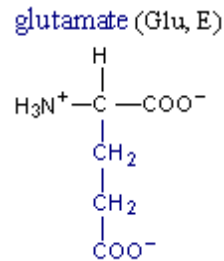
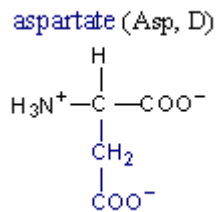


Lecture 7

Proteins

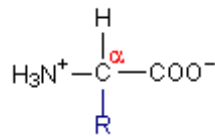
1. Which amino acids are considered as acidic amino acids and why?

Answer: Glutamic acid and Aspartic acid are the acidic amino acids due to presence of extra carboxyl group in their side chain. At physiological pH acidic amino acids are negatively charged.



2. What is the zwitter ionic form of α -amino acid?

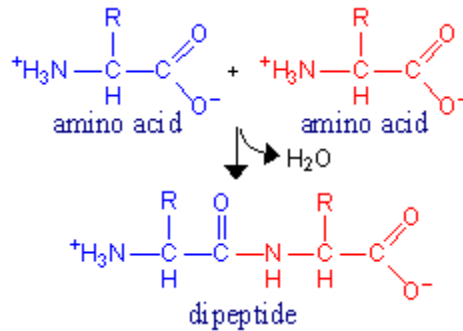
Answer: Zwitter ion of α -amino acid is ionic form with a positive and a negative formal charge on different atoms and a total net charge is zero.



Zwitter ion

3. What is peptide bond?

Answer: A peptide bond is a covalent bond that is formed between two amino acid molecules when the carboxyl group of one molecule reacts with the amino group of another molecule, releasing a molecule of water.



4. Differentiate between the use of Ion-exchange chromatography and Gel permeation chromatography in respect to protein purification?

Answer: Most chromatographic separations are based on the chemical interaction between the target molecule and the separation medium which includes IEC, HIC, Affinity chromatography etc. Gel permeation chromatography is exception as separation is based on the physical properties i.e. separation on the basis of the molecular size. Protein are composed of amino acid having non polar, polar, aromatic and charged residues which play a significant role during purification. Protein exist in highly organized form with only a fractions of amino acid exposed on the surface, and nature of amino acid at the surface will aid in purification The presence of charged amino acid at surface is essential if the purification is done by ion exchange chromatography to develop

a columbic interaction during separation, while for gel permeation chromatography no such requirement is there, as protein are separated according to their molecular size.

5. Which salt is used for precipitation of proteins?

Answer: Neutral salts are mainly used for the precipitation of protein as protein doesn't get denatured and activity is recovered upon redissolving the pellet. Several salts can be considered but effectiveness depends upon the nature of anion and cation. Monovalent cation and multicharged anion are more effective in precipitating protein. Ammonium sulphate is the most commonly used salt as it is cheap and sufficiently soluble. Other salts which can be used are ammonium acetate, sodium sulphate, and sodium citrate.

6. How to differentiate between secondary and tertiary structure of proteins?

Answer: Tertiary protein structure refers to the complete three dimensional folding of a protein. Stabilization of a protein's tertiary structure may involve interactions like hydrogen bonds, Van der Waals interactions, ionic bonds, disulfide bonds between amino acids located far apart along the primary sequence. While in secondary structure segments of polypeptides often fold locally into stable structures that include α -helices and β -pleated sheets by forming hydrogen bonds.

7. By which technique, protein quantification can be done?

Answer: Protein Quantification can be done by several ways which includes:

1) Ultraviolet absorption protein assay

2) Bradford dye binding protein assay

3) Lowry protein assay

4) Bicinchoninic acid protein assay

5) Colloidal gold protein assay