

## Properties of Amino Acids and Proteins

### Chapter Overview

Amino acids and proteins are fundamental structural and functional components of living organisms. Amino acids form proteins, which ensure virtually all vital functions—from structural (collagen, keratin), through transport (hemoglobin, albumin), to enzymatic and regulatory roles.

This chapter focuses on the chemical and physical properties of amino acids and proteins, their structure, behavior under different conditions, laboratory methods of their determination, and their clinical significance.

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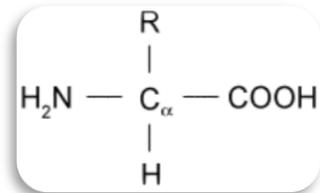
# 1 Amino Acids: Structure and Classification

The topic of amino acids, their structure, and classification is covered in the chapter “*Amino Acids, Peptides, Proteins; Separation Methods.*” Here, we will focus directly on the chemical properties of amino acids and the methods related to them.

## General summary of amino acids:

Amino acids are the basic building blocks of proteins, which determine their structure and thus their biological functions in the organism. A common feature of all amino acids is the presence of a central  $\alpha$ -carbon, to which four different groups are attached:

- **Amino group (-NH<sub>2</sub>):** behaves as a base, can accept protons
- **Carboxyl group (-COOH):** behaves as an acid, can release protons
- **Hydrogen atom (-H)**
- **Side chain (R):** the part that differs among amino acids and is key to their chemical and biological properties (polarity, charge, hydrophobicity)

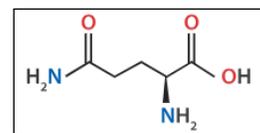


## Classification of amino acids:

- **By polarity of the R-group:**
  - **Non-polar (hydrophobic)** – found inside proteins (glycine, alanine, valine, etc.)
  - **Polar non-ionizable** – form hydrogen bonds, increase solubility (serine, threonine, etc.)
  - **Acidic** – carry a negative charge at physiological pH (aspartate, glutamate)
  - **Basic** – carry a positive charge (lysine, arginine, histidine)
- **By dietary requirement:**
  - **Essential** – cannot be synthesized by the body, must be obtained from the diet (e.g., leucine, lysine, tryptophan)
  - **Non-essential** – can be synthesized by the body (e.g., alanine, glutamate, glycine)



**Question:** Which three amino acids have basic side chains? Identify the following amino acid. Why is it so important for our body?

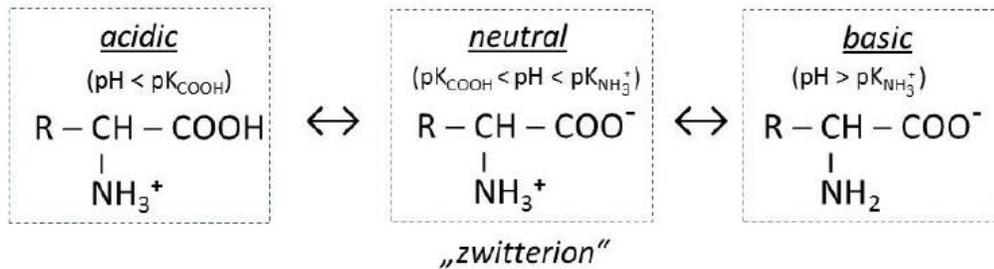


## 2 Chemical Properties of Amino Acids

The structure of the amino acid side chain determines the chemical and physical properties of amino acids. Amino acids do not absorb visible light; proteins are known for their absorption of UV radiation at around 280 nm, which is due to the presence of tryptophan. The side chain also affects their solubility in water. Aliphatic (Ala, Val, Leu, Ile) and aromatic (Trp, Tyr, Phe) amino acids are hydrophobic. Other amino acids are hydrophilic.

