

Qualitative tests of amino acids

Amino acids

An amino acid ($R-CH-NH_2COOH$) contains an amino group and a carboxylic acid group as side chains. It is the building block of all proteins and is linked with other amino acids as a chain by the peptide bonds ($CONH-$) to form the primary structure of a protein, see Figure 1.

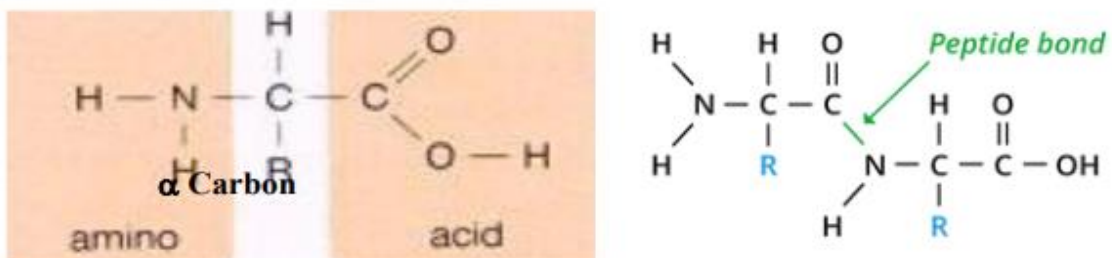
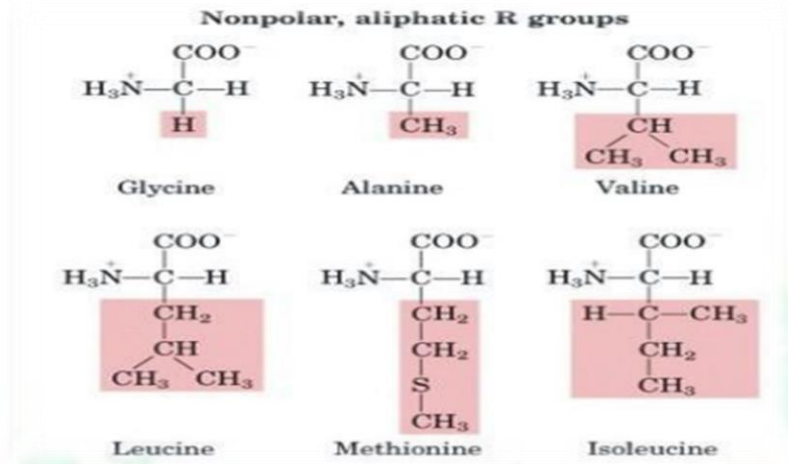
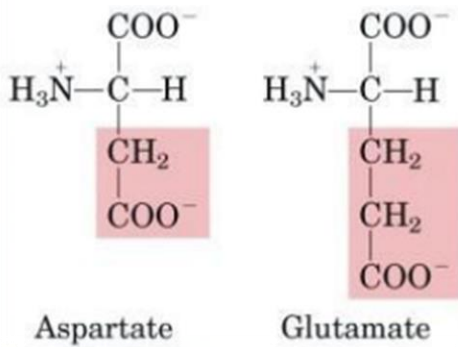


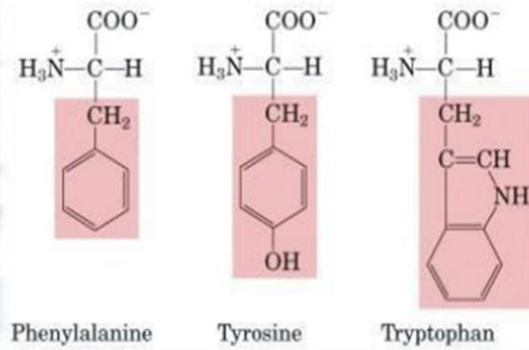
Fig 1. Structure of amino acids. The right-hand side figure shows the peptide bond.



