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Diploma in Pharmacy 2nd Year

Biochemistry & Clinical Pathology

Experiment

To perform qualitative analysis of proteins and amino acids.

Aim:

To perform qualitative analysis of proteins and amino acids.

Reference :

‘ Dr. Gupta G.D. , Dr. Sharma Shailesh, Kaur Manpreet, “Practical Manual of Biochemistry & Clinical Pathology” Published by Nirali Prakashan, Page no 5 - 9

Materials Required

Stirrer, dropper, water bath, distilled water, bunsen burner, test tube holder, clean and dried test tubes, food sample, nitric acid, ninhydrin reagent, etc

Theory :

Amino acids are the building blocks of all proteins. There are 20 amino acids commonly found in protein. These 20 amino acids are linked together through peptide bond, forming peptide chains and proteins. The chains containing less than 50 amino acids are called peptides, while those containing greater than 50 amino acids are called proteins. An amino acid chain with more than 25 (10-50) amino acids is called a polypeptide, e.g., insulin, hormones, etc. A protein consists of a long polypeptide chain or several polypeptide subunits, e.g., interferon, clotting factors, antibodies, enzymes, etc.

The word Amino Acid itself is made of two words amino + acid. This means the chemical structure of amino acids contains two important functional groups:

- 1) Amine group (-NH₂ group), and
- 2) Acid group or carboxylic acid group (-COOH group).

For the identification of proteins following tests are performed :

- 1) **Ninhydrin Test** : Like amino acids and peptides, proteins also give blue to violet colour with ninhydrin solution
- 2) **Xanthoproteic Test** : This test is given by proteins and peptides which have phenylalanine or tyrosine or tryptophan (aromatic amino acids) in their molecules. On treatment with concentrated nitric acid, these amino acids undergo nitration in the aromatic ring. These nitro compounds (particularly nitrophenols) are yellow in colour. This is the same reaction which occurs when skin comes in contact with concentrated nitric acid in the laboratories.
- 3) **Millon's Test** : When proteins or peptides are treated with Millon's reagent (a solution of mercuric and mercurous nitrate in nitric acid), a white precipitate is formed. When this is heated, a red coloured precipitate is formed. This test is given only by proteins containing tyrosine in their molecules, forming red mercury complexes with the nitrophenol formed. The test is also given by other phenolic compounds and is therefore not specific to proteins.

Procedure

- 1) **Ninhydrin Test** : A clean and dry test tube should be taken. The food samples and 1-2 ml of ninhydrin solution should be added into the test tubes and it should be shaken. The mixture should be boiled and the change should be observed. The presence of protein is confirmed if deep blue or purple color appears.
- 2) **Xanthoproteic Test** : A clean and dry test tube should be taken. The food samples and few drops of concentrated sulphuric acid should be added to test tube and it should be shaken. Then the test tube should be heated gently on a Bunsen burner. The presence of protein is confirmed if there is a formation of yellow precipitate.
- 3) **Millions Test** : A clean and dry test tube should be taken. The food samples and 2-3 drops of Millon's reagent should be added into the test tubes and it should be shaken. The presence of protein is confirmed if there is the formation of white precipitate or if the sample changes to brick red on heating

Result :

Qualitative analysis of proteins and amino acids was performed.

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