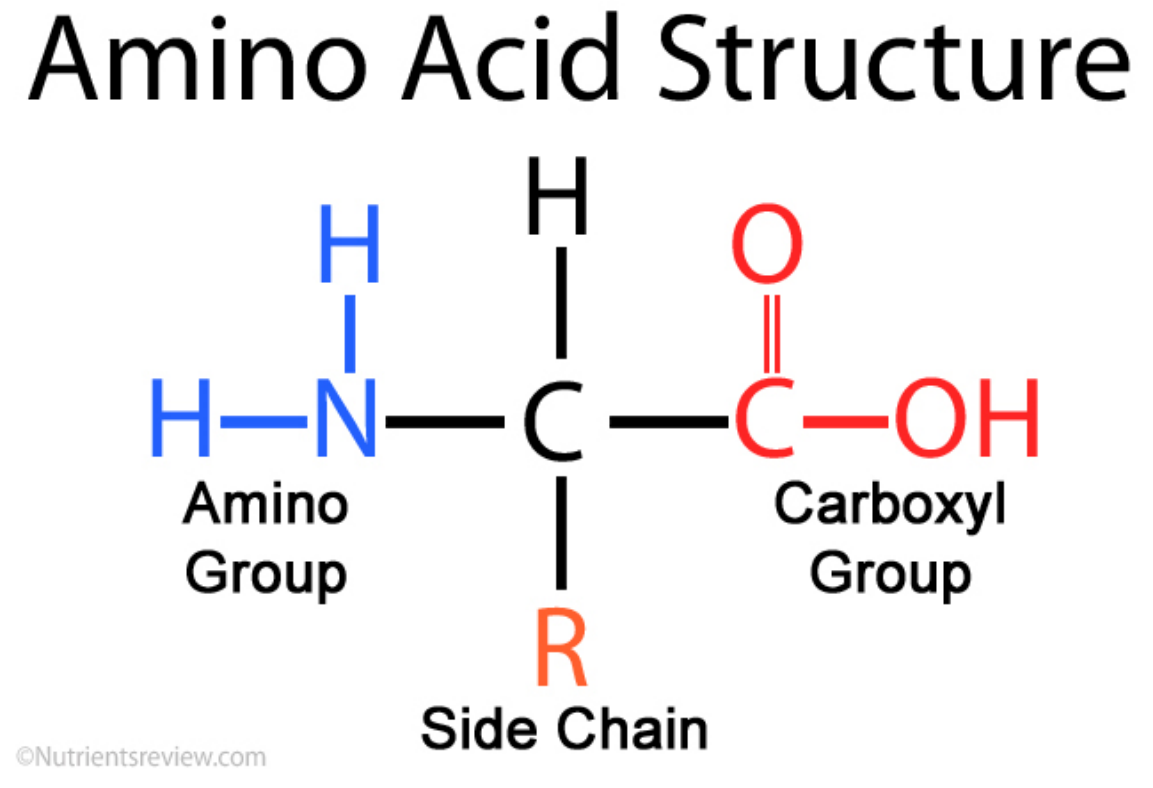
Aminoacid composition of proteins

The aim:

The estimation of quality of protein based on chemical identification of essential aminoacids in protein samples

The structure of aminoacid –

aminoacids are compounds containing carboxyl and amino group

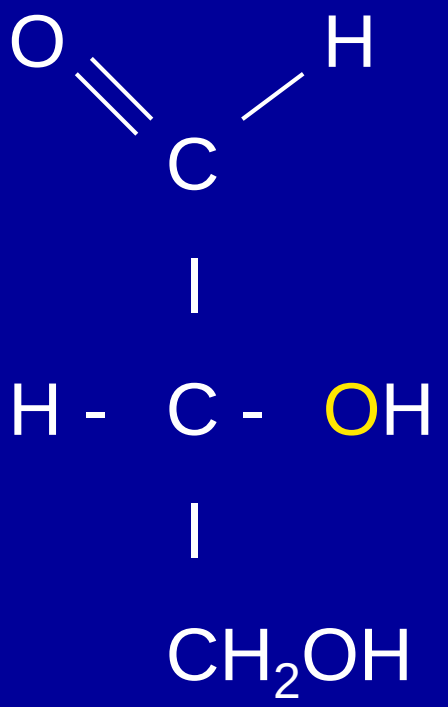


Criteria of aminoacid division

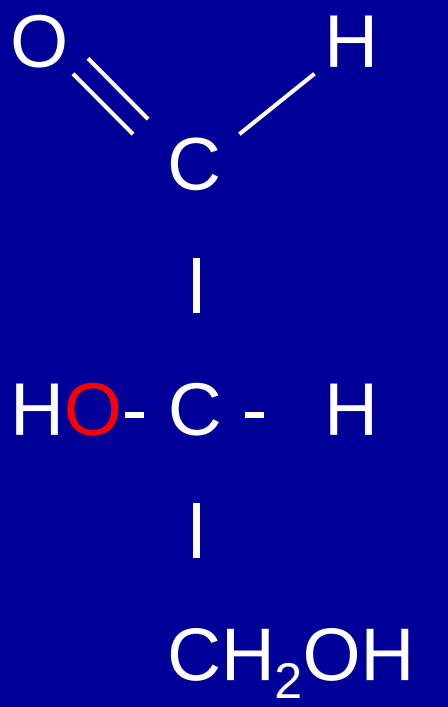
- Chemical structure: aminoacids of L and D configuration, α and β, containing sulfur, containing aromatic ring, containing hydroxyl group, cyclic
- The presence in proteins: protein aminoacids, non protein aminoacids
- The participation in metabolic pathways: glucogenic and ketogenic aminoacids
- The division in accordance to Karlson taking into account the participation of particular aminoacid groups in the formation of secondary protein structure (based on chemical properties of side chains)