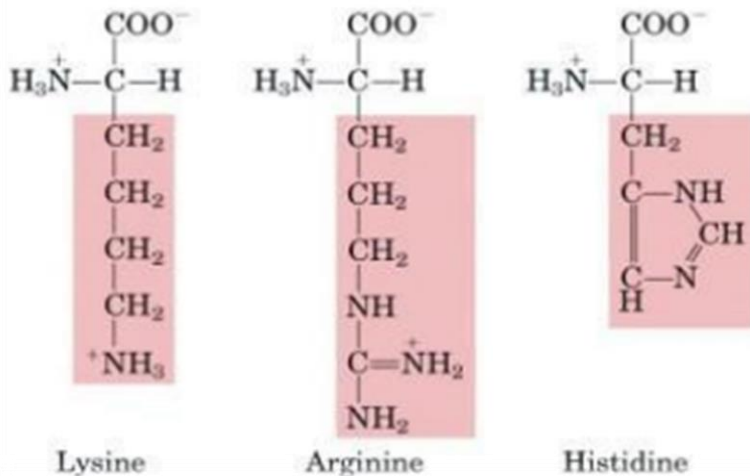
Negatively charged R groups

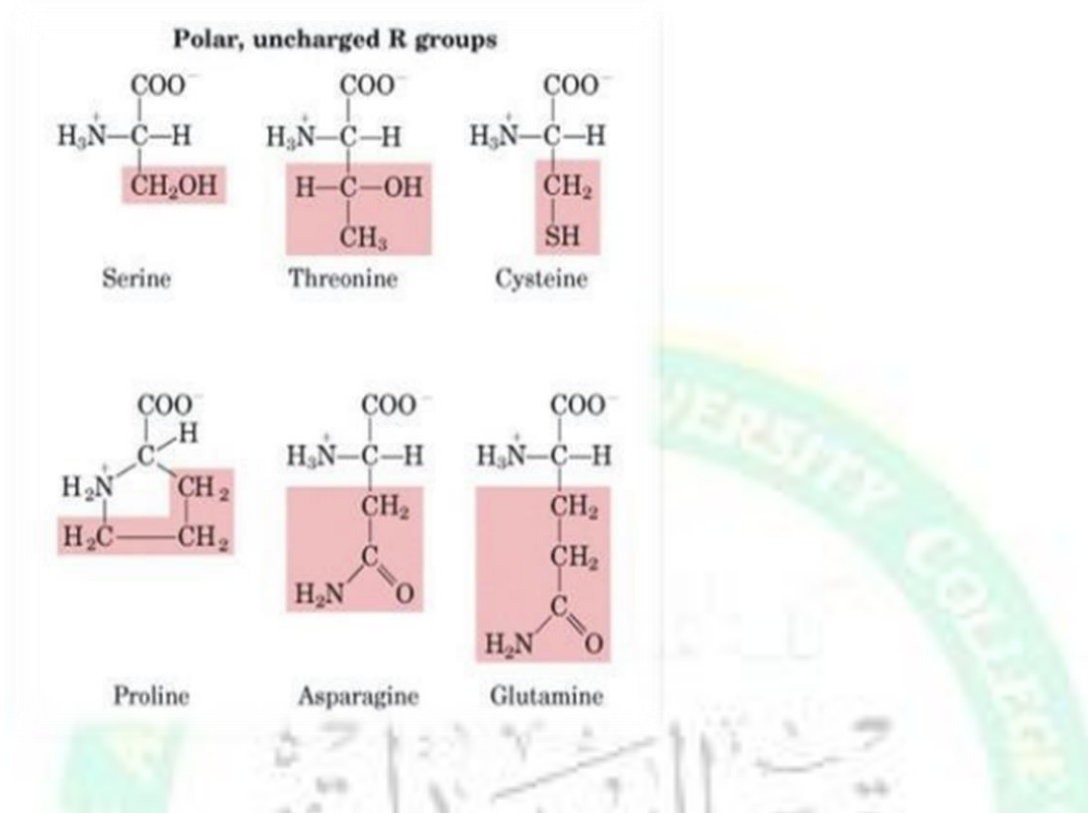


Aromatic R groups



Positively charged R groups





Classification of amino acids: -

Amino acids or side chain of them can be classified to different classes depending on their chemical features like: -

- Hydrophobic or hydrophilic character.
- Polar or non polar nature.
- Presence or absence of ionizable group.
- According to the nutrition.

Classified nutritionally into three groups: -

1. Essential amino acids (8 amino acids): these are not synthesized in the body and must be taken in diet in amount adequate to support the infant growth or to maintain health in adults, like: -

(Valine, Leucine, Isoleucine, Phenylalanine, Tryptophan, Lysine, Threonine, Methionine).

2. Non essential amino acids (12 amino acids): they can be synthesized by the body.

3. Semi – essential amino acids: these are growth promoting factors since they are not synthesized in sufficient quantity during growth.

