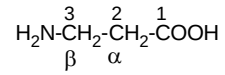
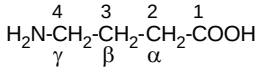
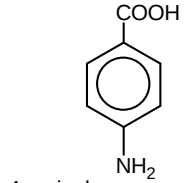
№ 40. AMINO ACIDS. CHARACTERISTICS, CLASSIFICATION. STANDARD α-AMINO ACIDS – ISOMERISM, PHYSICAL PROPERTIES. AMPHOTERIC AND CHEMICAL PROPERTIES OF AMINO ACIDS. PEPTIDE BOND, PEPTIDES.

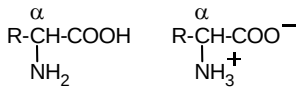
Biopolymers and their monomers. The three classes of biopolymers are nucleic acids (DNA, RNA), proteins, and carbohydrates. Polymers, including biopolymers, are made of repetitive units called monomers. The monomeric units of nucleic acids are pyrimidine and purine nucleotides, of proteins – α-amino acids, and of carbohydrates – monosacharides. A major but defining difference between polymers and biopolymers can be found in their structures. Synthetic polymers are with simpler, random structure. Biopolymers often have a well defined structure. Many biopolymers spontaneously fold into characteristic compact shapes, which determine their biological functions. There is strict relationship structure–function of a biopolymer. Understanding diseases at molecular level is the modern medicine.

Amino acids. **I. Classification**. Amino acids are molecules containing an amino group, a carboxylic acid group and a hydrocarbon (side) chain that varies between different amino acids. The nature of the hydrocarbon chain is one criterion for amino acids classification. There are aliphatic (saturated and unsaturated) and aromatic amino acids. The number of COOH and NH_2 groups can vary. According to their number distinguished are, for instance, monoamino or diamino monocarboxylic acids, monoamino or diamino dicarboxylic acids, etc. The presence of a heterocyclic ring gives a group of heterocyclic amino acids. Amino acids are classified also as α-, β-, γ-, and so on, according to the location of the amine group on the carbon chain that contains the carboxylic acid function. The Greek letters are always used with trivial names. Biologically relevant (proteinogenic which means building proteins) are the α-amino acids. While more than 700 different amino acids are known to occur naturally, chemists and biochemists recognize a group of 20 of them as deserving of special attention. These are the amino acids that are normally present in proteins. They are called **standard α-amino acids**. All the amino acids from which proteins are derived are α-amino acids, and all but one of these contain a primary amino function and can be described by the general formula $H_2NCHRCOOH$, where R is an organic substituent and by the structure:

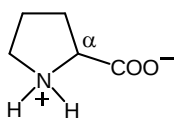

3-aminopropanoic acid
β-aminopropionic acid

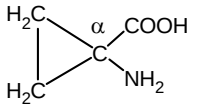

4-aminobutanoic acid
γ–aminobytiric acid (GABA)


4-aminobenzenecarboxylic acid
p-aminobenzoic acid

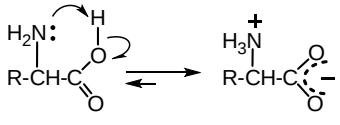


The 20 standard amino acids are genetically encoded and included in variety of proteins. Proline (2-pyrrolidinecarboxylic acid) is a secondary amine in which the amino nitrogen and the substituent R are involved in same ring. Small rings can be found in other non-proteinogenic amino acids, for example 1-aminocyclopropane carboxylic acid which is precursor of ethylene (CH_2=CH_2) in plants.


proline


1-aminocyclopropane-carboxylic acid

The simplest proteinogenic α-amino acid, glycine is a very polar substance, much more polar than would be expected on the basis of its formula H_2NCH_2COOH. It is crystalline solid that does not melt on heating but decomposes. It is very soluble in water and practically insoluble in organic solvents. These properties are not in accord with the formula H_2NCH_2COOH but are attributed to an inner salt. Every α-amino acid contains highly basic amino group (NH_2) that is a proton acceptor, and a carboxylic acid group (COOH) that is a proton donor (acidic group). In an acid-base reaction a proton transfer occurs internally, within the same molecule. This proton transfer is indicated with **zwitterionic** form of the amino acid (in German "*Zwitter*" means hybrid, hermaphrodite). The molecule contains both positive and negative charges which compensate each other. The proton transfer occurs through rearrangement of electronic density, as shown with curved arrows. The equilibrium expressed by the equation lies overwhelmingly to the side of the zwitterion. Zwitterions are the dominant form of standard amino acids in biological conditions (pH about 7).



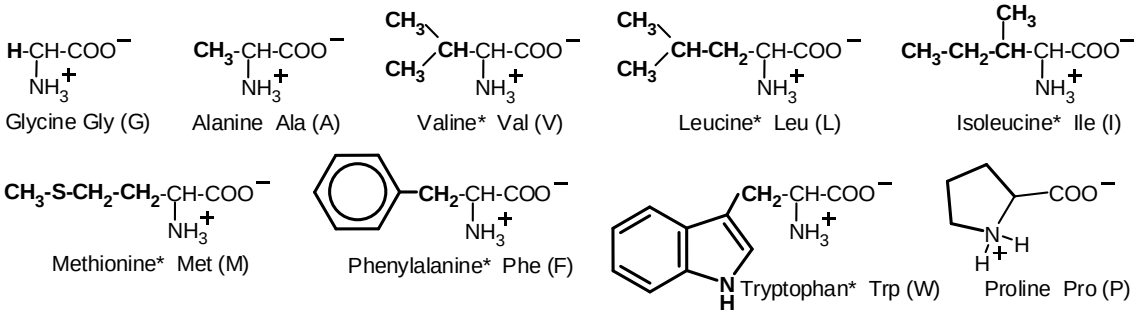
Classification of the standard α-amino acids. Besides the classification of amino acids by chemical structural features, there are subdivisions of the standard amino acids made on biological bases. While humans possess the ability to biosynthesize some of the amino acids shown below, they must obtain certain of

the others from their food. Those amino acids that **can not be biosynthesized** from other compounds at the level needed for normal growth and must be included in our dietary requirements are called **essential amino acids**. The rest are non-essential amino acids.

