Biochemistry of Proteins BCH 303 [Practical]

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

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Proteins

- The development of <u>biochemistry</u> and the study of proteins was assisted by analysis their <u>composition</u> and <u>structure</u> by *Heinrich Halsiwetz* and *Josef Hambermann* around **1873**.
- **Proteins** are made up of smaller units called **amino acids**.
- Amino acid molecules in proteins are covalently joined together through a amide linkage, termed a <u>peptide bond</u>.

How peptide bond formed?

- ➔ By <u>removal</u> of the elements of H_2O (dehydration) from the α-carboxyl group of one amino acid and the α-amino group of another.
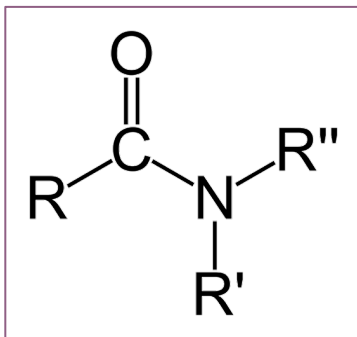

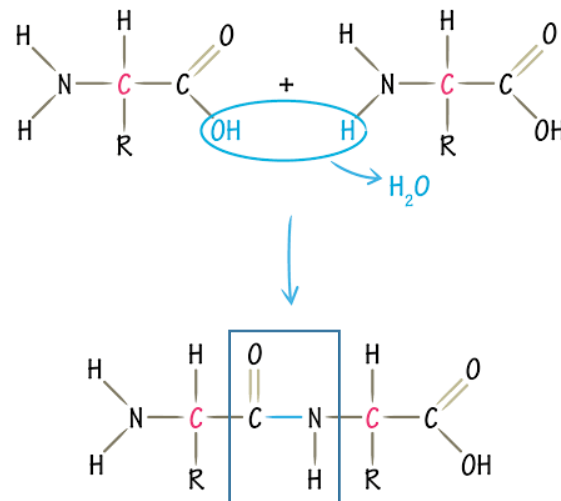
Fig.1. Amide linkage

Fig.2. Peptide bond formation

Protein precipitation

- **Protein precipitation** is the process of <u>separating</u> a protein from a <u>solution</u> as a <u>solid</u> by altering the protein solubility with <u>addition of a reagent</u>.

Applications: 1- To concentrate proteins **?** 2- Purify them from various contaminants **?**

Factors affecting protein solubility: pH, temperature, salts, heavy metal salts and others.

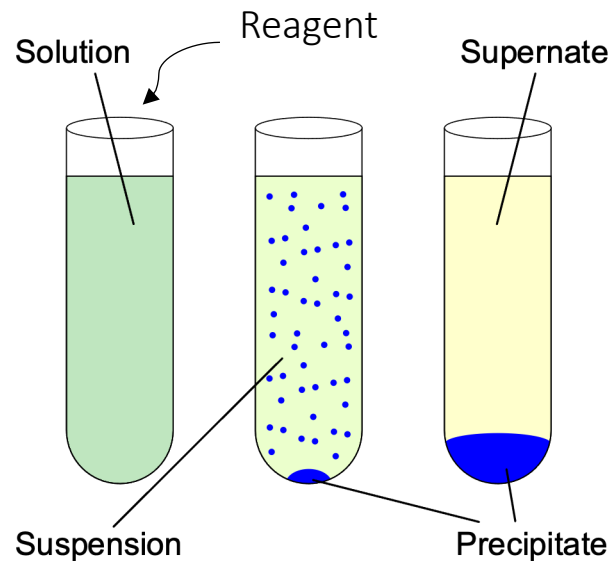- The change of one of these factors will lead to protein <u>precipitation</u> and/or <u>denaturation</u>.

Fig.3. Mechanism of precipitation

Protein denaturation

▪ **Denaturation** is a process in which the protein lose its <u>quaternary</u> structure, <u>tertiary</u> structure and <u>secondary</u> structure.

Factors: such as a strong acid or base, an organic solvent (e.g., alcohol or chloroform), or heat.

▪ No alteration on the molecule's <u>primary structure</u>, i.e., without cleavage of any of the primary chemical bonds that link one amino acid to another.

Characteristics: 1- Conformational change 2- Loss of solubility 3- Aggregation due to the exposure of hydrophobic groups.

▪ Denatured proteins lose their 3D structure and therefore cannot function.