- This type includes some standard amino acids like (Arginine, Histidine, also Tyr, Cys, Gly, and Glu).
- They become essential in growing children, pregnancy and lactating women.
- Polar (hydrophilic) amino acids are: -
Arg, Asp, Asn, Cys, Glu, Gln, Gly, His, Lys, Ser and Thr
- Non polar (hydrophobic) amino acids are: -
Ala, Ile, Leu, Met, Phe, Pro, Trp, Tyr, Val

Note/ amino acids are classified into three groups depending on their reactions: -

1. Neutral: aliphatic, aromatic, cyclic and hydroxyl or sulfur containing amino acids:

(Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Pro)

2. Acidic: this class contain 4 standard amino acids: -

(Asp, Asn, Glu, Gln).

3. Basic: this class contain only 3 standard amino acids: -

(His, Lys, Arg).

- 20 amino acids called “Standard amino acids” occur in almost all proteins & are coded in the DNA.
- Some amino acids may become chemically modified after being assembled in proteins, which called the **unusual L – α amino acids** by **post-translation processing**. The process

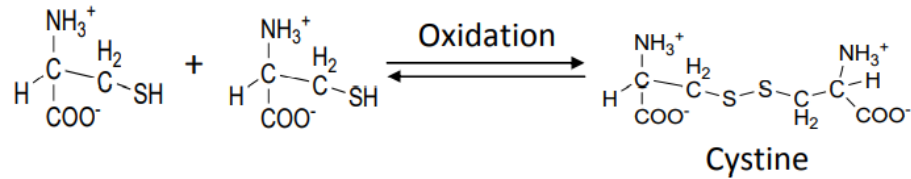
that occurs after formation of the polypeptides backbone, like: -

(Oxidation, phosphorylation, methylation, carboxylation, formylation and acetylation)

- These modifications are important for protein function and structure.

Examples: -

1. **Cystine** which formed by oxidation of the SH groups of the two Cysteines to (– S – S –) disulfide bond.

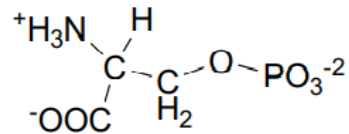


Note: oxidation is lose of H from SH

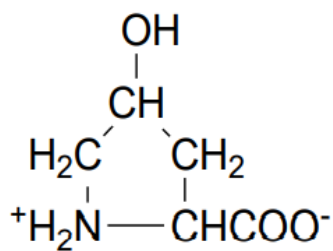
Cysteine → standard

Cystine → unusual

2. **O – PhosphoSerine:** unusual amino acid from standard Serine (Ser).



3. **4 – HydroxyProline:** This amino acid is important for stability of collagen structure.



temperature. This reaction is then followed by condensation between the reduced ninhydrin molecules, released ammonia, and the second molecule of ninhydrin. By the end of the reaction, a diketohydrin complex is formed which has a deep purple color. In amino acids like proline and hydroxyproline, this test yields an iminium salt, which is yellow-orange in color. Similarly, proteins with a free amine group like asparagine, react with the ninhydrin reagent to form a brown colored product.

Procedure:

- ❖ Take 1 ml of protein and carbohydrate solution in one test tube
- ❖ Add 4-6 drops of ninhydrin reagent to both the test tubes.
- ❖ Place the test tubes in the water bath for 5 minutes and then allow cooling to room temperature.
- ❖ color changing is a positive indication of a Amino acids.

Biuret test

Biuret test is a general test for compounds having a peptide bond. his test is given by compounds containing two or more peptide bond (CO-NH group). Since all proteins and peptides possessing at least two peptide linkage.

Objectives of Biuret test:

Biuret test used to detect the presence of proteins in biological fluids.

Principle:

In alkaline medium, copper (II) sulphate (CuSO_4) reacts with peptide bond nitrogen of peptides and proteins to form a violet coloured complex. The reaction is so named since this reaction is given by the substances biuret, which is obtained by condensation of 2 molecules of urea when heated to 180°C .

This reaction occurs only if the peptide molecule contains at least two peptide bonds (3 amino acids). Free amino acids and dipeptides do not undergo this test.

Procedure:

- ❖ Add 1ml of protein solution.
- ❖ Add $\frac{1}{2}$ ml of 40% NaOH.
- ❖ Add 2 drops of CuSO_4 solution.

A violet color indicated appositve results