Biochemistry

Amino acids

Chemical reactions of amino acids: -

The chemical reactions of amino acids are determined by the functional group like: -

- α – NH_2.
- α – COOH.
- Functional group: in R – side chain like **imidazol** in the His "play important role in the enzymatic catalysis", also gaundin in Arg, Indole ring in Trp.

– COOH group

Carboxyl group in Asp, Glu side chain and α – COOH can be participating in formation of ester, peptide bond (amides bond) also (acid anhydrides, oxidation and reduction, decarboxylation). These reactions used for detection for amino acids.

– NH_2 group

NH_2 group in side chain of Lys and the α – NH_2 group can ionization, acylation and esterfication. These processes are important in the **[detoxification]**.

– SH

SH group can be oxidation, alkylation. This important to stabilizes proteins.

– OH

OH group can be esterfication.

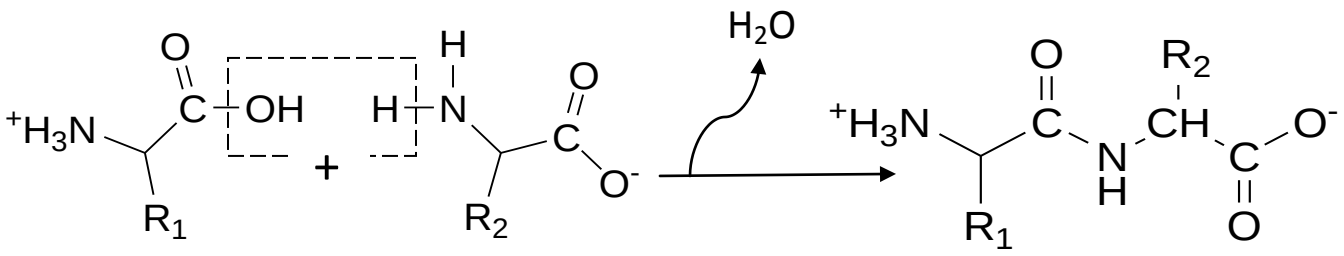
In general, ionized group (or charged group) in amino acid stabilize protein configuration by formation of [salt bonds].

Example:

Rupture and formation salts bonds accompany oxygenation and deoxygenation of Hb.

The most important reaction of amino acids is peptide bond formation.

- The reactions involve removal of water molecule between α – NH_3^+ group of amino acid and COO^- group of second amino acid.



- This reaction favors peptide bond hydrolysis, so to synthesis peptide bond, the COOH group must be first activated.
- Chemically this done by conversion to an acid chloride.
- Biologically done by condensation with ATP forming an amino acyladenlate.

There are different reactions for detections amino acids in general like ninhydrin test or fluorescamine, or for detection specific amino acids like: -

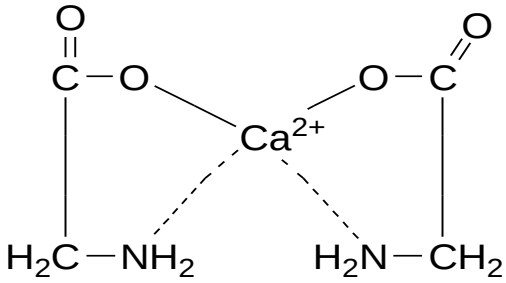
Millon test → for Tyr

Salkguchi test → for Arg

Nitroprusside test → for Cys

Hopkin test → for Trp

- There are reactions for free α amino group like: {Sanger reaction and Edman reaction}, these used to detect the first amino acid in primary structure of protein.
- Some peptides of amino acids are due to both – NH_2 and – COOH group together like chelating of amino acid with certain heavy metals and other ions like Cu^{2+}, Co^{2+},Mn^{2+} and Ca^{2+}.



Calcium diglycinate (soluble calcium complex)

This chelating may be used to remove Ca from bones and teeth and could development dental caries.

Different technique for separation of amino acids: -

Chromatography: -

This technique has stationary and mobile phase, where the molecules partitioned between these two phases.

- Thin layer chromatography (TLC).
- Paper chromatography (PC).
- Column chromatography or ion exchange chromatography.
- Electrophoresis.

Notes:-

1. The separation depends on the polarity or charge.
2. In the TLC and PC the separation depends on polarity.

Producer: -

The drop of mixture of amino acids is applied on filter paper strip. Then placed in vessel and its end contacts a solvent, after migration of solvent through the paper, the strip dried and treated with ninhydrin to reveal the position of amino acid (pink color).

Note: typically a water saturated low molecular weight alcohol containing acid or base like: -

[Butanol, acetic acid, H_2O]

- The more polar amino acid associated with the polar – OH group of the cellulose, so the more non polar of mixture; therefore, more farther than polar amino acid.

Direction of solvent migration

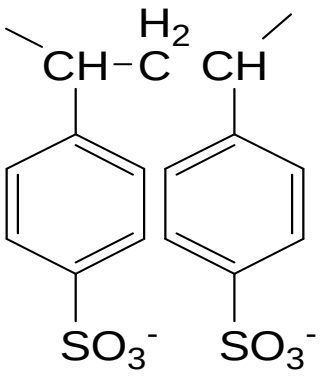
- Lys
- Asp
- Gly
- Pro
- Val
- Phe
- Ile
- Leu

(Solvent: butanol: acetic acid: H_2O)

Ion exchange chromatography: -

Separation depend on the charge, column packing with resin, sulfonated polystyrene dowex (contain exchanger).

The positive amino acid associated with resin then eluted with buffer at specific pH.



Q_1/ a solution containing Asp, Gly, Thr, Leu Lys at pH 3 was applied to Dowex 50, the column was eluted with buffer, in what order will the five amino acids elute from the column?

Asp, Thr, Gly, Leu, Lys

More polar

More non polar

Q_2/ for each pair of amino acids listed determine which will be eluted from an cation exchange using pH 7.0 buffer

Asp & Lys

Arg & MET

Glu & Val

Gly & Leu

Ser & Ala

- A rapid estimate of effective charge on an amino acid can be made by comparing its pI with the pH of buffer used.

  $\Delta p = pI - pH$

  If the Δp is (+), the amino acid carries a net (+) charge and amino acids with a greater Δp will stick more brightly to a cation – exchange resin than a lower Δp.

Chromatography analysis of amino acid on cation – exchange resin at pH = 7.4

Electrophoresis: -

The separation depends on charge of amino acid by different mobility in an electric field.

The media used may be paper or polyacrylamide gel, electrophoretic mobility of amino acids on a buffered solid support depends approximately on the (charge / mass) ratio, and this can be expressed mathematically as: -

$$mobility \propto pH - pI/Mw$$

Q_3/A mixture of Gly, Leu, Asp, Glu, Lys at pH 4.7; what is the electrophoresis mobility?