Most amino acids have no net electrical charge at neutral pH in solution; they form a **dipolar ion** (zwitterion, amphion), where the amino group is protonated ( $-\text{NH}_3^+$ ) and the carboxyl group is ionized ( $-\text{COO}^-$ ). Thus, the number of positively charged groups balances the number of negatively charged groups, and the protein behaves externally as electrically neutral, not moving in an electric field.

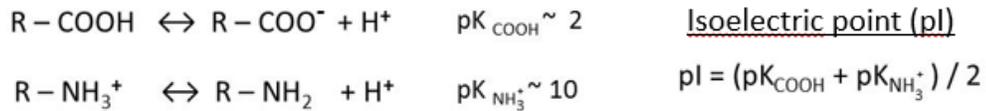
The solubility of proteins at the **isoelectric point (pI)** is lower than at any other pH value. If the  $\text{H}^+$  concentration in the solution increases (pH decreases) to the extent that dissociation of the carboxyl group is suppressed, the amino acid appears positively charged. In alkaline solutions, the opposite is true. A more complex situation arises in amino acids with two carboxyl or amino groups. However, in general, at neutral pH, acidic amino acids (Asp, Glu) are negatively charged, while basic amino acids (Lys, Arg, His) are positively charged.



### 2.1 Isoelectric Point (pI)

Every amino acid contains both an acidic and a basic group, and depending on the pH of the environment, it may act as an acid or as a base. However, there is a certain pH at which the amino acid has an equal number of positive and negative charges—the net electrical charge is therefore zero. This pH is called the isoelectric point (pI).

- At the isoelectric point, the solubility of an amino acid in water is at its lowest, because the molecules more easily aggregate and precipitate.
- The value of pI depends on the nature of the side chain:
  - Acidic amino acids have a pI lower than 6
  - Basic amino acids have a pI higher than 7

amphoteric character

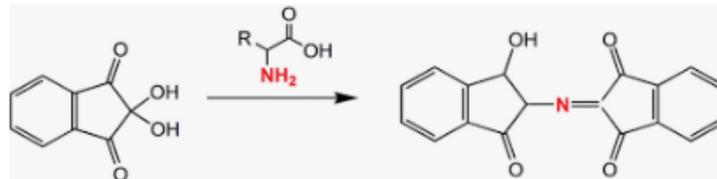
The isoelectric point is important in both biochemistry and medicine—for example, in **protein electrophoresis**, where the movement of molecules in an electric field depends on their charge at a given pH.

## 2.2 Selected Chemical Reactions of Amino Acids

Due to their structure, amino acids participate in a wide range of chemical reactions. Some of these are used in analytical chemistry for their qualitative detection:

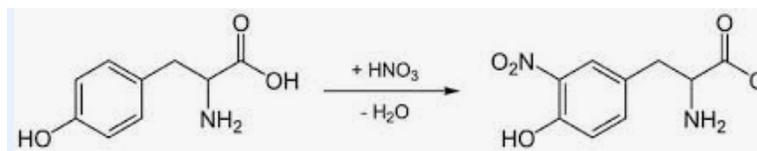
- **Reaction with ninhydrin**

Used to detect **free amino acids**. Ninhydrin oxidizes the amino group to an imino acid, which hydrolyzes to produce ammonia. The ammonia then reacts with two molecules of ninhydrin to yield a strongly **blue-violet product**. This reaction is used, for example, in amino acid chromatography or in forensic science for fingerprint detection.



- **Xanthoproteic reaction**

Specific for aromatic amino acids (tyrosine, partly tryptophan and phenylalanine). In the presence of concentrated nitric acid, nitration of the aromatic ring occurs, producing **yellow nitro derivatives**. After addition of a base, the color intensifies. Historically, this reaction was one of the first tests for proteins.

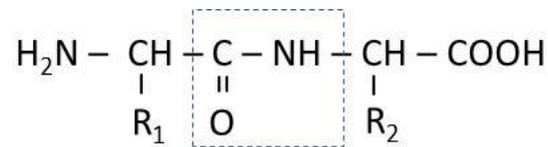


### 3 Structure of Proteins

The topic of peptides, proteins, and their structure is also discussed in the chapter *“Amino Acids, Peptides, Proteins; Separation Methods.”* Here, we will again focus directly on their chemical properties and related methods.

