

EXPERIMENT :2

Protein

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Qualitative chemical reaction of functional group of a.a protein

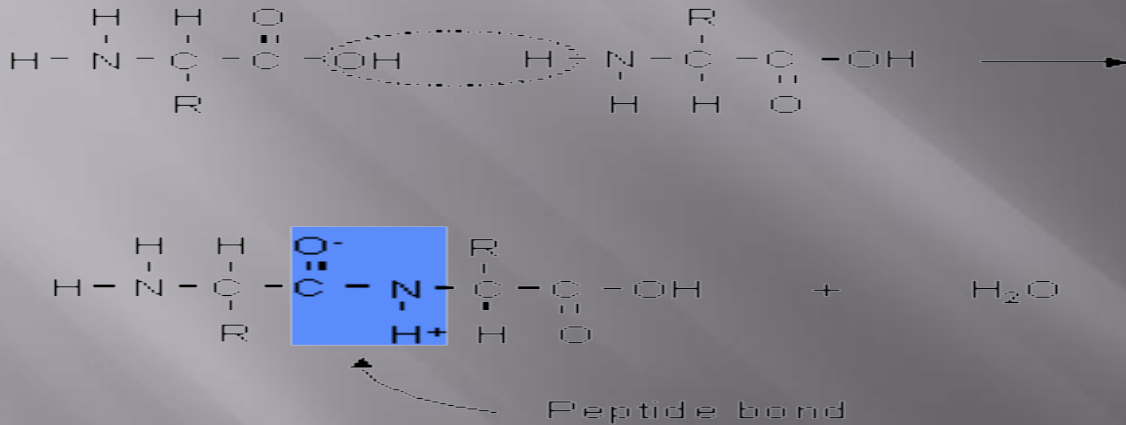
Certain chemical can react to specific functional group in protein ,

We will identified the the one qualitative test detect the presence of certaine functional group in protein {Biuret test}

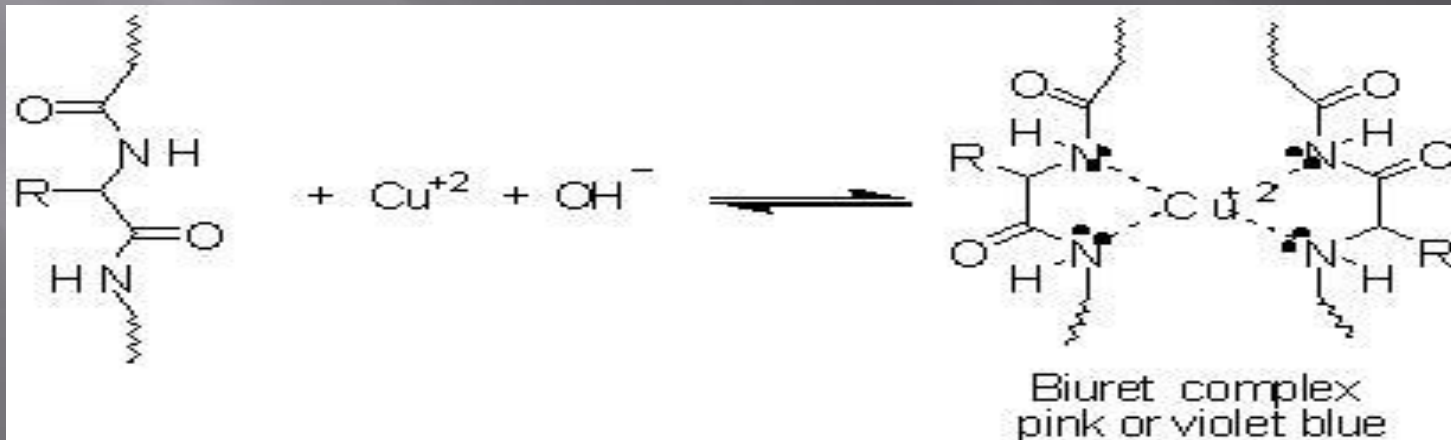
[1]Biuret test

This test is specific for the peptide bond. Substances (protein) containing not less than two peptide linkages give this test.

Principle:



Why the test is called biuret test?



Method:

1- add 1ml of protein(Albumin & Casein)

Each protein in one test tube.

2- Add 1 ml in each tube 3M NaOH

3- Add 0.5 ml of CuSO₄ and mix well.

protein	Observation	Comment
Albumin		
Casein		

[2]Precipitation of the protein:

C

Precipitation of protein by
salts of heavy metals

A

Effect of salt Concentration
on the protein solubility
"Salting out"

B

Effect of strong acid on
protein

{A} Effect of salt Concentration on the protein solubility “Salting out”

Objective:

This test used to separate different protein

Principle

high salt concentration solution or solids (dissolved in the reaction medium up till saturation solutions) causes the protein to precipitate; Because the salt ions effect on protein solubility . By the salt ions will compete with the protein molecules in binding wate molecules.[Dehydration]

Each protein can precipitate at different salt concentration.