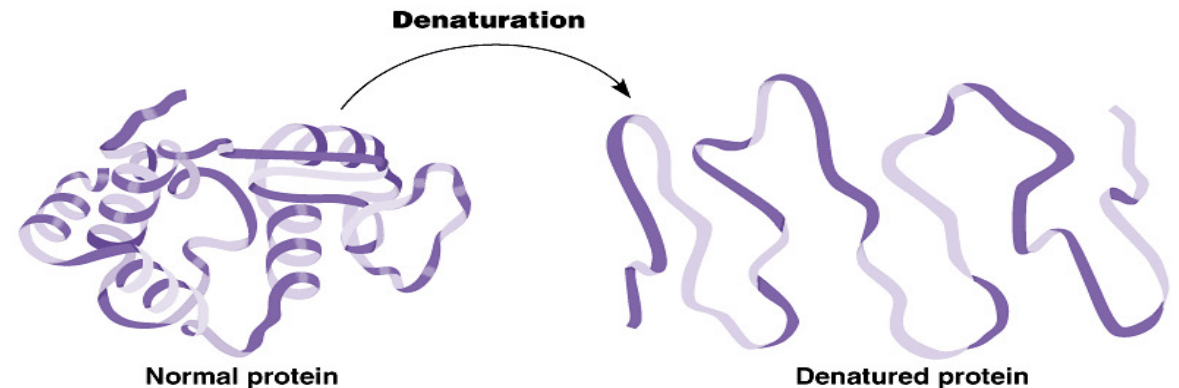💡 PAUSE AND THINK What is the difference between protein precipitation and denaturation?

Denaturation

Normal protein

Denatured protein

Fig.4. Protein denaturation

Practical part 🧪

Tests of proteins

1. Effect of salt concentration on the protein solubility.

2. Effect of strong acids on protein solubility and structure.

3. Effect of salts of heavy metals on protein solubility and structure.

4. Effect of heat on protein solubility and structure.

Experiment (1): Effect of salt concentration on the protein solubility

Objective:

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.

Notes:

1. Each protein can be precipitated at specific salt concentration. So?

2. It is reverse process, the protein can again become soluble when we add water.
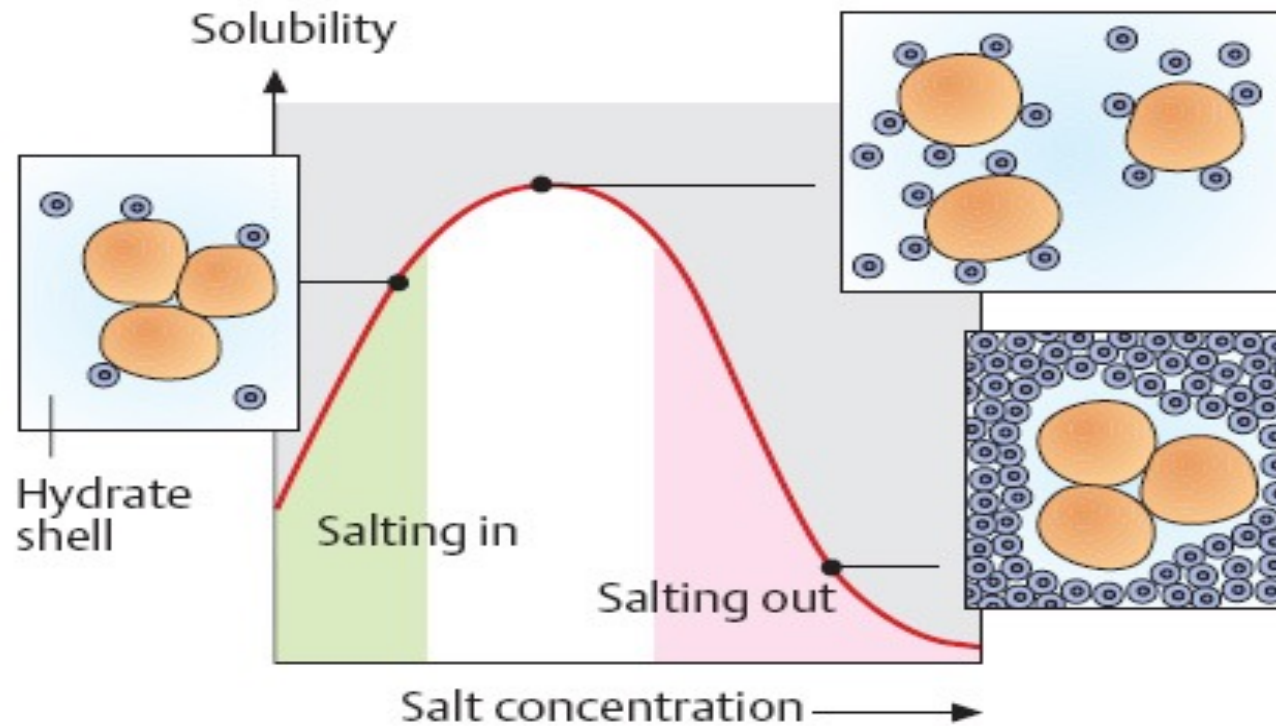
- The process of "salting out" is a purification method that relies on the basis of protein solubility.
- High salt concentration causes the protein to precipitate (decrease the solubility) since salt ions, in this case, compete with the protein molecules in binding water molecules.
- The low salt concentration 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.