General information about peptides, proteins, and structure:

Proteins are the fundamental building material of the body (collagen, keratin), but also function as hormones, enzymes, and antibodies. They are composed of 20 basic amino acids linked by peptide bonds. Shorter chains form peptides (e.g., insulin, oxytocin, glutathione), while longer chains form polypeptides and proteins.



**Protein structure:**

- **Primary** – amino acid sequence determined by the genetic code.
- **Secondary** – arrangement into  $\alpha$ -helix or  $\beta$ -pleated sheet (hydrogen bonds).
- **Tertiary** – three-dimensional shape stabilized by disulfide bonds, ionic bonds, and hydrophobic interactions.
- **Quaternary** – association of multiple subunits (e.g., hemoglobin).

**Protein classification:**

- **By shape:** fibrous (collagen) and globular (albumin, hemoglobin).
- **By composition:** simple (only amino acids) and conjugated (lipoproteins, hemoproteins, glycoproteins, metalloproteins).

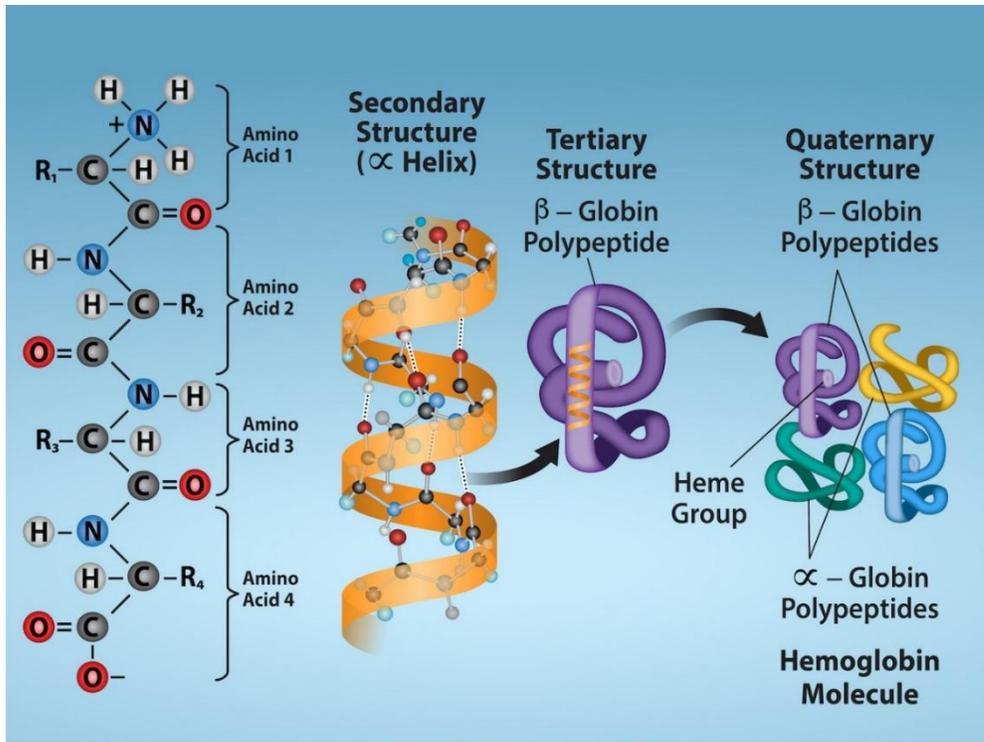


**Interesting fact:** The bright red color of oxygenated blood and the darker shade of deoxygenated blood correspond to conformational changes in hemoglobin.