Chemical structure: aminoacids of L and D configuration, α and β, containing sulfur, aromatic ring, functional groups, cyclic

comparative patterns of D and L configuration



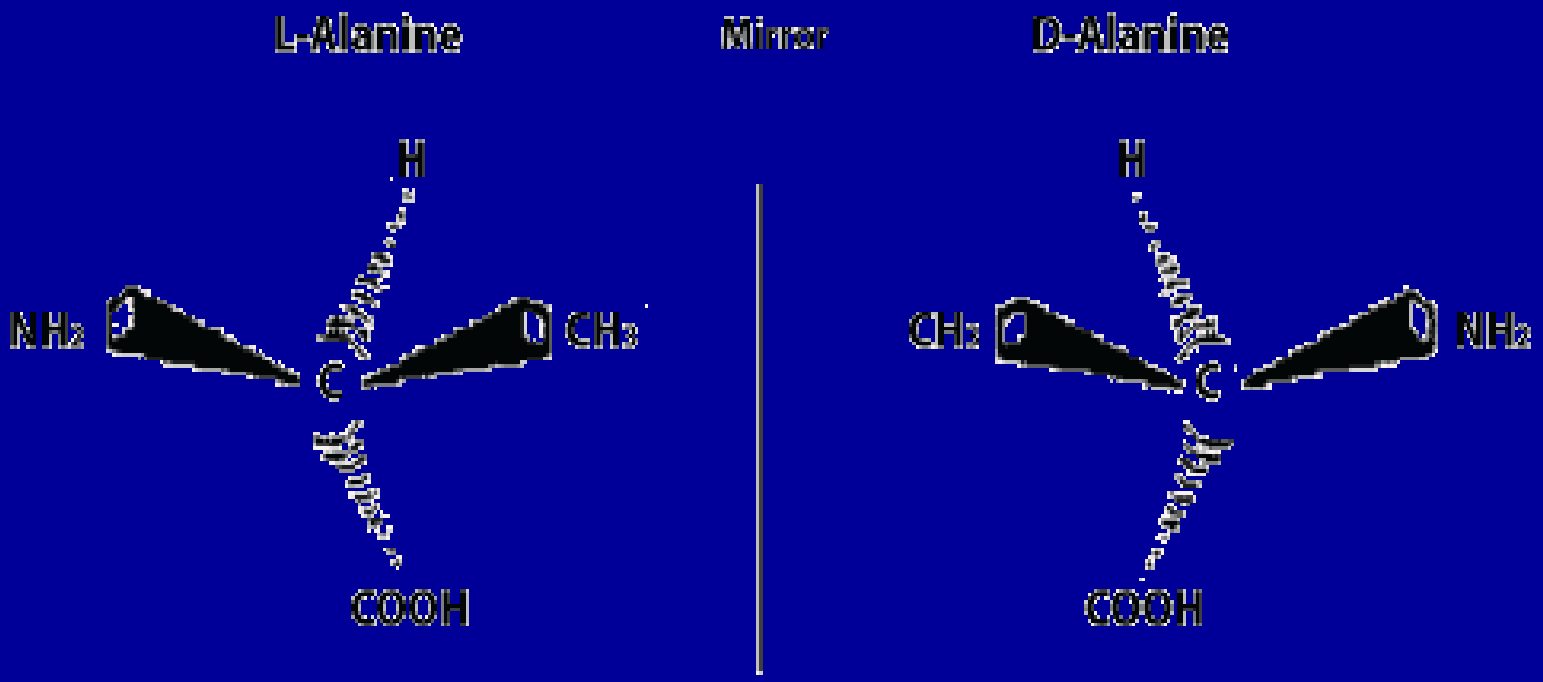
glycerine D-aldehyde



glycerine L-aldehyde

Commonn Amino Acids are Stereoisomers

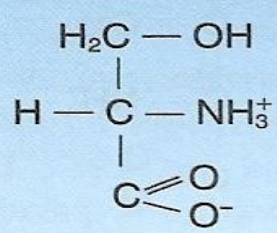
They have a Chiral α Carbon Center



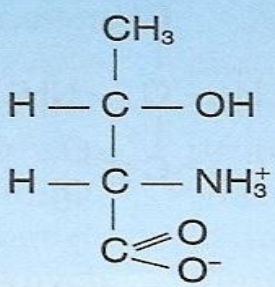
Amino acids can exist in either the D or L configuration.

However, all chiral amino acids in proteins have the L configuration.