Of particular importance in describing the proteins' structure is the polarity of the side chain and its acid-base properties. Depending on the polarity of the side chain, amino acids vary in their hydrophilic or hydrophobic character. These properties are important in protein structure and protein–protein interactions. The importance of the physical properties of the side chain comes from the influence this has on the amino acid residues' interactions with other structures, both within a single protein and between proteins. The standard amino acids are classified according to the polarity, acidic or basic properties of the side chain into four groups: (1) amino acids (AA) with nonpolar side chains; (2) AA with polar, uncharged side chains; (3) AA with anionic (negatively charged) side chains; (4) AA with cationic (positively charged) side chains (at pH ~ 7).

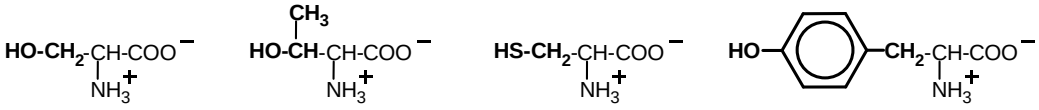
When writing a protein structure is more convenient to use abbreviations with three letters or the newer one-letter code. Essential amino acids are indicated with (*) on their trivial name.

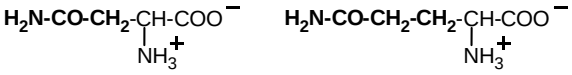
(1) Amino acids with nonpolar side chains:



Proline is the only amino acid containing secondary α-amino group incorporated in five membered pyrrolidine ring. An aromatic residue is always separated from the α-carbon by CH_2 group.

(2) amino acids with polar, uncharged (nonionized) side chains:



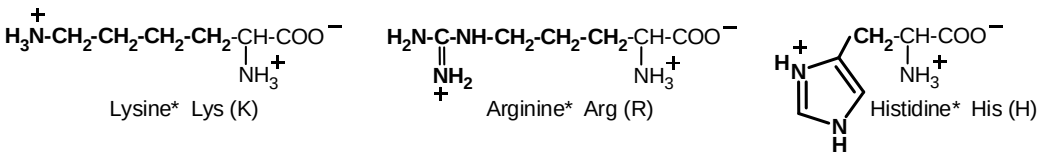


Cysteine and tyrosine may be considered also as AA having acidic side chains because of higher acidity of a thiol and phenol hydroxyl group *vs*. alcohol OH. Threonine and isoleucine are the only two AA with second chiral center besides the α-carbon.

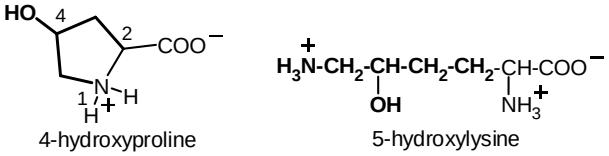
(3) amino acids with acidic (negatively charged) side chains:

They exist as anions at pH ~ 7.

(4) amino acids with basic (positively charged) side chains:



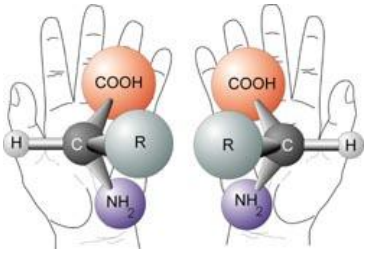
They exist as cations at pH ~ 7.



Some α-amino acids, although found in significant amounts in living organisms including humans, are not standard AA. Examples are 4-hydroxy-

proline and 5-hydroxylysine. They are created by modification of a standard amino acid **after** the protein synthesis (the so called post-translational modification). Therefore they are not considered proteinogenic; they are not DNA-encoded. Both of these AA are found in collagen, a fibrous protein in connective tissues.

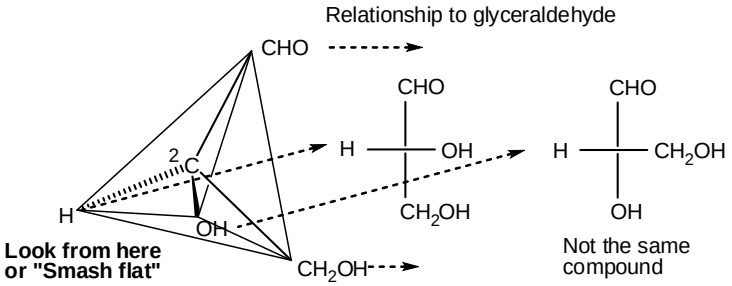
II. Stereochemistry of amino acids. **Chirality**. The term chiral is used to describe an object that is non-superposable on its mirror image. Achiral (not chiral) objects are objects that are identical to their mirror image. Left and right hands of a human are chiral because no matter how the two hands are oriented, it is impossible for all the major features of both hands to coincide. A chiral carbon atom carries four different substituents. Such carbon atom is a stereogenic center. Stereogenic center may also be an atom of other element fulfilling the definition for chirality. Chirality is the property of the object not to coincide with its mirror image. In chemistry, chirality usually refers to molecules. Two mirror images of a chiral molecule are called **enantiomers** or optical isomers. The connectivity (topology) in both enantiomers is identical, however they **differ by configuration**. Configuration of a chiral center is the spatial arrangement of atoms or groups around the stereogenic center. Designators for configuration are usually L-/D- (older system) and R/S but not l/d or +/– which describe optical rotation. **All natural amino acids in proteins have L-configuration** of their α-carbon atoms. Glycine (aminoacetic acid) is the only standard amino acid that is achiral. Threonine and isoleucine are the only two AA with second chiral center besides the α-carbon. These amino acids have four stereoisomers each but only one is incorporated in proteins.



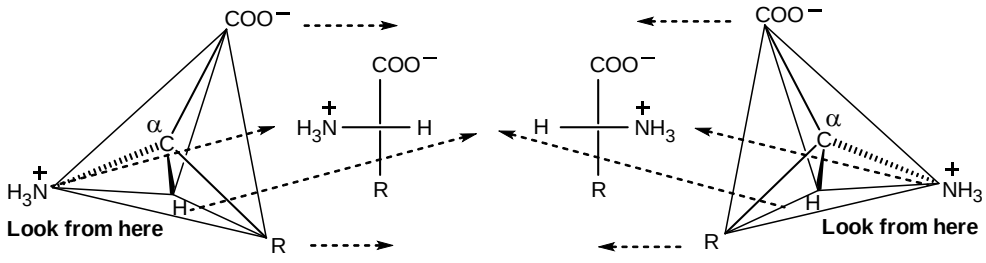
(**L**) R = CH_3 (**D**)
two alanine enantiomers

Chirality can make enormous difference at the molecular level of life. One asparagine enantiomer tastes sweet and the other tastes bitter. All biopolymers are chiral. Their relationship to two enantiomers of other chiral molecules is different. For instance, an enzyme performing chemical transformation on its substrate must have first the substrate tightly bound to the active center. Since the enzyme is composed of chiral amino acids, the active center could accommodate only one of two (or more) possible steric arrangements of the substrate. Fitting an L-amino acid to active site of enzyme, e.g. aminoacyl tRNA synthase, is like fitting glove to hand. The substrate must possess matching chirality to the enzyme. Molecules with opposite chirality can't fit.