**Question:** Which types of interactions contribute to the tertiary structure of proteins, and why are they important?

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(Source: <https://raider.pressbooks.pub/biology1/chapter/21-protein-structure-and-function/>)

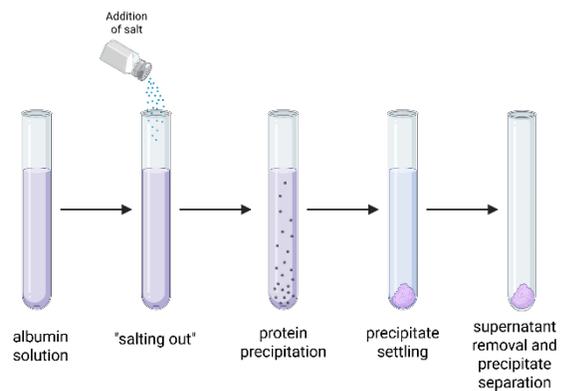
## 4 Physical and Chemical Properties of Proteins

The diversity of proteins is determined by the chemical character of amino acids and their relative proportions within proteins. Proteins exhibit a range of specific physicochemical properties related to their structure. These properties influence their function, behavior in different environments, and the possibilities of laboratory analysis.

### 4.1 Solubility

Protein stability and solubility in aqueous solution are influenced by several factors: the amino-acid composition (number of ionizable and hydrophobic residues), molecular size and shape, pH (distance from the isoelectric point, pI), ionic strength (salts), temperature, and co-solutes such as organic solvents.

At low ionic strength (0.15 M NaCl, physiological saline), many proteins show salting-in: ions screen excessive electrostatic interactions, help maintain the hydration shell and hydrogen-bond network around the protein and thereby increase solubility.



At high ionic strength salting-out occurs. Salts compete with proteins for water and strip the hydration shell; charge screening also reduces repulsive forces between proteins. These effects promote hydrophobic interactions and aggregation of molecules of proteins (precipitation). Ammonium sulfate, which is highly soluble in water and yields three hydrated ions upon dissolution, is most used to precipitate proteins from solution. By gradually adding it, individual protein fractions can be isolated from a mixture.



**Interesting fact:** Protein supplements—*whey protein* and *casein*—differ in digestibility and solubility, which results from differences in their structure.

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## 4.2 Denaturation

Protein denaturation is the process in which higher structural levels of proteins (secondary, tertiary, and quaternary) are disrupted without altering their primary structure (the amino acid sequence). As a result, the protein loses its biological activity. Denaturation can be caused by physical factors such as elevated temperature, ultraviolet radiation, ionizing radiation, or mechanical action, as well as chemical factors such as extreme pH, organic solvents, chaotropic agents (urea, guanidinium chloride), or heavy metals forming complexes with cysteine sulfhydryl groups. Denaturation may be reversible, where the protein partly regains its structure after the removal of the causative factor, or irreversible, when permanent changes and precipitation occur. This phenomenon is crucial in biology and practice—it explains the loss of enzyme activity, is applied in sterilization, and plays an important role in the food industry, such as protein coagulation during egg cooking or cheese production.

## 4.3 Size and Charge

Proteins are amphoteric macromolecules that carry different net charges depending on pH, due to the presence of ionizable amino acid side groups. The charge affects their behavior in an electric field—proteins become positively charged in acidic environments and negatively charged in alkaline environments. In addition, the size and shape of protein molecules play a major role in determining their movement in solution or gel. These properties are practically exploited in methods that separate proteins based on charge and size—the classic example is electrophoresis, which enables protein mixtures to be separated and provides valuable information about their composition.

