b) characterize all the levels of the spatial organization of hemoglobin;
c) draw the formulas of amino acids located in the position 6 of hemoglobin A (normal) and hemoglobin S (abnormal), and describe their properties;
d) explain how valine affects the properties and function of the hemoglobin molecule.

5. In his Explanatory dictionary of the living great Russian language Vladimir Dahl gives the following definition of the word «угорать» («burn away», meaning poisoning by the fumes of coal): «burning coal forms with oxygen of the air a gas which «burns people away», that is, it causes headaches, nausea, vomiting, people lose consciousness and even die.» What is such action of carbon monoxide (CO) connected with? To answer this question:

a) name the protein which has the high affinity to CO;
b) describe normal functioning of this protein;
c) explain why people get poisoned at high concentration of CO in inhaled air.

6. When examining the patient it was revealed that he had Prasad's disease, severe hereditary anemia caused by impaired zinc (Zn^{2+}) absorption. Concentration of Zn^{2+} in the patient's blood was 12.7 μmol/l, whereas normal is 10,7–22,9 μmol/l. That lead to the reduction of the activity of Zn^{2+}-dependent enzymes, such as carbonic anhydrase, DNA polymerases, and so on. Why did the deficiency of Zn^{2+} in the body cause the anemia? To answer this question:

a) draw the schematic diagram and describe the functioning of hemoglobin;
b) name the Zn^{2+}-dependent enzyme ensuring the realization of Bohr effect and draw the reaction that it catalyzes;
c) explain why the decrease in the speed of the reaction leads to the development of anemia.