The **L-configuration** of standard amino acids means that **in a Fischer projection** the **amino group is on the left-hand side**. Fischer projections were described already for lactic acid. Fischer projections are always generated the same way: the molecule is oriented so that the vertical bonds at the chiral carbon are directed away from you and the horizontal bonds point toward you. Since these projections are related to glyceraldehyde and are widely used for amino acids and carbohydrates, the most oxidized carbon is placed on the top of the projection. A projection of the bonds onto the page is a cross. The chiral carbon atom lies at the center of the cross but is not explicitly written. Two groups in a Fischer projection cannot switch places because the result is enantiomer, and projection formulas cannot be rotated at 90°, 270°, etc. but can be rotated at 180°.

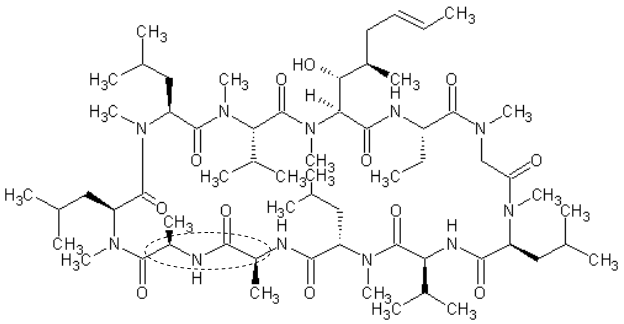


In order to apply Fischer projections to α-amino acids, the tetrahedron of the α-carbon is oriented in such way that the carboxyl group points upward and the side chain (R) is downward, both pointing away from an observer. The observer looks towards the imaginary line connecting the α-H with α-NH_2. If the observer sees, and the Fischer projection shows, the amino group on the left – then the amino acid has L-configuration. If the amino group is on the right – the amino acid is D-amino acid.



All proteinogenic amino acids are L-amino acids.

D-Amino acids are not only known, obtained synthetically, but also found in Nature. Many D-amino acids occur naturally, notably in bacterial walls. An astonishing example is Ciclosporin which is a cyclic peptide containing eleven amino acids. Initially the substance was isolated from soil sample. The chemical structure reveals seven N-methylated and one D-amino acid, all rarely encountered in nature. The oval in the shown structure encircles the connected D-Ala–Ala fragment. Ciclosporin is an immunosuppressant drug widely used in post-organ transplant to reduce the activity of the patient's immune system. With such important action, Ciclosporin decreases significantly the risk of organ rejection. The compound has been studied in transplants of skin, heart, kidney, liver, lung, pancreas, bone marrow and small intestine.

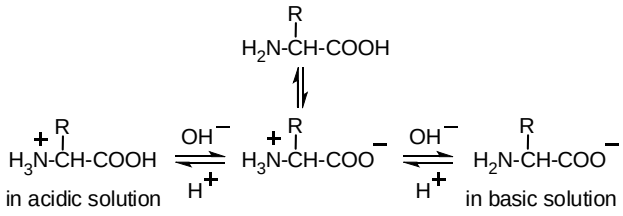


The important point is that D-amino acids are not constituents of proteins.

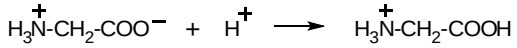
III. Physical properties. The α-amino acids physical properties are determined by the presence of ionized amino (cationic) and carboxyl (anionic) groups. The substances exist as internal salts. Usually they are colorless solids that have ionic crystal lattice. The α-amino acids have high melting points and normally decompose on heating without melting (unusual for an organic compound). They are highly soluble in water and in very polar solvents. Amino acids have low solubility in alcohol, ether and organic solvents.

The other characteristics, including chemical properties, are not additive of those for acids and amines.

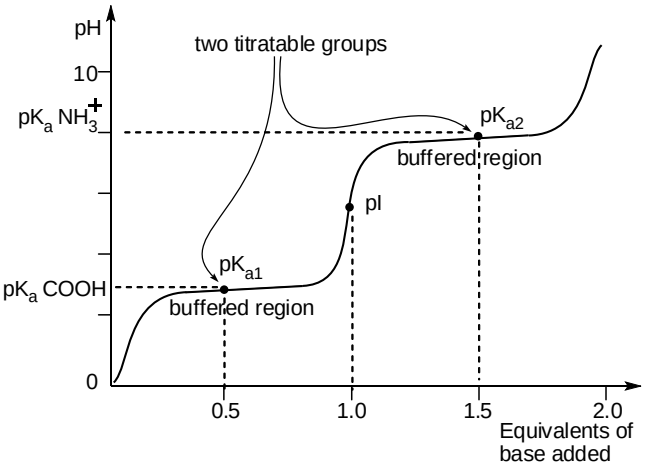
IV. Amphoteric properties of amino acids. Since amino acids contain both an acidic and a basic functional group they possess both acidic and basic properties, i.e. they are amphoteric compounds. The acidic functional group is the ammonium ion (NH_3^+); the basic functional group is the carboxylate ion (COO^-).