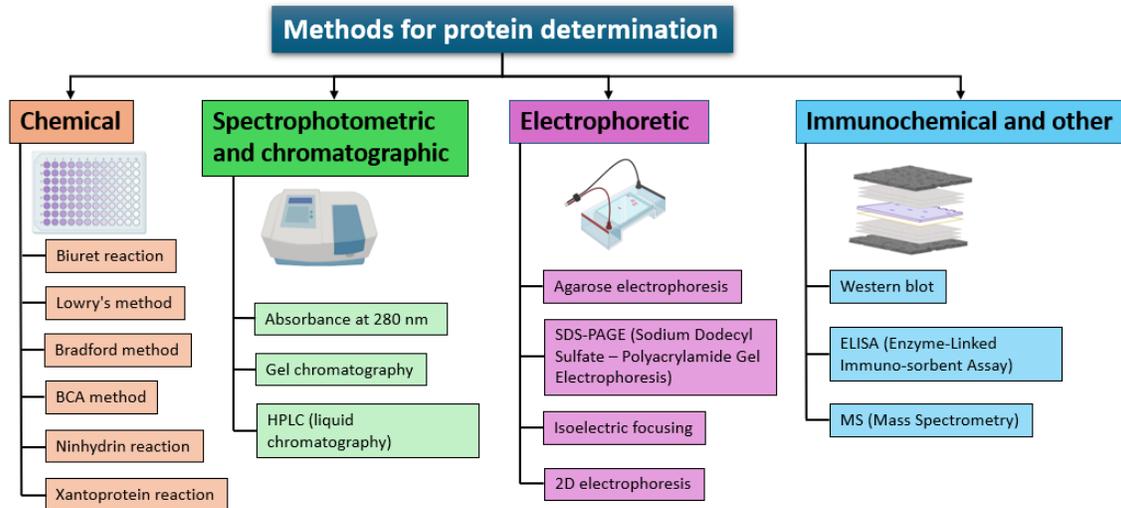
## 4.4 Sorption and Buffering Properties

Proteins have strong sorption properties, due to functional groups capable of forming hydrogen bonds, electrostatic, or hydrophobic interactions. This enables them to bind various molecules and ions on their surface, which is important in biological processes as well as in protein purification.

At the same time, proteins also exhibit buffering properties, as they contain ionizable groups (carboxyl, amino, imidazole, etc.) that can accept or release protons. This allows them to contribute to maintaining stable pH in biological systems, for example in blood plasma.

? **Question:** How does pH affect the overall electrical charge of proteins, and how is this property used in practice?

## 5 Methods for Protein Determination



### 5.1 Chemical Methods

#### Biuret reaction

The basic colorimetric method for detecting proteins. In an alkaline environment, copper ions ( $\text{Cu}^{2+}$ ) form a complex with peptide bonds. A characteristic violet color appears, and its intensity is directly proportional to the protein concentration.

#### Lowry method

One of the most sensitive classical methods for protein determination. It combines the biuret reaction with the formation of a colored complex after reduction of the Folin–Ciocalteu reagent (a mixture of phosphomolybdate and phosphotungstate). The result is a blue color that can be measured precisely by spectrophotometry.

#### Bradford method

Based on the binding of the dye Coomassie Brilliant Blue G-250 to basic amino acids (mainly arginine, partly lysine and histidine) and to aromatic side chains (phenylalanine, tryptophan, tyrosine). After binding to protein, the dye shifts from its red form to a blue form. This method is fast, simple, and very popular for determining proteins in biological samples.

#### BCA method (bicinchoninic acid)

Similar to the Lowry method, it is based on the complexation of copper ions after their reduction by proteins. In an alkaline environment, a violet-colored complex forms with high

sensitivity, evaluated spectrophotometrically. Its advantages include good color stability and reproducibility of results.

## 5.2 Spectrophotometric Methods

Proteins naturally absorb ultraviolet (UV) light at around 280 nm. This is due to the presence of aromatic amino acids—primarily tyrosine, tryptophan, and partly phenylalanine—whose aromatic rings strongly absorb in this spectral region.

**Advantages:** the method is simple, fast, and requires no additional reagents—the protein solution is measured directly. The absorbance at 280 nm is proportional to protein concentration and can be quantified using the Lambert–Beer law.

**Disadvantages:** relatively low specificity—if other substances that absorb in the UV region are present in the sample (e.g., nucleic acids or certain metabolites), false high values may occur. Therefore, this method is often combined with other approaches to verify results.

