Dr.May Jaleel

Biochemistry
Week 3 Practical
" Chemistry of Proteins"

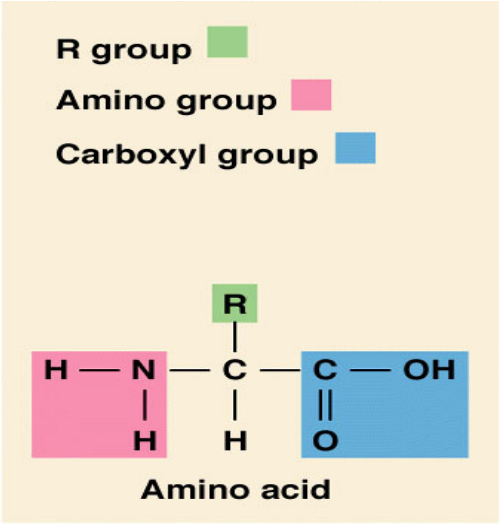
The word "protein" is derived from the Greek word "proteios", which means "of primary importance". In fact, proteins plays an important role in all biochemical and physiological body processes; they act as enzymes, hormones, receptors, antibodies and are required for the structural integrity of cells.

The aim of this practical session is to:

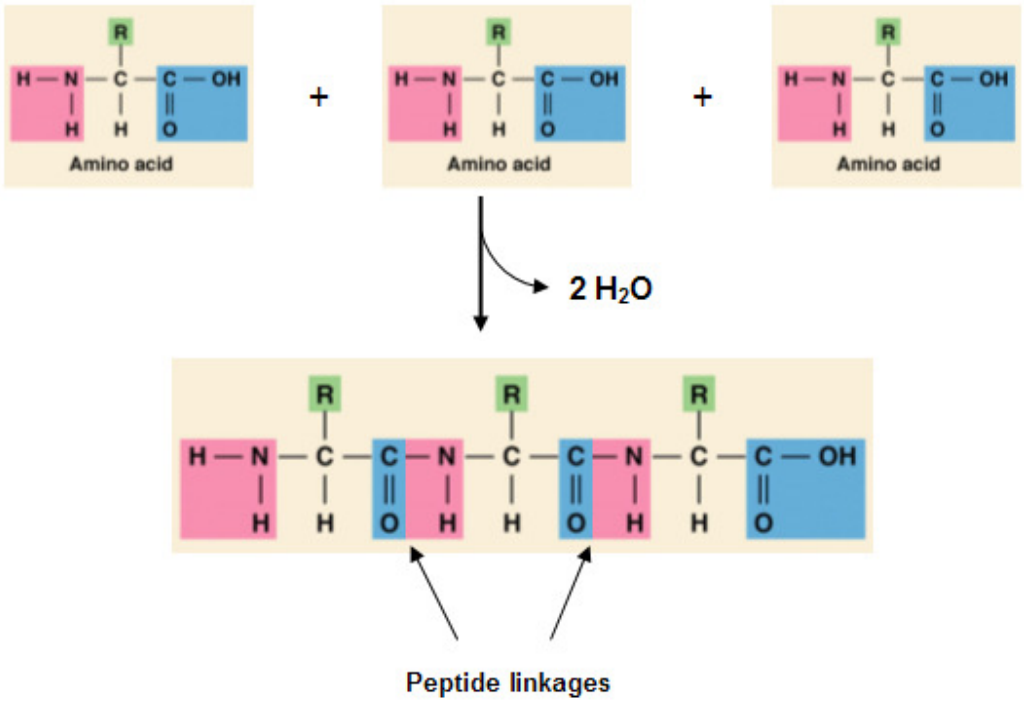
1. Obtain a simplified knowledge about protein structure.
2. Practically apply this knowledge by performing some protein color and precipitation reactions.

Protein structure

Proteins are organic compounds made of "amino acids" joined together by "peptide linkages".



These pepetide linkages are obtained by condensation reactions (removal of water) between carboxylic & amino groups of two adjacent amino acids.



Essential and non-essential amino acids:

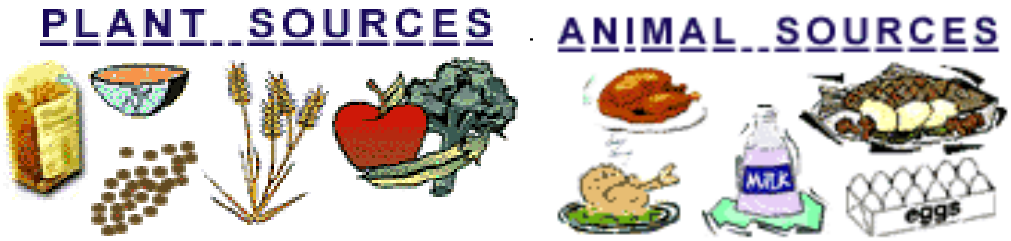
There are 20 standard amino acids which differ in their side chain (R).