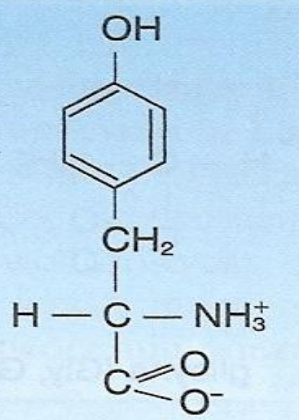
The examples of aminoacids containing different functional groups



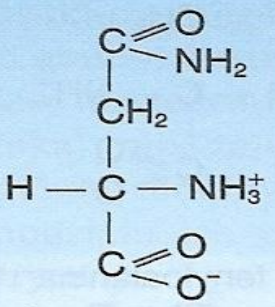
seryna (Ser, S)



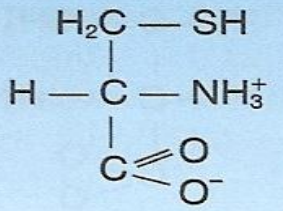
treonina (Thr, T)



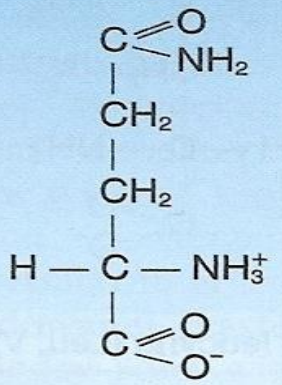
tyrozyna (Tyr, Y)



asparagina (Asn, N)



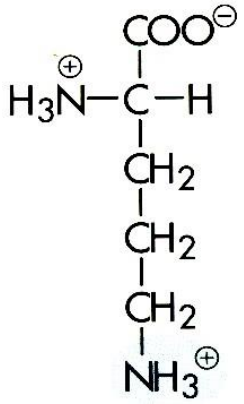
cysteina (Cys, C)



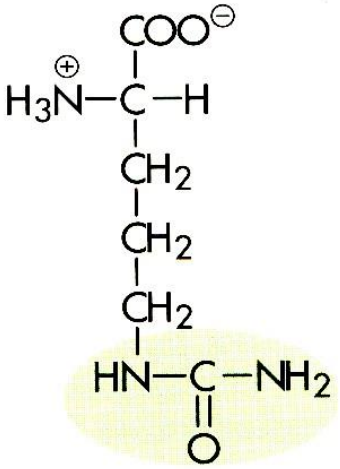
glutamina (Gln, Q)

Around 300 aminoacids are detected in surrounding world but only 20 participate in the structure of plant and animal proteins. That is why proteins can be divided into protein and non protein aminoacids

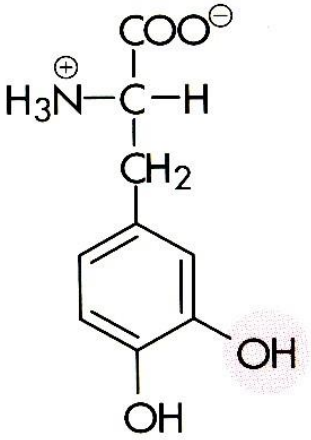
- Non protein aminoacids can be involved in many metabolic functions eg. participate in urea cycle, are biologically active metabolites eg. DOPA



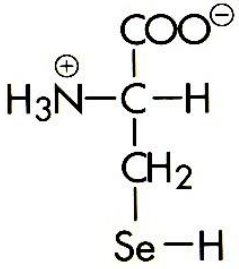
Ornityna



Cytrulina



L-DOPA



Seleno-
cysteina

Karlson divided protein aminoacids in accordance to physico-chemical properties of side chains into 4 groups:

- Nonpolar (hydrophobic) and uncharged aminoacids

Ala, Ile, Leu, Met, Phe, Pro, Trp, Val

- Polar (hydrophilic) and uncharged aminoacids

Asn, Cys, Gln, Gly, Ser, Thr, Tyr

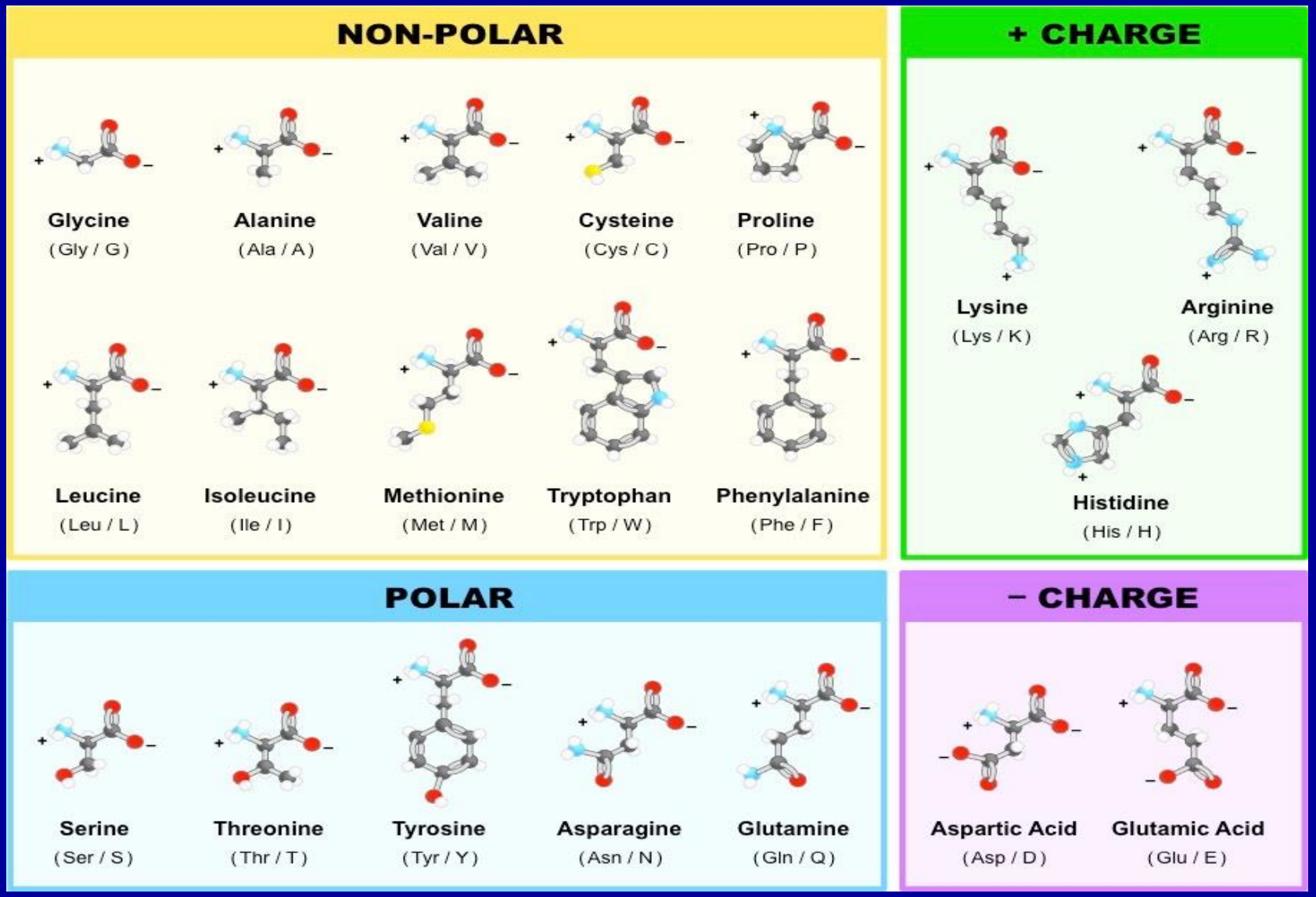
- Acidic aminoacids (monoaminodicarboxylic)

Asp, Glu

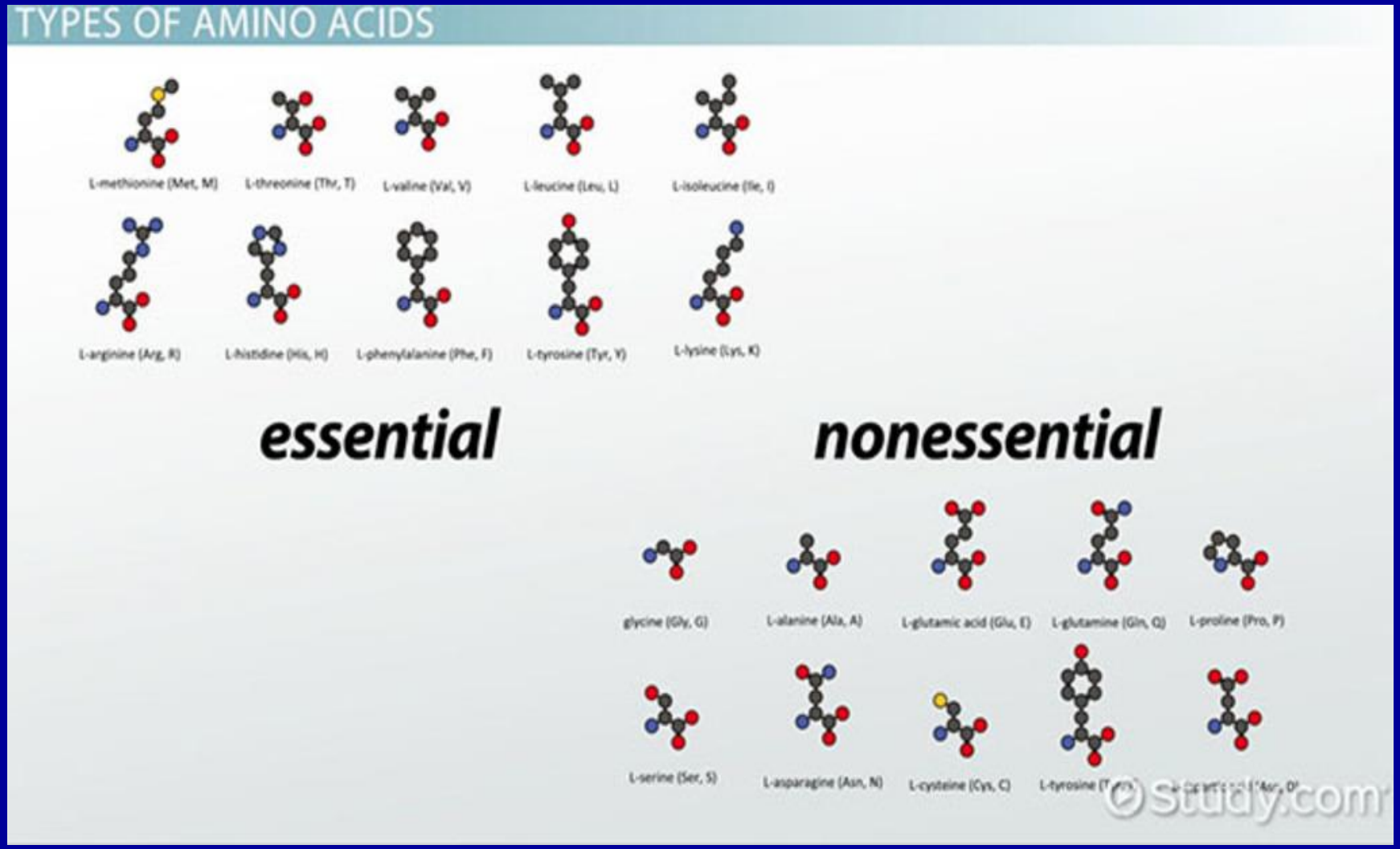
- Basic aminoacids (diaminomonocarboxylic)