## 5.3 Immunochemical Methods

Immunochemical methods make use of the high specificity of antibodies, which bind to the target protein. This allows not only detection of its presence but also precise quantification. These methods are now fundamental tools in both clinical and research biochemistry.

### ELISA (Enzyme-Linked Immunosorbent Assay)

ELISA is one of the most commonly used immunochemical techniques for the determination of proteins, hormones, antibodies, or other analytes. It is based on the specific binding between antigen and antibody, which is then translated into a measurable color signal through an enzymatic reaction.

#### 1. Capture of the analyte by the primary antibody

A specific primary antibody against the analyte is immobilized on the surface of an inert carrier (usually a 96-well microtiter plate). Samples and calibrators are pipetted into the wells. If the sample contains the analyte, it binds to the antibody. Unbound components are then washed away.



#### 2. Binding of the secondary antibody with enzyme

A secondary antibody is added, which binds to another epitope of the analyte. This antibody is covalently linked to an enzyme (e.g., horseradish peroxidase – HRP). After binding, excess unbound antibody is removed by washing.

**3. Enzymatic reaction with substrate**

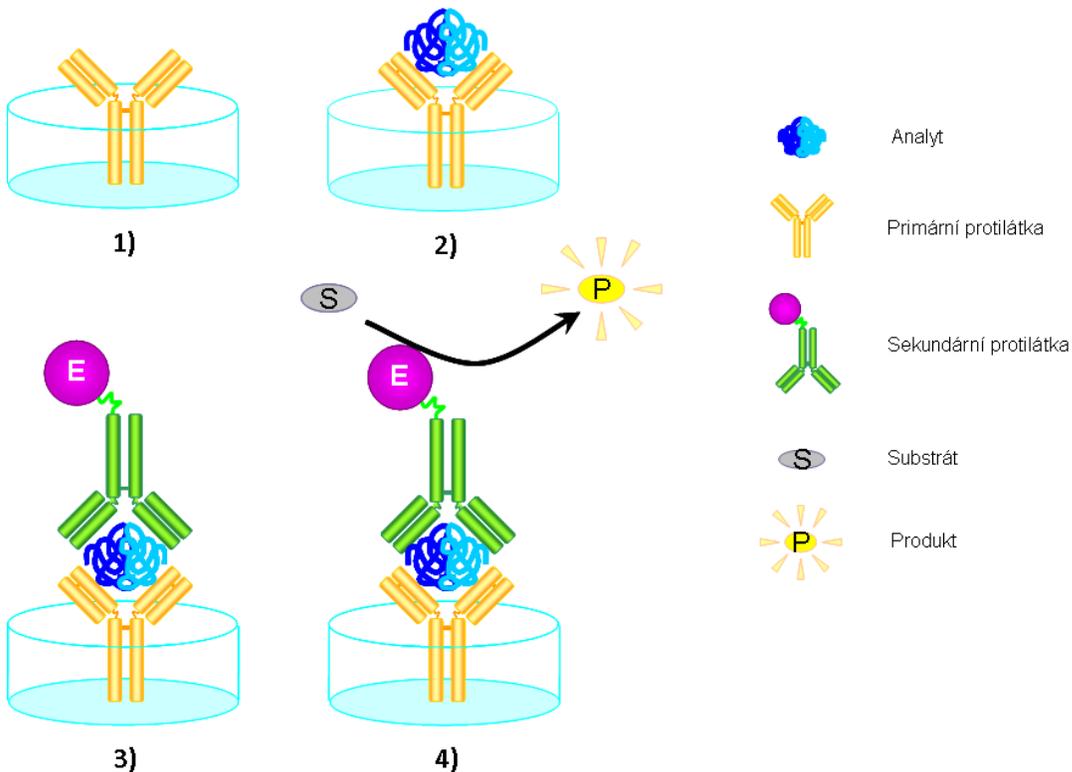
A suitable substrate for the enzyme is added. The enzyme catalyzes a reaction that produces a colored product. The intensity of the color is directly proportional to the amount of analyte in the sample.

