Color Reactions of Proteins

Objective:

To idelitify the different proteins and amino acids by color reaction tests

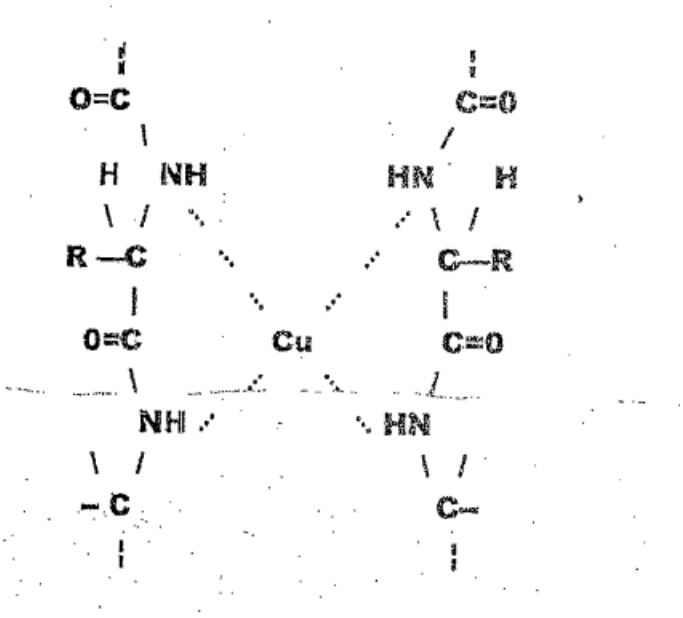
Introduction:

The chemistry of the living things involves a variety of large and complex molecules be connected to form long chains or rings. This result in a vast array of molecules. The structure of each molecules is related to it's function. Changes in the structure may result in abnormal function which we call disease. Some of the most common types of organic molecules found in living things are proteins, carbohydrates, lipids and nucleic acids,

Proteins are polyamides and have molecular weights above 5000. Polyamides of molecular weight below 5000 are usually referred to as polypeptides. Amino acids are the bullding blocks of proteins. An amino acid consists of an amino group, a carboxyl group, a hydrogen atomı, and adistinctive R group boun. It is based on the chemistry of the carbon atom and the fact that carbon atoms can ded to a carbon atom. Color producing tests as the Biuret, Ninhydrin, Milion's, Hopkins Cole, and Unoxidized suitur tests are used to detect proteins in biological mixtures. Some of these reactions [Biuret and Ninhydrin] are general tests that give positive results with all proteins and amino acids.

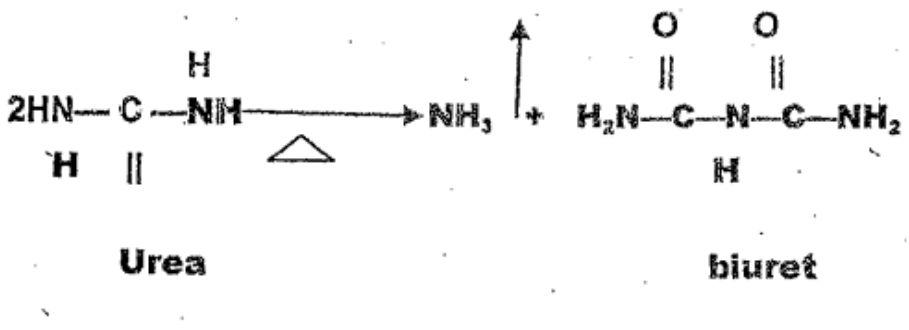
1. Biuret Test:

The biuret test depends upon the reaction of cupric ions Cu^{+2} in an alkaline solution with peptide linkage of the protein to produce a purple color. This color is apparently caused by the coordination complex of the copper atom and nitrogen atoms of two peptide chains as shown below:



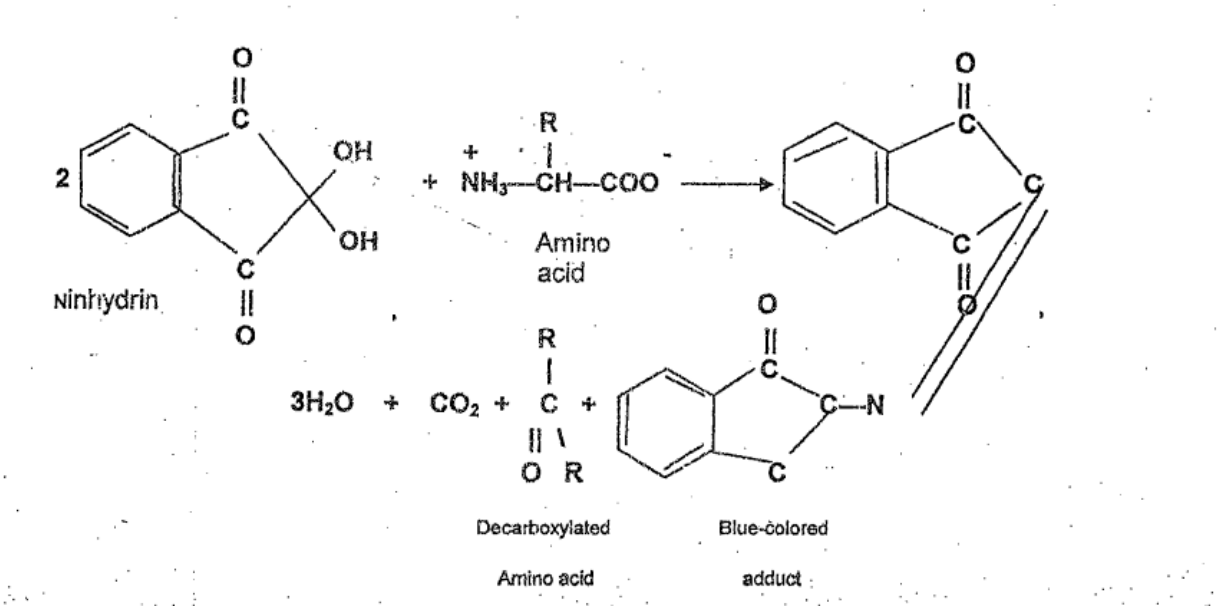
Why we called this reaction as a biuret test?

A biuret compound is formed when the urea is heated. If alkaline cupric ions are added to biuret solution, a violet color is produced. This is a characteristic color of not only biuret but also protein and peptides which contain a structure similar to biuret. The biuret test requires the presence of at least two peptide linkages per molecule to be positive. The biuret reaction can be said to be polypeptide specific since proteins are about the only compounds found in nature that have the polypeptide character required for biuret assay, therefore the biuret test is remarkably specific for proteins.



2. Ninhydrin Test:

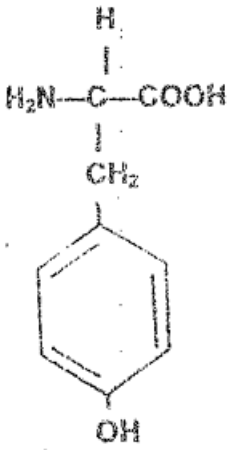
Ninhydrin reacts with a amino acids to yield a characteristic blue violet products [decarboxylation]. The overall reaction is shown below, for the sequence of steps involved:



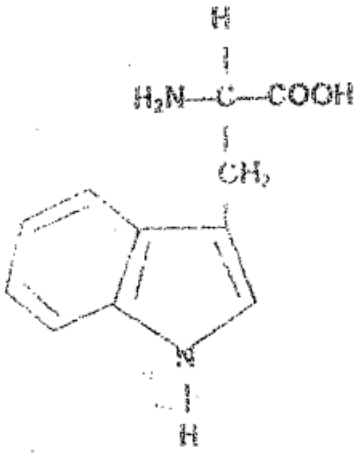
Most araino acids give the same color expect proline makes a pale-yeliow product with Nirhydrin. This is due to the fact that proline is an imino acid instead of having traditional a-amino acid structure.

384. Milton's and Hopkins-Cole Test

These tests are actually specific for certain functional groups of amino-acids and not for the amino acide themselves pillow's testis specific for the phenolic hydroxyl group of tyrosine and Hopkins-Coletest specific for the indole group of Tryptophan.

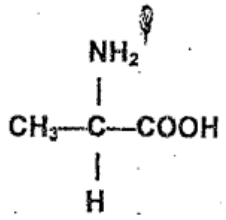


Tyrosine

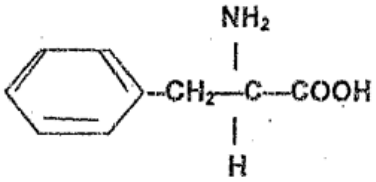


Tryptophan

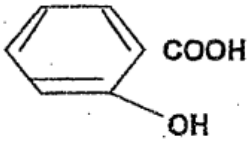
Amino acids are not the only chemical components in the sample which contain the particular functional group which gives the positive reaction Milon's test will be conducted with one compound which is not an amino acid nor a protein. This compound is salicyic acid which is simply a 2-hydroxy benzoic acid. These three compounds are tested with Milon’s reagent.