Arg, His, Lys

The division in accordance to Karlson



Division of aminoacids



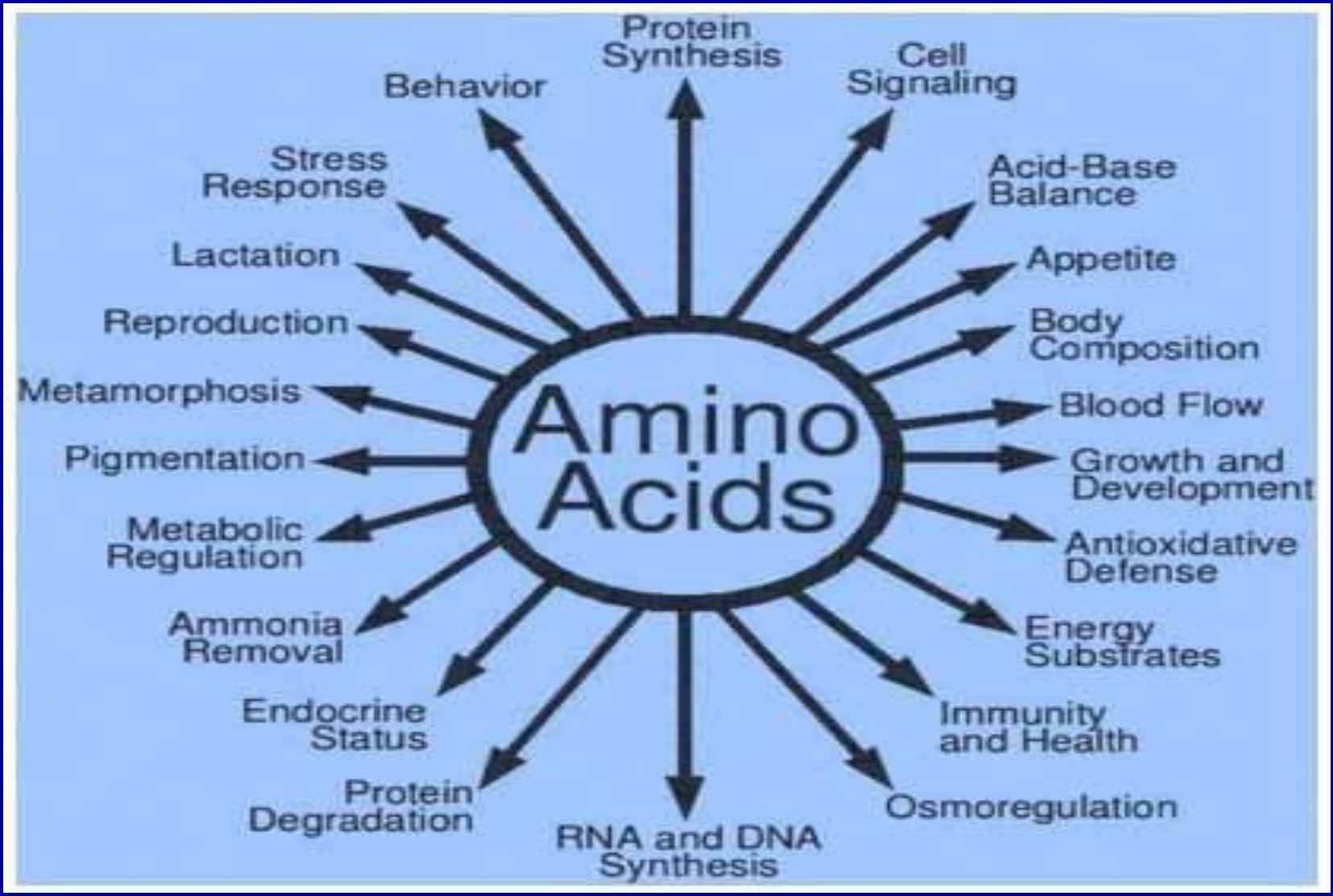
Properties of aminoacids

- Water soluble
- Positive and negative
- Isoelectric point
- Properties characteristic for particular function groups eg. hydroxyl, SH, aromatic

