Lab (3): Effect of various factors on protein solubility and structure

Introduction:

The development of biochemistry and the study of proteins was assisted by analysis their composition and structure by Heinrich Halsiwetz and Josef Hambermann around 1873. They recognize that proteins were made up of smaller unites called **amino acids**. [1] Amino acid molecules in proteins are covalently joined together through a amide linkage, termed a peptide bond. Such a linkage is formed by the removal of water (dehydration) from the α-carboxyl group of one amino acid and the α-amino group of another (Figure 1). [2]

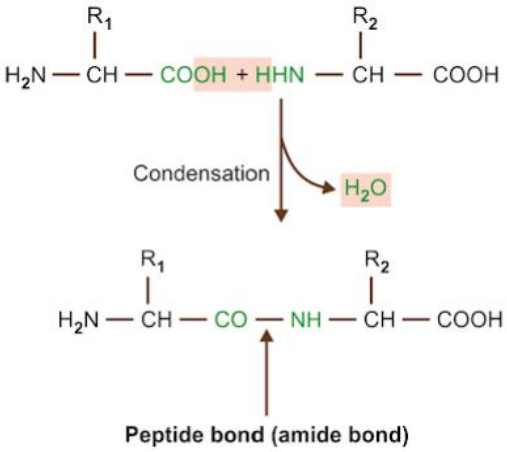

Figure 1. Formation of peptide bond. [2]

Protein precipitation is the process of separating a protein from a solution as a solid by altering the protein solubility with addition of a reagent. [3] Proteins precipitation is widely used in downstream processing of biological products in order to concentrate proteins and purify them from various contaminants. [4] The solubility of proteins is affected by various factors including pH, temperature, salts, heavy metal salts...etc. [3] The change of one of these factors will lead to protein precipitation and/or denaturation.

Denaturation is a process in which the proteins losing its quaternary structure, tertiary structure and secondary structure, by application of some external factor or compound such as a strong acid or base, an organic solvent (e.g., alcohol or chloroform), or heat. [5] No alteration on the molecule's primary structure, i.e., without cleavage of any of the primary chemical bonds that link one amino acid to another. Denatured proteins can exhibit a wide range of characteristics, from conformational change and loss of solubility to aggregation due to the exposure of hydrophobic groups. Denatured proteins lose their 3D structure and therefore cannot function. [6]

Experiment (1). Effect of salt concentration:

Aim:

- To investigate the effect of different salt concentration on protein solubility.

Principle:

The solubility of globular proteins increases upon the addition of low salt concentration (<0.15 M), an effect termed salting-in. At higher salt concentrations, protein solubility usually decreases, leading to precipitation; this effect is termed salting-out.[7] Each protein can be precipitated at specific salt concentration. It is reverse process, the protein can again become soluble when we add water.

The process of "salting out" is a purification method that relies on the basis of protein solubility. High salt concentration causes the protein to precipitate (decrease the solubility) since salt ions, in this case, compete with the protein molecules in binding water molecules. The low salt concentration solutions make protein solubility increased using the attraction of salt ions to the functional groups of the protein "this called salting in", i.e. the salt ions interact with oppositely charged group on the protein, forming double layer of ionic groups, thus leading to decrease the electrostatic interaction between proteins molecules cause more protein solvation. [8]

Materials:

Chemical

0.1 M NaCl, egg albumin, solid ammonium sulfate $(NH_4)_2SO_4$, distal water.

Equipment and Glassware

Test tubes, rack, pipette, pipette pump.

Protocol:

1. In a tube add 2 ml of albumin.
2. Add drops of 0.1M NaCl solution, concentrate your vision on the tube while adding.
3. Record your results.
4. In the same tube add few amounts of 100% solid $(NH_4)_2SO_4$, shake it well.
5. Record your results.
6. Compare between the two results.

Results:

Tube	Observation
Albumin + o.1 M NaCl	
Albumin+100% solid $(NH_4)_2SO_4$	

Experiment (2). Effect of strong acids:

Aim:

- To investigate the effect of strong acids on protein solubility and structure.

Principle:

This test depend on affecting solubility of the protein as a function of changes in pH. The pH of an aqueous solution can affect the solubility of the solute. By changing the pH of the solution, you can change the charge state of the solute. [9] In highly acidic media, the protein will be positively charged, which is attracted to the acid anions leading to protein precipitation and denaturation as a result of disrupting the salt bridges. [10,11]

There are many applications of this test in laboratories, i.e. in the detection of small amounts of protein in urea sample, also in the separation and purification of proteins or to stop the enzymatic action of an enzyme.

Materials:

Chemical

Concentrated nitric acid, trichloroacetic acid (TCA), egg albumin, distal water.

Equipment and Glassware

Test tubes, rack, pipette, pipette pump.

Protocol:

1. Label two tubes **A** and **B**.
2. **In tube A:** add 3 ml of conc. nitric acid (HNO_3) CAREFULLY.
3. Then, using a dropper add drops of albumin on the inner wall of tube A to form a layer up the acid.
4. **In tube B:** Add 3 ml of the albumin solution.
5. Then add 5-7 drops of TCA solution CAREFULLY.
6. Record your results.

Results:

Tube	Observation
Albumin + conc. nitric acid	
Albumin+ TCA	

Experiment (3). Effect of salts of heavy metals:

Aim:

- To identify the effect of heavy metal salt on protein solubility and structure.

Principle:

Heavy metal salts act to denature proteins in much the same manner as acids and bases. Heavy metal salts usually contain Hg^{+2}, Pb^{+2}, Ag^{+1} Tl^{+1}, Cd^{+2} and other metals with high atomic weights. Since salts are ionic they disrupt salt bridges in proteins (heavy metal salt will neutralize the protein). The negative charge of protein will bind with positive charge of metal ion which cause the protein to precipitate as insoluble metal protein salt. (11)

Materials:

Chemical

$AgNO_3$, $HgCl_2$, egg albumin, distal water.

Equipment and Glassware

Test tubes, rack, pipette, pipette pump.

Protocol:

1. Label two tubes **A** and **B**.
2. In tube A and B add 1 ml of Albumin sample.
3. **In tube A:** using a dropper add few drops of $AgNO_3$.
4. **In tube B:** add few drops of $HgCl_2$.
5. Record your results.

Results:

Tube	Observation
Albumin + $AgNO_3$	
Albumin+ $HgCl_2$	

Experiment (4). Effect of high temperature:

Aim:

- To identify the effect of high temperature on protein solubility and structure.

Principle:

Heat can be used to disrupt hydrogen bonds and non-polar hydrophobic interactions (non-covalent bonds). This occurs because heat increases the kinetic energy and causes the molecules to vibrate so rapidly and violently that the bonds are disrupted leading to protein precipitation and denaturation.

The proteins in eggs denature and coagulate during cooking. Other foods are cooked to denature the proteins to make it easier for enzymes to digest them. Medical supplies and instruments are sterilized by heating to denature proteins in bacteria and thus destroy the bacteria. (11)

Materials:

Chemical

Egg albumin, distal water.

Equipment and Glassware

Test tubes, rack, pipette, pipette pump, water bath.

Protocol:

1. Take 3 ml of protein Albumin.
2. Place it in a boiling water bath for 5-10 minutes
3. Remove aside to cool to room temperature.
4. Note the change.

Results:

Tube	Observation
Albumin + heating	

References:

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