Aside from the physical properties cited in the previous theme, the acid-base properties of any standard amino acid require such conclusion. As simplest example, consider the glycine behavior in proceeding from strongly acidic solution to one that is strongly basic. In acidic solution glycine electroneurtal molecule is protonated, exists as cation that is glycine conjugate acid according to equation:

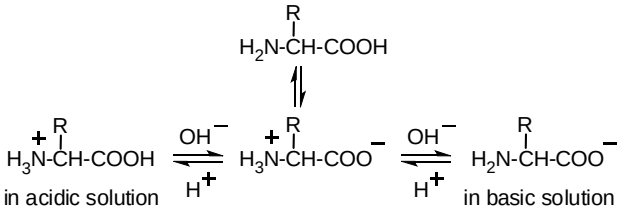


Upon gradual addition of solution of a base, e.g. NaOH, which is a titration, the pH rises and a proton is gradually removed from this species. Is the proton removed from the positively charged nitrogen or from the carboxyl group? Based on the relative acid strengths of RNH_3^+ and RCOOH the conclusion is that the proton is removed first from the carboxylic group which is the stronger acid. A typical ammonium ion has $pK_a \approx 9$, and a typical carboxylic acid has $pK_a \approx 5$. The titration curve for glycine shows measured $pK_a = 2.34$ for the most acidic site in the conjugate acid of glycine, a value much more in accord with that expected for deprotonation of the carboxyl group, which is stronger acid than in acetic acid ($pK_a = 4.76$). The reason for this enhanced acidity of glycine is the large inductive effect of the NH_3^+ which stabilizes the corresponding anion. On continual addition of base, pH rises more and a second deprotonation step is observed. It corresponds to removal of proton from nitrogen in the zwitterion. The pK_a associated with this step is 9.60, much like that of typical alkylammonium ions. Thus, glycine is characterized by two pK_a values: the one corresponding to the more acidic site is designated pK_{a1}, the one corresponding to the less



acidic site is designated pK_{a2}. The qualitative titration curve of glycine is shown in the above graphic.

The quantitative description of the processes during titration of glycine requires consideration of all species in equilibria at each point of the titration and use of Henderson-Hasselbalch equation. The possible species present in solutions with different pH are:



They are involved in the following equilibria described with corresponding dissociation constants:

$$H_3\overset{+}{N}\text{-}CH_2\text{-}COOH \overset{K_{a1}}{\rightleftharpoons} H_3\overset{+}{N}\text{-}CH_2\text{-}COO^- + H^+ \qquad K_{a1} = \frac{[H^+] \cdot [H_3N^+\text{-}CH_2\text{-}COO^-]}{[H_3N^+\text{-}CH_2\text{-}COOH]}$$

$$H_3\overset{+}{N}\text{-}CH_2\text{-}COO^- \overset{K_{a2}}{\rightleftharpoons} H_2N\text{-}CH_2\text{-}COO^- + H^+ \qquad K_{a2} = \frac{[H^+] \cdot [H_2N\text{-}CH_2\text{-}COO^-]}{[H_3N^+\text{-}CH_2\text{-}COO^-]}$$

At the beginning of the titration, the medium is strongly acidic. Addition of small amount of strong base (hydroxide) generates some zwitterion which is the conjugate base of a weak acid. Therefore, the conditions for obtaining a buffer system are met and the pH rises slowly during following base addition. The buffer resists big increase of pH.

When exactly half equivalent of hydroxide is added, half of the protonated glycine is neutralized and

$$[H_3N^+\text{-}CH_2\text{-}COOH] = [H_3N^+\text{-}CH_2\text{-}COO^-]$$

From the expression for the first dissociation constant follows:

$$K_{a1} = \frac{[H^+] \cdot [H_3N^+\text{-}\cancel{CH_2}\text{-}COO^-]}{[H_3N^+\text{-}\cancel{CH_2}\text{-}COOH]}$$

At this point the pH is equal to the pK_{a1} value, 2.34.

$$K_{a1} = [H^+]_1 \qquad -\lg K_{a1} = -\lg[H^+]_1$$
$$\text{at 0.5 eq} \qquad pK_{a1} = p[H^+]_1$$

Similarly, when the titration is progressed to more than one equivalent of base, second buffered region is obtained. In it the predominant species in equilibrium are the zwitterion and the anion of glycine ($H_2N\text{-}CH_2\text{-}COO^-$). When exactly 1.5 equivalents of base are added, the half of glycine (neutral as zwitterion) is neutralized and:

$$[H_3N^+\text{-}CH_2\text{-}COO^-] = [H_2N\text{-}CH_2\text{-}COO^-]$$

From the expression for the second dissociation constant follows:

$$K_{a2} = \frac{[H^+] \cdot [H_2N\text{-}\cancel{CH_2}\text{-}COO^-]}{[H_3N^+\text{-}\cancel{CH_2}\text{-}COO^-]}$$

At this point the pH is equal to the pK_{a2} value, 9.60.

$$K_{a2} = [H^+]_2 \qquad -\lg K_{a2} = -\lg[H^+]_2$$
$$\text{at 1.5 eq} \qquad pK_{a2} = p[H^+]_2$$

Third, important point on the titration curve is when exactly 1.0 equivalent of base is added. At this point the major species in solution is the zwitterion, as if glycine itself is dissolved in water. The pH corresponds to a solution in water of uncharged glycine (or any other amino acid). The electrical conductivity of the solution is at minimum since no ions are present at appreciable concentration. This characteristic for every amino acids pH value is called **isoelectric point** (**pI**). The isoelectric point is the pH at which the amino acid bears no net charge. Quantitatively the relevant concentrations at the isoelectric point are:

At isoelectric point: $[H_3N^+\text{-}CH_2\text{-}COOH] = [H_2N\text{-}CH_2\text{-}COO^-]$ **at minimum**

The concentrations of cationic and anionic glycine can be expressed from the dissociation constants as follows:

$$[H_3N^+\text{-}CH_2\text{-}COOH] = \frac{[H^+] \cdot [H_3N^+\text{-}CH_2\text{-}COO^-]}{K_{a1}}$$

$$[H_2N\text{-}CH_2\text{-}COO^-] = \frac{K_{a2} \cdot [H_3N^+\text{-}CH_2\text{-}COO^-]}{[H^+]}$$

Therefore at the isoelectric point:

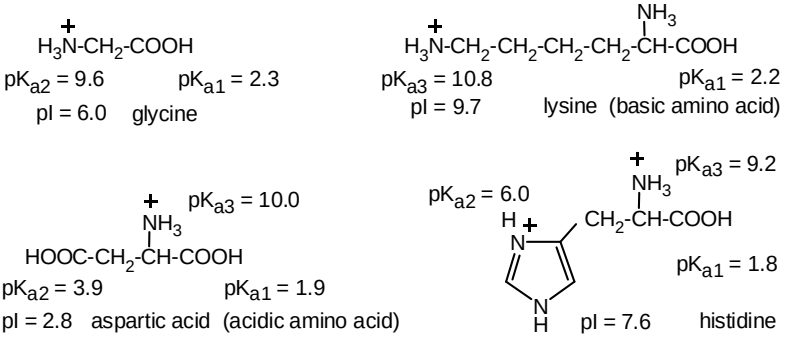
$$\frac{[H^+]_i \cdot [H_3N^+\text{-}\cancel{CH_2}\text{-}COO^-]}{K_{a1}} = \frac{K_{a2} \cdot [H_3N^+\text{-}\cancel{CH_2}\text{-}COO^-]}{[H^+]}$$

$$[H^+]_i^2 = K_{a1} \cdot K_{a2} \qquad \mathbf{pH_i} = \frac{pK_{a1} + pK_{a2}}{2} = \mathbf{pI}$$