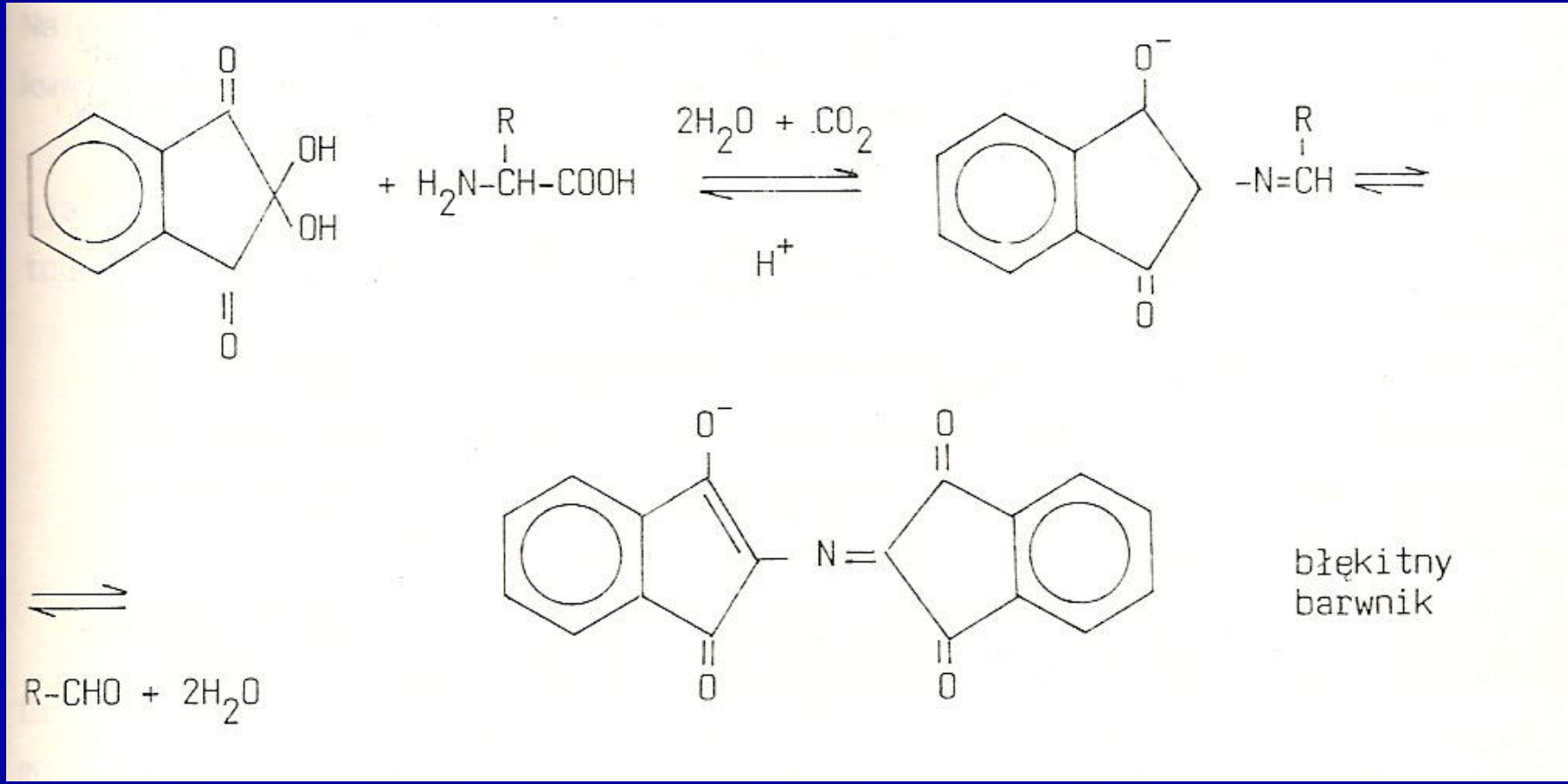
Meaning of aminoacids

- Structural elements of peptides, proteins,
- Neurotransmitters – glycine, glutamate, aspartate
- Precursors – biogenic amines, ketoacids, glucose, heme, creatine, nitrogen bases
- Donors – NH2