**4. Detection and evaluation**

The color signal is measured with a spectrophotometer (the so-called ELISA reader). The result is a relationship between analyte concentration and absorbance at a selected wavelength. Using a calibration curve, the concentration of the analyte in individual samples can be precisely determined.

**Advantages:** very high sensitivity and specificity, ability to analyze many samples simultaneously.

**Applications:** diagnosis of infectious diseases, determination of hormones, tumor markers, or antibodies.



## Western Blot

A combined method: proteins are first separated by size using electrophoresis, then transferred onto a membrane and subsequently detected with specific antibodies.

**Advantages:** enables not only quantification but also identification of a specific protein among many others.

**Applications:** research, diagnosis of certain infectious diseases (e.g., HIV), confirmation of ELISA test results.

## Immunonephelometry and Immunoturbidimetry

In both methods, turbidity caused by the formation of antigen–antibody immune complexes are measured.

- **Immunonephelometry:** measures light scattering produced by immune complexes.
- **Immunoturbidimetry:** measures the direct decrease in transmitted light intensity caused by sample turbidity.

**Applications:** determination of serum protein concentrations (e.g., immunoglobulins, C-reactive protein, complement).

## 5.4 Electrophoretic Methods

**Electrophoresis** is a group of methods that exploit the movement of charged molecules in an electric field. Proteins are separated according to their charge, size, or isoelectric point, providing highly detailed information about their properties.

### Agarose and acetate electrophoresis

Proteins migrate in a porous support (agarose or acetate gel) under the influence of an electric field. Separation is determined by their overall charge and size.

**Use:** classical method in clinical diagnostics, e.g., for separating serum proteins into fractions (albumin,  $\alpha$ -,  $\beta$ -, and  $\gamma$ -globulins). Important in diagnosing paraproteinemias (e.g., multiple myeloma).

### SDS-PAGE (Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis)

Before separation, proteins are treated with the detergent SDS, which denatures them and imparts a uniform negative charge. Thus, in an electric field they are separated mainly according to molecular weight.

**Use:** research biochemistry and molecular biology—analysis of sample purity, determination of protein size, control of recombinant proteins.

### **Isoelectric focusing (IEF)**

Proteins are separated in a pH gradient and migrate in the electric field until they reach their isoelectric point (pI). At this pH, their net charge is zero and migration stops.

**Advantage:** extremely high resolution, even between proteins that are otherwise very similar.

**Use:** detailed protein characterization, detection of mutations and subtle structural changes.

### **2D electrophoresis (2D-PAGE)**

Combines isoelectric focusing (1st step) and SDS-PAGE (2nd step). Proteins are first separated by pI, then by size. The result is a two-dimensional gel with very high resolution.

**Use:** a key method in proteomics, allowing mapping of hundreds to thousands of proteins in a cell or tissue and comparing their changes under different conditions (healthy vs. diseased).

## **5.5 Other Modern Methods**

In addition to classical colorimetric, spectrophotometric, immunochemical, and electrophoretic methods, modern biochemistry also employs highly sensitive instrumental techniques that allow detailed analysis of protein composition and quantity.

### **Mass spectrometry (MS)**

Mass spectrometry enables precise determination of the molecular weight, composition, and structure of proteins. The principle is ionization of proteins or their fragments, followed by measurement of their movement in an electric and magnetic field according to their mass-to-charge ratio (m/z).

**Advantages:** extremely high sensitivity, ability to identify even very small amounts of substance, capability to distinguish various protein modifications (e.g., phosphorylation, glycosylation).

**Applications:** proteomics, disease biomarker discovery, analysis of complex mixtures.

### **High-Performance Liquid Chromatography (HPLC)**

HPLC is a technique for separating and quantifying proteins or peptides based on their interactions with the stationary and mobile phases. Proteins migrate through a chromatographic column at different speeds depending on their physicochemical properties (e.g., hydrophobicity, size, charge).