For an amino acid the pI value is the average of the pK_a values of the protonated amino and the carboxylic acid groups (the two titratable groups). In the case of glycine the isoelectric point is 5.97. All α-amino acids that have neutral acid chains have similar pK_{a1} and pK_{a2} values, from which their pI values are also similar, around 5.5-6.0. These values aren't exactly at neutral pH 7, because carboxyl groups are stronger acids in aqueous solution than amino groups are bases.

In summary: In acidic solution, the carboxylate and amine functional groups of an amino acid are in their conjugate acid forms, an overall cation. In basic solution, the groups are in their base forms, an overall anion. In neutral solution zwitterionic form is present. This pH where the overall charge is zero is the isoelectric point, pI. When 0.5 equiv of NaOH is added to protonated amino acid, the first deprotonation is 50% done; when 1.0 equiv of NaOH is added, the first deprotonation is complete and the isoelectric point is reached; when 1.5 equiv of NaOH is added, the second deprotonation is 50% done; and when 2.0 equiv of NaOH is added, the second deprotonation is complete.

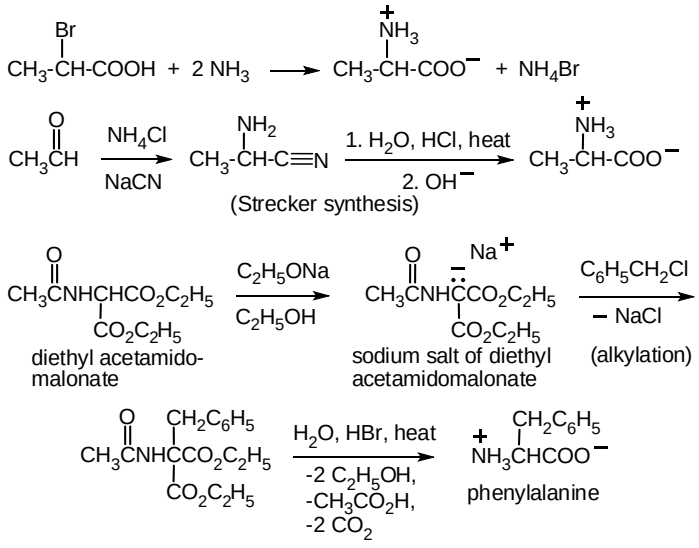
The value of pI depends on the side chain nature. As shown before, some amino acids bear acidic or basic group. These amino acids are characterized by three pK_a values. The "extra" pK_a value reflects the nature of the functional group present in the side chain. The isoelectric points are midway between the pK_a values of the monocation and monoanion and are well removed from neutrality when the side chain has carboxyl or basic amino function. The 15 amino acids with hydrocarbon chains or containing thiol, hydroxyl groups have pI = 5.0 to 6.5 (average of the pKa's). Aspartic and glutamic acids have acidic side chains and a lower pI. Arginine, lysine, and histidine have basic side chains and higher pI.



Histidine is the only amino acid with pK_a near neutrality. In a histidine proton shuttle, the imidazole nitrogen is used to quickly shuttle protons. Histidine can do this by abstracting a proton due to relatively low pK_{a2} and then transferring it to another molecule, also favorable, because of this intermediate pK_{a2} value.

The concept of pI is applicable to proteins as well. The isoelectric point is important for protein separation.

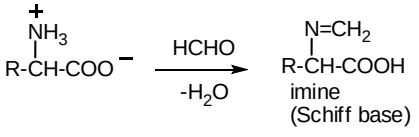
V. **Chemical properties of amino acids**. Amino acids can be obtained in a laboratory by several classical methods, such as nucleophilic displacement of halogen for amino group in α-halodenoacids; Strecker synthesis, where the introduction of N and C as cyano group is simultaneous; malonate synthesis relying on the high C-H acidity of β-dicarbonyl derivative which is alkylated with desired side chain.



Reactions of amino acids.

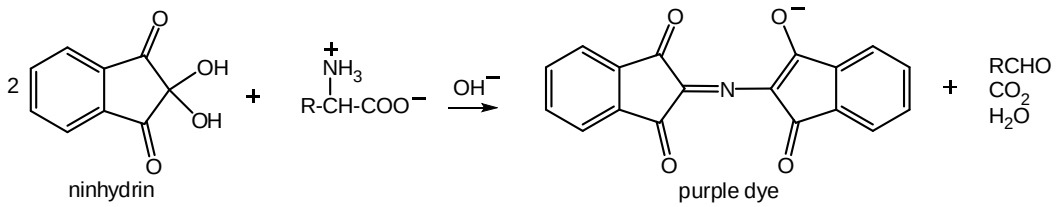
Typical are all reactions for carboxylic acid and amino group, e.g. esterification, acylation but only when proper measures are taken not to engage the second functional group in side reaction.

Titration of an amino acid with potassium hydroxide using typical indicators is done in the presence of formaldehyde. The chemical reaction gives corresponding methyleneimines in which NH_2 is blocked by condensation with HCHO (Sørensen formol titration).



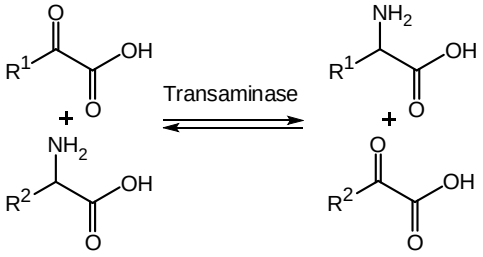
Ninhydrin reaction. Ninhydrin is used to detect ammonia or primary and secondary amines, as in amino acids (without proline). The positive reaction gives a deep blue or purple color. Ninhydrin is most commonly used to detect fingerprints, since amines left over from peptides and proteins react with ninhydrin.