Biological meaning of aminoacids

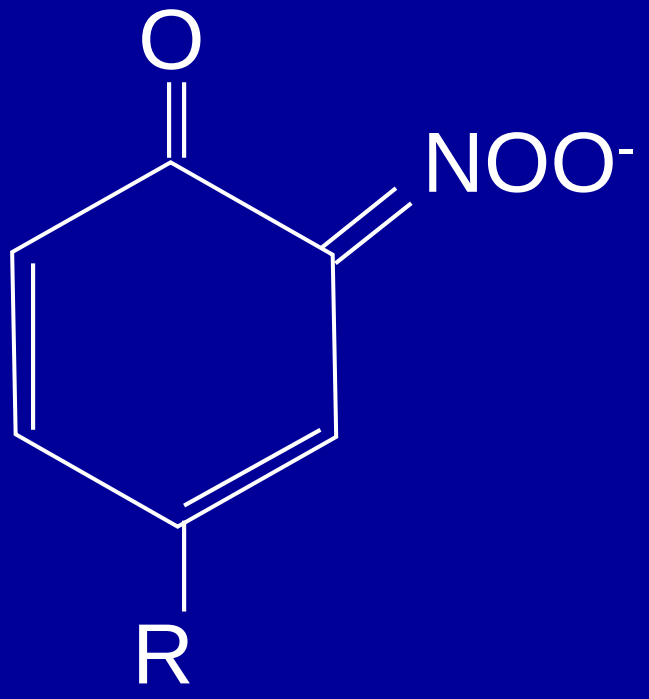


The reaction of $-NH_2$ group with ninhydrin

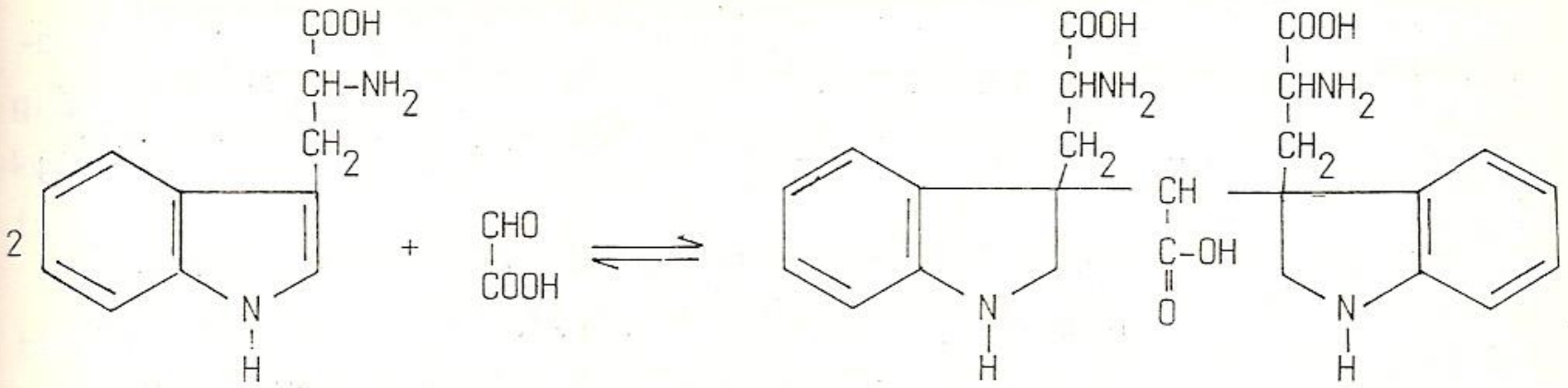


Characteristic reactions of side chains

- Xantoprotein reaction
- Millon reaction
- Cystein reaction
- Condensation with aldehyde group

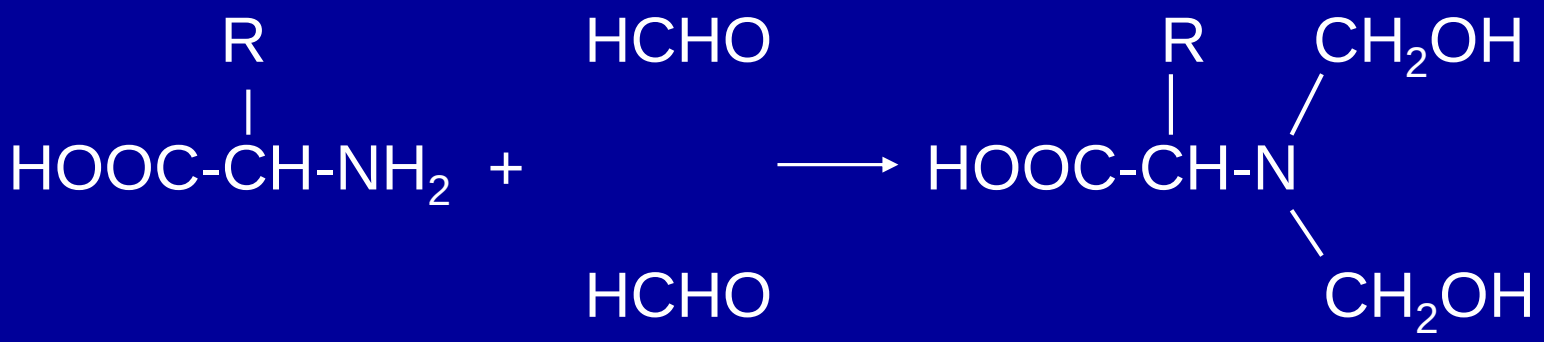


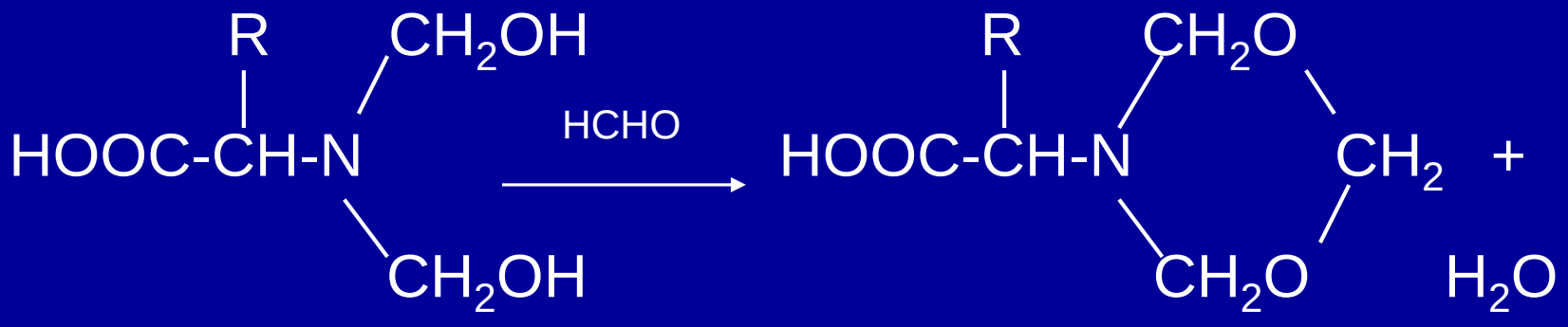
Adamkiewicz-Hopkins reaction



Condensation of amino group with aldehyde

Formol titration in accordance to Sorensen





Task 1. Biuret test

The aim of the test is to demonstrate the presence of protein in solution based on the colour reaction resulting from the combination of copper ions with peptide bond.