Some of them are considered "essential" since they cannot be synthesized in our body and must be therefore provided in the diet (e.g. tryptophan & phenylalanine), while others are "non-esential" and can be synthesized in the body (e.g. alanine & cysteine).

Functions of dietary proteins:

Proteins are necessary components in our diet.

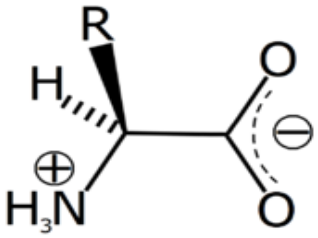
Through the process of digestion, proteins are hydrolyzed into amino acids that can be used for the synthesis of different body proteins (enzymes, hormones, antibodies,…etc), tissue repair and growth. Deficiency of proteins can cause general weakness, protein malnutrition diseases, and decreased resistance to infection.



Amphoteric nature of amino acids:

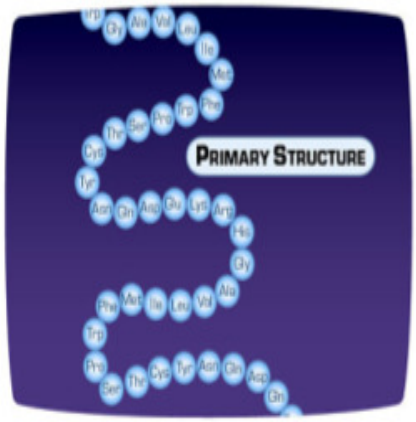
As amino acids have both an "amino" gp and a "carboxylic" gp, they are considered as both "base" and "acid", i.e. they are amphoteric.

At a certain pH, the amino group can become protonated gaining a positive charge, and the acid group can become deprotonated gaining a negative charge. The resulting doubly charged ion is known as "zwitterion".

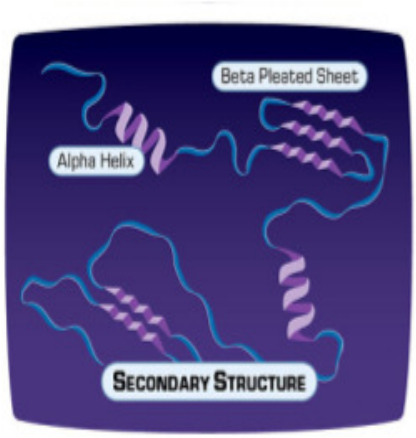


Aspects of protein structure:

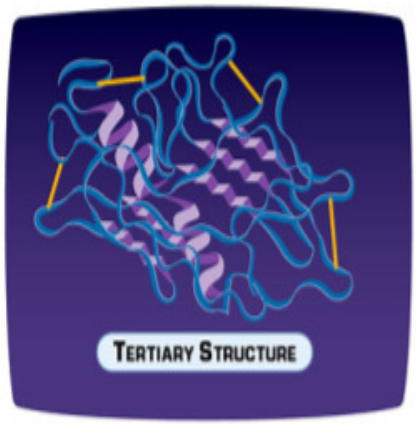
There are 4 aspects to describe a protein structure: 1°ry, 2°ry, 3°ry & 4°ry structures.



Amino acid sequence



Hydrogen bonding to give alpha helical or beta pleaded structures



Overall shape (globular or fibrous)

Assembly of protein subunits

Practical

Using the provided solutions of albumin (egg white), casein (milk protein) and gelatin (animal collagenous material), perform the following:

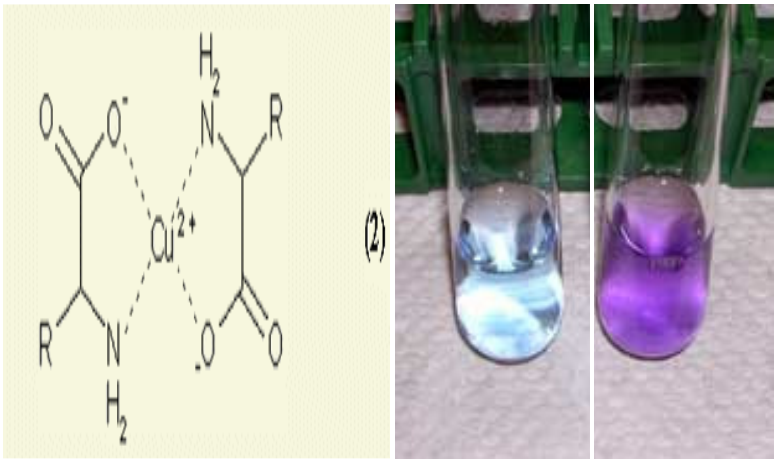
A. General tests
B. Color reactions
C. Precipitation reactions