**Advantages:** high separation efficiency, possibility of quantification, compatibility with detection methods (e.g., UV, fluorescence, MS).

**Applications:** determination of protein purity, mixture analysis, preparation of protein samples for further methods (e.g., MS).

## 6 Clinical Significance of Proteins and Amino Acids

**Proteins and amino acids** are not only the fundamental structural and functional components of the organism but also important indicators of health and disease. Their levels in biological fluids (especially blood and urine) are used in clinical biochemistry for diagnosis, monitoring the course of disease, and assessing the nutritional status of the patient. Changes in concentration often reflect pathological processes, metabolic disorders, or inflammatory reactions.

### 6.1 Clinically Significant Changes in Proteins

**Hypoalbuminemia** refers to a decreased concentration of albumin in serum. The most common causes include liver failure (reduced protein synthesis), nephrotic syndrome with increased urinary loss, or severe malnutrition and malabsorption. Clinically, hypoalbuminemia is important primarily because it leads to decreased oncotic pressure and the development of edema. It also serves as a marker of disease severity.

**Paraproteinemia** is a condition where abnormal monoclonal immunoglobulins, known as paraproteins, appear in the blood. It is typically encountered in multiple myeloma. It is a significant diagnostic and monitoring marker in hematological malignancies.

**Acute phase reactants** are proteins whose serum concentration increases during inflammation or infection. The most important include **C-reactive protein (CRP), haptoglobin, and transferrin**. These proteins allow rapid evaluation of the presence and intensity of an inflammatory process and are among the basic laboratory markers in clinical practice.

**Specific proteins** are primarily important in the diagnosis and monitoring of particular diseases. For example, **prostate-specific antigen (PSA)** is used in screening and monitoring prostate cancer.  **$\beta_2$ -microglobulin** is a marker of certain hematological malignancies and is also used in nephrology to assess kidney function.

### 6.2 Amino Acid Metabolism disorders

Disorders of amino acid metabolism are often rare hereditary metabolic diseases that can have serious consequences for a child's development and health. For this reason, they are now part of newborn screening.

**Phenylketonuria (PKU):** a genetic disorder of phenylalanine metabolism caused by deficiency of the enzyme phenylalanine hydroxylase. This results in accumulation of phenylalanine, which is toxic to the nervous system. Without treatment, it leads to severe intellectual disability. The cornerstone of therapy is early dietary management with restricted phenylalanine intake.

Other inherited disorders include **homocystinuria, tyrosinemia, and MSUD (Maple Syrup Urine Disease)**. All of these conditions are caused by genetically determined enzyme defects in

amino acid metabolism. Without treatment, they may lead to serious neurological disorders, delayed psychomotor development, or organ damage. Thanks to newborn screening, these diseases can be detected early and appropriate treatment initiated.

### Summary

Amino acids are the building blocks of proteins, whose properties determine the behavior and function of the entire protein. A key concept is the **isoelectric point**, which influences protein charge and solubility. Protein structure is described at four levels—from the **primary sequence** of amino acids to **quaternary organization**. Proteins are amphoteric, possess sorption and buffering properties, and undergo denaturation under physical or chemical factors.

Protein determination is performed using a wide range of methods—from simple **colorimetric assays** (Biuret, Bradford) to highly sensitive **immunochemical methods** (ELISA, Western blot) and modern **instrumental techniques** (MS, HPLC). In clinical practice, proteins play a crucial role in diagnosing inflammation (CRP), cancer (PSA), immunological disorders (paraproteinemia), or inherited metabolic diseases (PKU, MSUD).

### Control Questions

1. Which three amino acids have basic side chains?
2. What is the isoelectric point, and why is it important for protein solubility?
3. What is the difference between the secondary and tertiary structure of proteins?
4. What is the principle of the ninhydrin reaction in protein determination?
5. Why do proteins absorb UV radiation at 280 nm?
6. What is paraproteinemia, and with which disease is it associated?
7. What is the principle of 2D electrophoresis?