Protocol: Add 4cm3 of the cooper reagent to 1cm3 of a 1% protein solution. The presence of the protein is evidenced by appearance of a violet or pink colour.

Task 2. Xanthoprotein test

The aim of the test is to demonstrate the presence of aromatic amino acids in test solution based on the colour reaction, whose products are nitro-derivatives of benzene rings which are present in aromatic amino acids.

Protocol: Add 1cm3 of concentrated HNO_3 to 1cm3 of 1% protein solution and heat a mixture for 30 seconds. The precipitate of sedimenting protein stains yellow.

Task 3. Millon test

The aim of the test is to demonstrate the presence of tyrosine in test solution based on the colour reaction, whose products are mercury-derivatives of nitrated tyrosine.

Protocol: Add a few drops of Millon's reagent to 1cm3 of 1% protein solution and heat a mixture carefully above the burner. Proteins with nitric acid form an insoluble precipitate, which turns red during heating. If the red colour doesn't appear, add few more drops of Millon's reagent and heat again.

Warning! With excess of the Millon's reagent, the colour disappears during heating!

Task 4. Adamkiewicz – Hopkins test

The aim of the test is to demonstrate the presence of tryptophan in examined solution based on the colour products of tryptophan's indole ring with glyoxylic acid condensation.

Protocol: Add 1cm3 of concentrated CH3COOH to 1cm3 of 1% protein solution. Mix the received solution and make a sublayer by slow pouring of 1cm3 of concentrated H2SO4 over the wall of the tube. A violet ring is formed on the border between two layers.

Task 5. Cysteine test

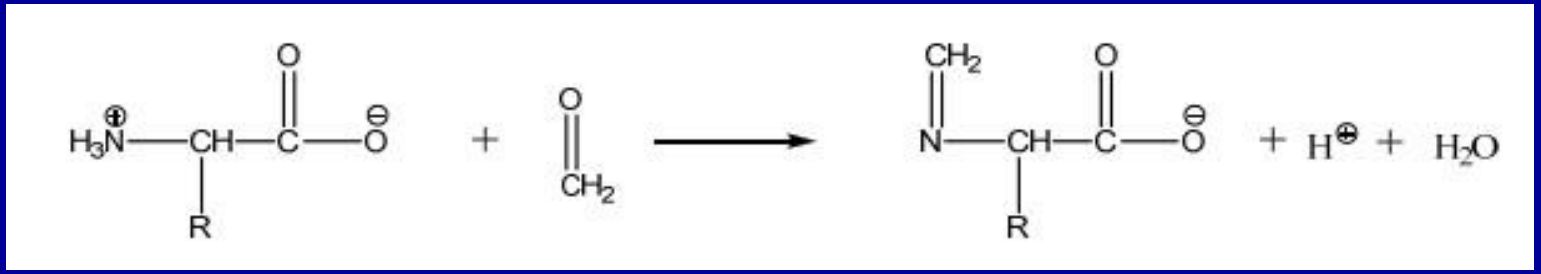
The aim of the test is to demonstrate the presence of cysteine in examined solution based on the formation of sulphur hydride (H_2S) and lead sulphide (PbS).

Protocol: To 1 cm^3 of 1% protein solution add 1cm^3 of 20% NaOH and a few drops of 2mol/dm^3 of lead (II) acetate and heat the mixture. The liquid darkens. Cool the mixture and add concentrated HCl carefully to it. Sulphur hydride is evolved.

Under the reaction conditions no sulphur hydride is formed from methionine and the test result is negative.

Task 6. Measurement of amino acids by formol titration using the Sörensen method

The aim of the test is to determine the content of nitrogen (amino acids) in the received sample. Formaldehyde (methyl aldehyde) reacts with amine groups or ammonium ion to give methylene derivatives. For each amine group or ammonium ion, a hydrogen ion is released and can be titrated with the base in the presence of phenolphthalein. The reaction of amino acids with formaldehyde proceeds according to the equation below:



Task 6. Measurement of amino acids by formol titration using the Sörensen method

Protocol: Take 5 cm3 of the amino acid solution to the beaker, add 0,5ml of phenolphthalein and carefully add dropwise 0,1mol/dm3 NaOH until it appears a pink colour, then add 5cm3 of phenolphthalein neutralized formaldehyde (formalin) solution. Mix. The solution turns discoloured. Titrate with 0,1mol/dm3 NaOH until the pink colour reappears.

Calculations: 1 cm3 of 0,1mol/dm3 NaOH used in titration responds to 1,4 mg of nitrogen. Calculate the nitrogen content in the received sample.

http://www.up.lublin.pl/4925/?rid=11910