A. General test for proteins

1. Biuret test:

Principle:

The biuret reagent (copper sulfate in a strong base) reacts with peptide bonds in proteins to form a blue to violet complex known as the "biuret complex". N.B. Two peptide bonds at least are required for the formation of this complex.



Procedure & observation:

- To 2 ml of protein solution in a test tube, add 3 drops of 10% sodium hydroxide solution and 3-6 drops of 0.5% copper sulfate solution.
- Mix well; a blue to violet color is obtained with albumin, casein & gelatin.

B. Color reactions of proteins

1. Reduced sulfur test:

Principle:

Proteins containing sulfur (in cysteine and cystine) give a black deposit of lead sulfide (PbS) when heated with lead acetate in alkaline medium.

Procedure & observation:

To 1 ml of protein solution in a test tube, add 2 drops of 10% sodium hydroxide solution and 2 drops of lead acetate.

Mix well and put in a boiling water bath for few minutes; a black deposit is formed with albumin, while a slight black turbidity is obtained with casein due to its lower content of sulfur. Gelatin gives negative result.

2. Xanthoproteic acid test:

Principle:

Nitric acid gives a color when heated with proteins containing tyrosine (yellow color) or tryptophan (orange color); the color is due to nitration.

Procedure & observation:

- To 2 ml of protein solution in a test tube, add 2 drops of concentrated nitric acid.
- A white precipitate is formed and upon heating in a boiling water bath, it turns yellow with "tyrosine" and orange with the essential amino acid "tryptophan" indicating a high nutritive value.

3. Millon's test:

Principle:

Millon's reagent (Hg/HNO3) gives positive results with proteins containing the phenolic amino acid "tyrosine".

Procedure & observation:

- To 2 ml of protein solution in a test tube, add 3 drops of Millon's reagent.
- Mix well and heat directly on a small flame.
- A white ppt is formed with albumin and casein (but not gelatin); the ppt gradually turns into brick red.

C. Precipitation reactions of proteins

1. Precipitation by heavy metals:

Principle:

Heavy metals (e.g. Hg^{2+}, Pb^{2+}, Cu^{2+}) are high molecular weight cations.

The positive charge of these cations counteracts the negative charge of the carboxylate group in proteins giving a precipitate.

Procedure & observation:

To 1 ml of protein solution in a test tube, add 1 drop of lead acetate; a white ppt is obtained.

To 1 ml of protein solution in a test tube, add 1 drop of 10% copper sulfate; a blue ppt is obtained.

2. Precipitation by alkaloidal reagents:

Principle:

Alkaloidal reagents (e.g. tannate & trichloroacetate) are high molecular weight anions. The negative charge of these anions counteracts the positive charge of the amino group in proteins giving a precipitate.

Procedure & observation:

- To 1 ml of protein solution in a test tube, add tannic acid drop wise until a buff ppt is obtained.
- To 1 ml of protein solution in a test tube, add 1 ml of trichloroacetic acid (TCA); a white ppt is obtained.

N.B. Precipitation of proteins by heavy metals and alkaloidal reagents indicates the presence of both negative and positive charges and hence the amphoteric nature of proteins.

3. Precipitation by denaturation:

Denaturation by heat (heat coagulation test):

Principle:

Heat disrupts hydrogen bonds of secondary and tertiary protein structure while the primary structure remains unaffected. The protein increases in size due to denaturation and coagulation occurs.

Procedure & observation:

- Put 2 ml of protein solution in a test tube, incline it and heat to boiling.
- A permanent clotting and coagulation is obtained with albumin only.

BCM 112
Biochemistry I
Week 3
"Chemistry of Protein"

Student Name:…….. Student ID:……

Laboratory exercise:

1. Using the provided solutions of albumin, casein and gelatin perform the tests in the table below and write down your observations.

	Albumin	Casein	Gelatin
Biuret test			
Reduced sulfur test			
Xanthoproteic acid test			
Heavy metal pptn			
Alkaloidal reagent pptn			
Heat coagulation test			

2. Is gelatin of high nutritive value? Why?

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