Alanine



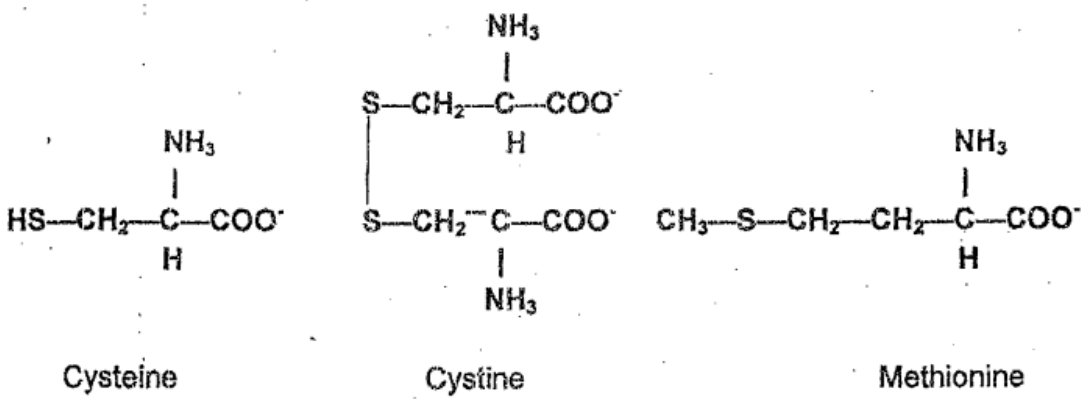
Phenylalanine



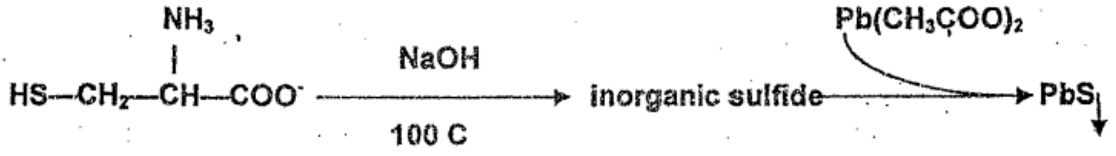
Salicylic acid

5. Unoxidized Sulfur Test:

This test is positive only in the presence of amino acids containing sulfhydryl [SH] or disulfide group [-S-S-]. Methionine is the third amino acid which contains sulfur but not in the form of SH or SS groups.



When proteins boiled in strong alkali, the SH and SS groups are converted to organic sulfide If we add lead acetate solution a black precipitate of lead sulfide [PbS] is formed:



Experimental Procedure:

1-Buiret Test:

Into three separate test tubes, add 2 ml of the following solution: 2% egg albumin 1% gelatin and 0.5% alanine one solution per tube.

-Into a fourth test tube add 20mg of powdered casein.

-Add 2ml of 10% NaOH into each of the four tubes and mix the contents.

-Report your observation.

-Add 5 to 10 drops of 0.5% $CuSO_4$, into each tube.

2-Ninhydrin Test

-Into three clean test tubes pipet 5ml of the following solution: 2% egg albumin, 0.5% glycine and water, one solution per tube.

-Add 1ml of 0.1% ninhydrin solution.

-Mix the contents of each tube and heat in a boiling water bath.

-Record your observation with each solution.

3-Millon's test

1-Test the solution list below: 2% egg albumin, 0.5% tyrosine, 0.5% alanine, 0.5% salicylic acid.

2- Into four clean test tubes pipet 4ml of each one solution per tube.

3-Add 5 to drops of fresh Millon's reagent into each tube.

4-Mix the contents of each tube and place them in a boiling water bath.

5-Note any changes in the test tubes, and record your observation.

4-Hopkins-Cole Test

1-add 2ml of 2% Ovalbumin, 1% tryptophan, 0.5% tyrosine, in separate test tubes.

2-Add 3ml of Hopkins-Cole reagent to each tube and mix (sof.A).

3-Add 3ml of conc. H_2SO_4, into separate test tube (sol.B).

4-Very carefully and cautiously pour solution B down the side of solution A.

Note: Work carefully so that conc. H_2SO_4 and solution A do not become mixed,

5-Note any color that develops at the interface of the tow liquids and record your observation

5- Unoxidized Sulfur Test

1-Add 2ml of each of the following solutions [2% ovalbumin, 0.5% cystyelne, 0.5% methionine] into three separate test tubes.

2-Add 5ml of 10% NaOH and 2 drops of 5% lead acetate, $Pb(CH_3COO)_2$, into all tubes.

3-Mix the contents of each tube and place them in a boiling water bath for 10 minutes.

4-Record your observation.