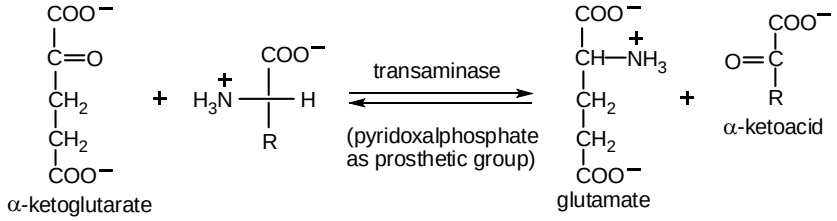
The ninhydrin test is also used for quantitative spectrophotometric analysis and for detection of amino-containing compounds in thin layer chromatography (TLC).



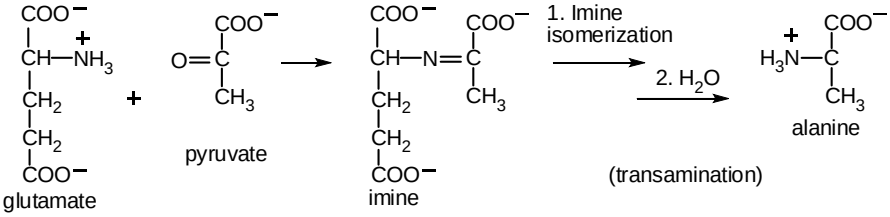
Some bio-chemically relevant reactions are transamination and enzymatic oxidation.

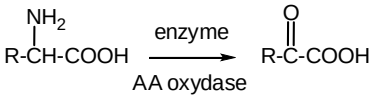
Transamination. The transamination (or aminotransfer) reaction is the reaction between an amino acid and an alpha-keto acid. The amino group is transferred from the former to the latter; this results in the amino acid being converted to the corresponding α-keto acid, while the reactant α-keto acid is converted to the corresponding amino acid. Transamination in biochemistry is accomplished by enzymes called transaminases or aminotransferases. This process is an important step in the synthesis of some non-essential amino acids that are not supplied from the diet. The chirality of the resulting amino acid is determined during transamination. The product of transamination reactions depend on the availability of alpha-keto acids. The products usually are alanine, aspartate or glutamate, since their corresponding alpha-keto acids are produced through metabolism of food. A very common α-keto acid is α-ketoglutarate, an intermediate in the citric acid cycle. Transamination of α-ketoglutarate gives glutamate. Most amino acids catabolize to only one product – glutamate.





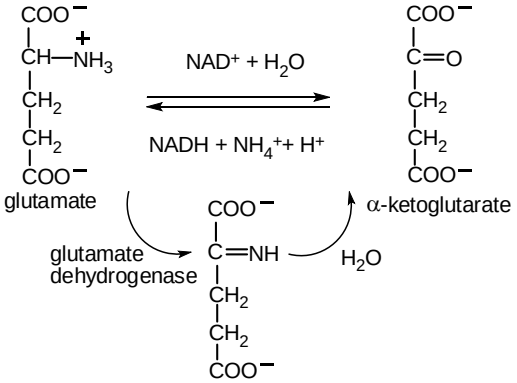
Example of glutamate involved in transamination with pyruvate to produce alanine is shown.



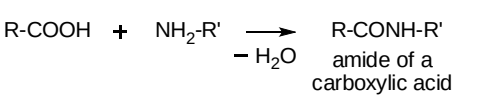


Oxidative deamination. This enzymatic reaction transforms an amino acid into an α-ketoacid with loss of amino group.

Ammonia is formed from glutamate in an oxidative deamination reaction. Glutamate is the only amino acid that undergoes rapid oxidative deamination. Glutamate is the major source of ammonia. Ammonia (as ammonium ion) is then excreted predominantly as urea, synthesised in the liver. Transamination can, thus, be linked to deamination, effectively allowing nitrogen from the NH_2 groups of amino acids to be removed, via glutamate as an intermediate, and finally excreted from the body in the form of urea.

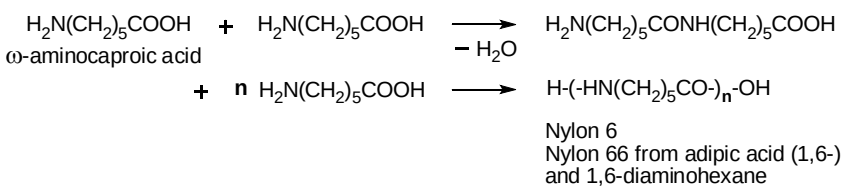


VI. **Peptides**. The chemical reaction between a carboxylic acid and amine is a condensation reaction (water is liberated) that gives an



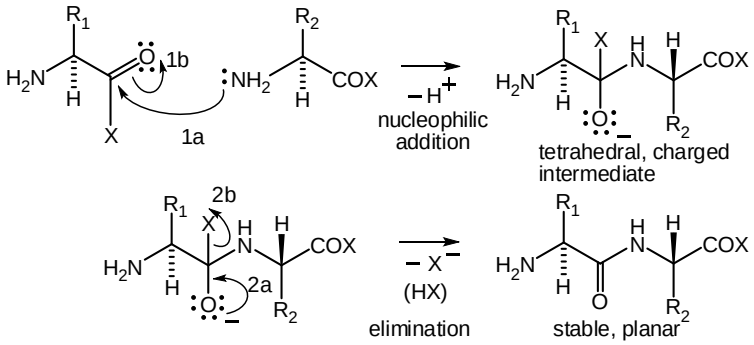
amide. In this example, the groups R and R' can also be residues in an amino acid. The newly created amide bond (-CO−NH-) between the amino group of one amino acid and the carboxyl of another is called a **peptide bond** (peptide linkage). The product is a peptide, for instance the product of condensation between glycine and alanine is a dipeptide. The same reaction, in principle, can lead to polymers. Nonproteinogenic amino acids, like 6-aminocaproic acid, produce long polymeric chains of a polyamide, Nylon 6, that has wide use in practice.

Nylons are condensation copolymers formed by reacting equal parts of a diamine and a dicarboxylic acid, so that amide bonds are formed at both ends of each monomer in a process similar to polypeptide biopolymers.