Experiment (1): Effect of salt concentration on the protein solubility

Method:

1. Label one tube as **A.**

2. Add 2 ml of albumin.

3. Add drops of **0.1M NaCl** solution, concentrate your vision on the tube while adding.

4. Record your results.

5. In the same tube add few amounts of 100% solid $(NH_4)_2SO_4$, shake it well.

6. Record your results.

7. Compare between the two results.

Results:

Tube	Observation
Albumin + 0.1M NaCl	
Albumin+100% saturate $(NH_4)_2SO_4$	

Experiment (2): Effect of strong acids on protein solubility and structure

Objective:

To investigate the effects of strong acids on the protein solubility.

Principle:

- This test depends 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.

- 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 <u>disrupting the salt bridges.</u>

Applications:
1. Detection of small amount of protein in urine sample.
2. The separation and purification of proteins
3. Stop the enzyme reaction.

Experiment (2): Effect of strong acids on protein solubility and structure
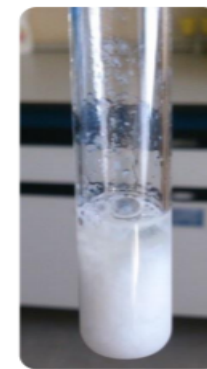
Method:

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. Record your results.
5. **In tube B:** Add 3 ml of TCA solution CAREFULLY.
6. Then add 5-7 drops of the albumin solution.
7. Record your results.

Results:

Tube	Observation
Albumin + HNO_3	
Albumin+TCA	
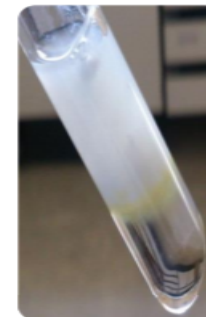
A   B

When handling acids:
- Wear appropriate protective eyeglasses or chemical safety goggles
- Wear appropriate protective gloves and clothing to prevent skin exposure.
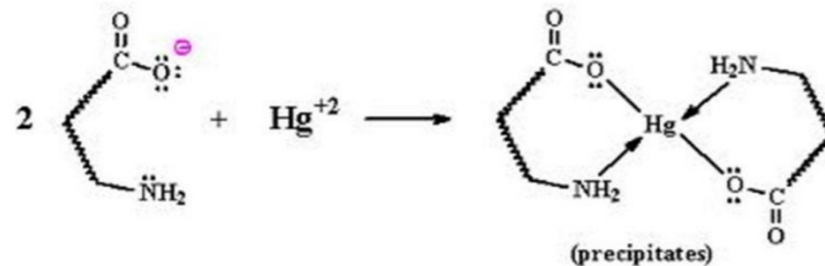
Experiment (3): Effect of salts of heavy metals on protein solubility and structure

Objective:

To identify the effect of heavy metal salt on protein.

Principle:

- Heavy metal salts act to denature proteins in much the <u>same manner as acids and bases.</u>

- 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**.

(precipitates)

Applications:

1. To eliminate the poisoning by palladium Pb^{++} and mercury salts Hg^{++}

Experiment (3): Effect of salts of heavy metals on protein solubility and structure

Method:

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 $AgNO_3$.
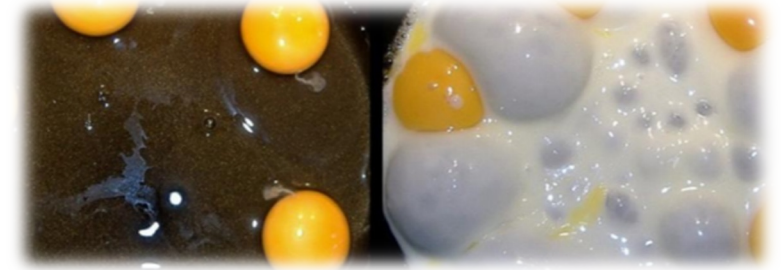
4. Record your results.

Results:

Tube	Observation
Albumin + $AgNO_3$	

Experiment (4): Effect of heat on protein solubility and structure

Objective:

To investigate the effect of high temperature on protein 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.

Application:

1. The proteins in eggs denature and coagulate during cooking.

2. Other foods are cooked to denature the proteins to make it easier for enzymes to digest them.

3. Medical supplies and instruments are sterilized by heating to denature proteins in bacteria and thus destroy the bacteria.

Experiment (4): Effect of heat on protein solubility and structure

Method:

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	

Homework

- How does protein denaturation lead to precipitation?

- How denatured protein can be retuned to its native form?