Salting out: used high salt concentration to separate different protein by using different salt concentration.

In salting out you must take into account the following:

- 1-The type of salt (ammonium sulfate , $(\text{NH}_4)_2\text{SO}_4$
- 2-The molecular weight (Mwt)of protein ,, the high Mwt will will precipitate first .

3- there is **inverse relationship** between the Mwt of protein and the concentration of salt..



High Mwt need low concentration salt

Low Mwt need high conc. Salt

4- it is Reverse process, the protein can again become soluble when we add water

5- Application , in separate mixture protein

Method:

1-separate globulin (high Mwt)from albumin(Low Mwt)

2- dissolve the globulin in 0.1 M NaCl ,, then take 1ml from globulin solution + add 1ml saturated $(\text{NH}_4)_2\text{SO}_4$ and record your observation

3- add 1ml egg albumine + add 1ml saturated $(\text{NH}_4)_2\text{SO}_4$,.... and record your observation,,,

then in same tube (egg albumin) add solid $(\text{NH}_4)_2\text{SO}_4$

step	Observation	Comment
Golbulin+NaCl		
Globulin sol. + saturated $(\text{NH}_4)_2\text{SO}_4$		
Albumin +saturated $(\text{NH}_4)_2\text{SO}_4$		
Albumin +Solid $(\text{NH}_4)_2\text{SO}_4$		

{B} Effect of strong acid on protein

Objective:

- Separation and purification
- Detection of small amount of protein in urea sample
- Stop the enzyme reaction

Principle:

By changing the pH value of protein, because the addition of acid will reduce the optimum pH of protein until it is equal to the pI (isoelectric point). $[+] = [-]$ in protein and the weak bonds will be affected, and this causes protein precipitation.

⋮

Method

1- add 1ml from protein (egg albumin, Caseine) each protein in separate tube .

2-Add few drop (2drop) 10% TCA

protein	Observation
Egg albumin	
Casein	

[C]Precipitation of protein by salts of heavy metals:

Heavy metal salt ; usually contain Hg^{+2} , Pb^{+2} , Ag^{+1} ,, and other metal ion with high atomic weight

Objective: to identify the effect of heavy metal salt on protein

Principle: heavy metal salt will neutralization the protein ,, By the negative charge of protein will bind with positive charge of metal ion . Then the protein will precipitate as insoluble metal protein salt.

Application:

To eliminate the poisoning by palladium Pb^+ , mercury Mg^+ How???

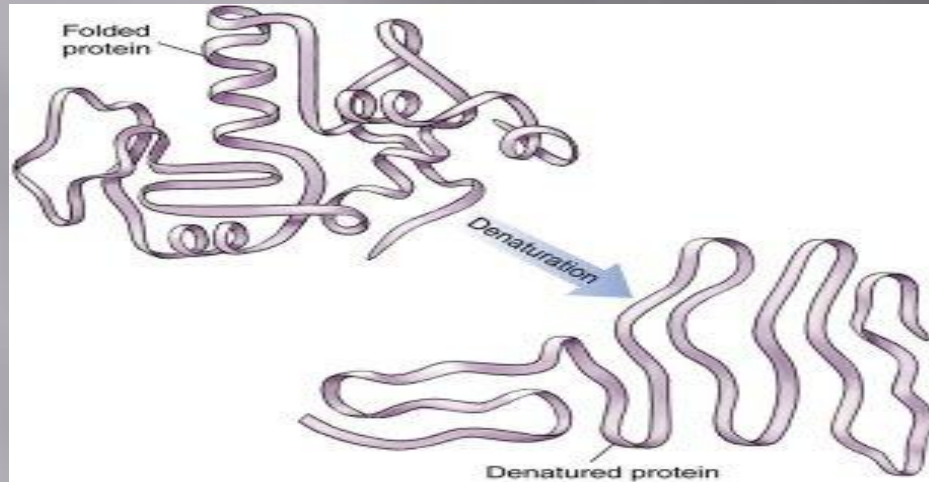
Method

1- Add 1ml from protein(Albumin , Casein)

2- Add 0.5 ml from Heavy metal salt silver nitrate $AgNO_2$

Protein	Observation	Comment
Albumin		
Casein		

[3]Protein denaturation



The 3-dimensional structure will change but the primary structure will not change

Denaturation Factors:

Heat , inorganic salt , organic solvent , irradiation , strong acid , strong base,

[3]Protein denaturation

Denaturation: is defined as a major change from the original native state without alteration of the molecule's primary structure

Objective:

To identify the denaturation factors and its effect on protein

Principle:

Denaturation factor(boiling) cause destroy weak bond and this will cause loss of three – dimensional structure and loss its biological activity function and lead to precipitation

Method

1- add 1ml from protein (albumin “prepared” , egg globulin)

2- put each tube in boiling water

protein	observation	comment
albumin		
Egg globulin		