The most common variant is Nylon 6-6 which refers to the fact that the 1,6-diaminohexane reacted with adipic acid (hexane-1,6-dicarboxylic acid) and each donate 6 carbons to the polymer chain. The difference between Nylon 6 and Nylon 6-6 is that in the former the amide bonds are regularly arranged (-CO–NH-) whereas the bonds in Nylon 6-6 are alternating (-CO–NH-,-NH–CO-). The natural polypeptides possess amide bonds oriented in the same direction.

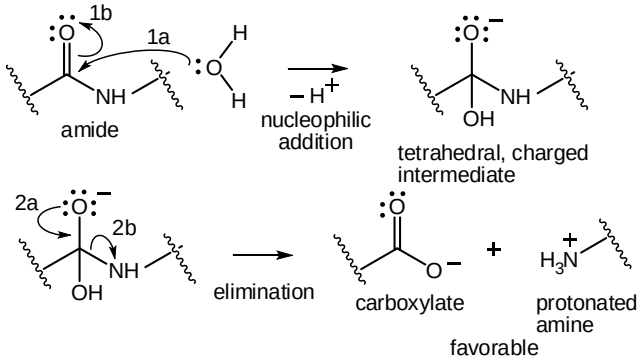
The peptide linkage synthesis proceeds via mechanism characteristic for other carboxylic acids (or their derivatives). The mechanism involves two stages: (1) nucleophilic addition to the carbonyl carbon, with development of a tetrahedral intermediate; (2) elimination of a group from this intermediate which can be the participating amine (no new bond formation) or the group originally attached to the carbonyl carbon. Using two general structural formulas of standard α-amino acids, the peptide bond formation is expressed as follows:



The group X need not to be OH but can be halogen, anhydride residue, etc. Human metabolic reactions use the same mechanism to synthesize proteins from amino acids.

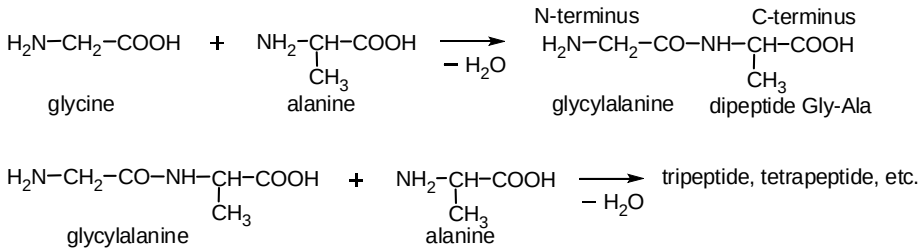
Peptides, also called polypeptides, usually contain from 2 to about 50 amino acid residues. Longer polypeptides are called with the group name **proteins**.

The peptide bond hydrolysis which is important for assimilation of food proteins proceeds via the same mechanism. The attacking nucleophile in a hydrolysis reaction is water molecule. The resulting compounds from protein hydrolysis are shorter proteins, peptides, and ultimately amino acids.



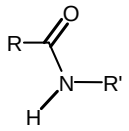
Unless the polypeptide is cyclic, it has in physiological conditions a free protonated amino group at one end (NH_4^+) and deprotonated carboxylic acid group at the other end (COO^-). By agreement, peptide structures are written so that the amino group is at the left, and this end is referred to as N terminus. At the right is the carboxyl group and this end is C terminus.

Nomenclature and naming of short peptides.



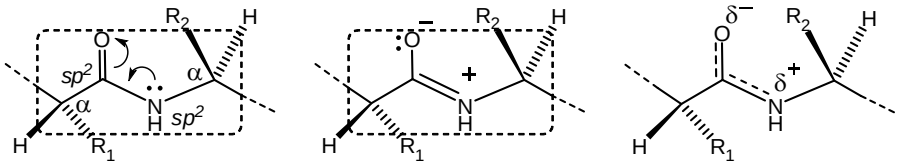
For instance, the product of condensation between glycine and alanine is the dipeptide glycylalanine. The base for naming a short peptide is the amino acid name at the C terminus. The rest of

amino acid residues are included as substituents beginning from the N terminus. The name of each amino acid residue ends on –yl. The name of a tetrapeptide formed from serine (as N terminus), valine, tyrosine and cysteine (at the C-end) is serylvalyltyrosylcysteine, or expressed with three-letter code: SerValTyrCys, or even shorter is one letter coded name: SVYC.



VI.1. Characteristic features of the peptide bonding in a peptide. The three dimensional structures of many short peptides and even large proteins are unequivocally determined by X-ray crystallographic analysis. The central feature of one such polypeptide chain is the succession of amide bonds separated by the α-carbons. Amides possess a conjugated system spread over the O, C and N atoms. This π-system consists of molecular orbitals occupied by delocalized electrons.

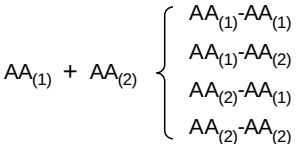
The characteristics of a peptide bond can be summarized:

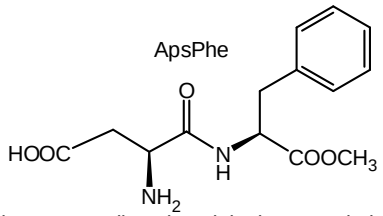


- The arrangement of six atoms is **planar**, at approximately 120°. The two C_α, NH, and C=O (included in dashed rectangle) lie in the same plane;
- The amide bond is **stabilized by delocalization** of N lone pair over the carbonyl O (by roughly 20 kcal/mol). This renders an amide less reactive than many similar groups (such as esters); amide bond is a strong bond and resists hydrolysis;
- The amide bond has **partial double bond character** between the carbonyl C – N;
- **Rotation about carbonyl C – N bond is hindered**. The rotation about the amide linkage is slow because delocalization of the unshared electron pair on nitrogen into the carbonyl group gives partial double character to the carbonyl C – N bond. The preferred form (conformation) of a peptide bond is with **anti to one another C_α** (and correspondingly – anti residues of amino acids). The general structure shows also this as ***trans*** location of C=O oxygen and N-H hydrogen;
- **The amide nitrogen atom is not basic**. The delocalization trend reduces the nitrogen basicity and the peptide group is uncharged at all normal pH values, but with an unusually large dipole moment.

All of these characteristics are relevant to the protein 3D structure in space.

Peptide bond synthesis is difficult in laboratory because the starting amino acids have two potential reacting sites, as illustrated in general for a mixture of two amino acids. In order to achieve even a desired dipeptide, is necessary to protect some of these sites. However, the enzymatic protein synthesis is entirely selective in a living organism. Currently the most adopted method for laboratory synthesis of peptides is an automated, computer-controlled synthesis on solid support, using protecting groups and activating groups.

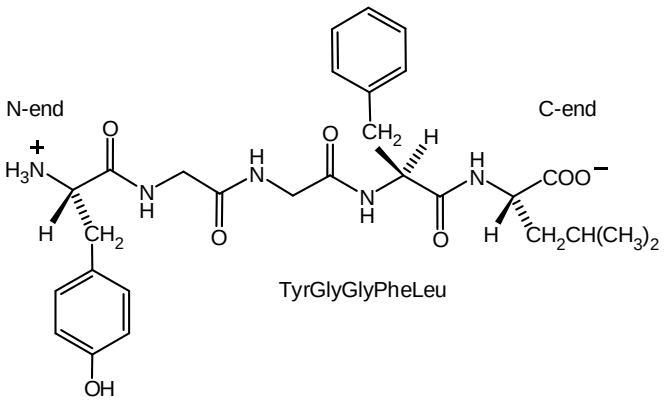




N-(L-α-aspartyl)-L-phenylalanine 1-methyl ester

Example of a widely used dipeptide is Aspartame ($AspPheCH_3$). Aspartame is an artificial, non-saccharide sweetener used as a sugar substitute in many foods and beverages. The sweetener contains residue of phenylalanine. This is a health hazard to people born with phenylketonuria, inherited disease that prevents phenylalanine from being properly metabolized.

Naturally occurring pentapeptides are members of enkephalin family. They are discovered recently and comprise two types: Leu-enkephalin (having leucine) and Met-enkephalin (having methionine). These compounds are opioid peptide neurotransmitters. They are found in the brain as body's own painkillers.

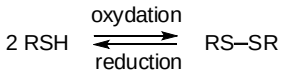


TyrGlyGlyPheLeu

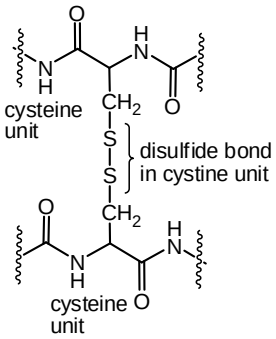
The presence of peptide bond can be detected by a characteristic qualitative reaction: the Biuret reaction. Alkaline solution of a peptide reacts with $CuSO_4$ to form a colored compound. Its color

varies from blue-violet to red-violet depending on the number of peptide groups. The reaction is applied for detection of peptides.

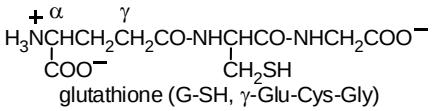
VI.2. Disulfide bond. Disulfide bonds are usually formed from the oxidation of sulfhydryl (-SH, thiols) groups, especially in biological contexts. Disulfide bonds in proteins are formed between the thiol groups of cysteine (methionine cannot form disulfide bonds). Disulfide bonds play an important role in the folding and stability of some proteins.

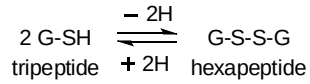


Prototypical is cystine. It is formed by oxidation of two cysteine units. They can be located in the same polypeptide chain but at large distance. When folded, the chain brings these units close to each other and they can be linked by disulfide bond. Within the same chain the disulfide bonds stabilize the secondary structure (folding, H-bonding). Disulfide bonds can connect two different protein chains, as found in insulin structure. Insulin is a peptide hormone composed of 51 amino acids, a number that is borderline with proteins. The hormone physiological action is to lower blood glucose levels.



Glutathione is a tripeptide with unusual linkage between cysteine and glutamate. Glutathione exists in reduced and oxidized states. It is an antioxidant that helps protect cells from reactive oxygen species such as free radicals and peroxides. By this action it is oxidized. Such a reaction is possible due to the relatively high concentration of glutathione in cells (up to 5 mM in the liver). Through direct conjugation (a connection via chemical bond), it detoxifies many foreign compounds. In effect, glutathione reduces any disulfide bond formed within cytoplasmic proteins to cysteines by acting as an electron donor.





VII. Protein structure. The order of amino acid residues in a protein molecule (its sequence) determines unambiguously the function of this protein in the living organism. Considerations of a protein structure are given to:

- Functional Conformation
- Principles of protein structure
- Primary structure (sequence)
- Secondary structure
- Tertiary structure
- Bonds contributing to tertiary structure (hydrogen bonds, -S-S- bonds)
- Hydrophobic effect
- Quaternary structure
- Four levels of protein structure
- Protein folding
- Back to function

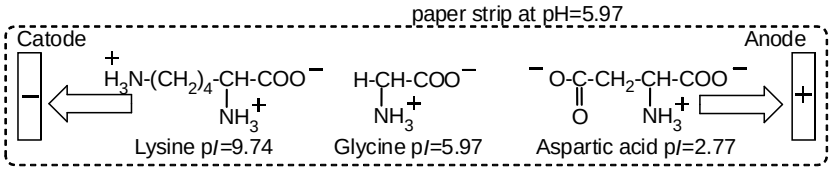
They will be studied in greater detail in biochemistry course.

Individual proteins are analyzed in modern chemistry by:

- X-ray crystallography is the primary method for determining the molecular conformations of biological macromolecules (particularly proteins and nucleic acids).
- Protein nuclear magnetic resonance spectroscopy (NMR) is a field of structural biology in which NMR spectroscopy is used to obtain information about the structure and dynamics of proteins.
- Calculations. Protein structure prediction is the prediction (*ab initio, de novo*-modeling) of the three-dimensional structure of a protein from its amino acid sequence.

Electrophoresis. The principle of isoelectric point (pI) is applicable to proteins as well. Proteins have an overall pI that depends on the net acidity/basicity of the side chains. The differences in pI of various proteins can be used for separating proteins on a solid phase permeated with liquid (paper, PAA gel).

For simplicity, if mixture of amino acids is loaded on the solid and electrical potential is applied, different amino acids will migrate at different rates, depending on their isoelectric points and on the pH of the aqueous buffer.



The same phenomenon occurs with mixture of proteins if their pI is different. The method is used in medicine for evaluation the relative amounts of groups of proteins in blood.

Electrophoreses of human blood serum

Band in a patient with multiple myeloma.