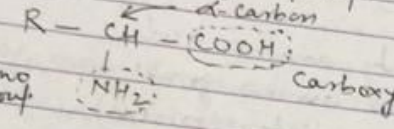
P.G. Semester 2nd - Zoology

Histochemical Demonstration of Protein

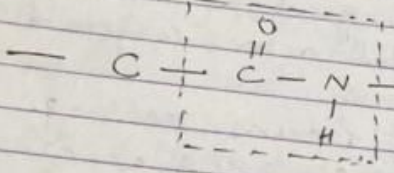
Ninhydrin Schiff's Method (long Question)

Introduction

The term Protein was suggested by Berzelius. Proteins are made up of large number of amino acid, ~~react with the~~ linked with each other through peptide bond. Peptide bonds are formed when the amino group of one amino acid react with the carboxyl group of another.



General formula of Amino acid



Showing Peptide bond.

Out of the two ~~ends~~ ends of a Protein one end has a free $-NH_2$ group and is called aminoterminal or N-terminus, the other end has a free $-COOH$ group and is called Carboxyl terminus or C-terminus.

Hence the Constituent elements of Proteins are Carbon, hydrogen, oxygen, nitrogen and very rarely sulphur. Proteins are most abundant intracellular macromolecules and Constitute over half of the dry weight of most organisms.

Some proteins participate in muscular Contraction and some are associated with the genes.

Classification - On the basis of composition and Solubility —

Protein

- Simple protein
- Conjugated Protein
- Derived protein.

Simple protein

→ Containing only amino acids
→ Additional non amino groups are absent.

Conjugated protein (Complex or Heteroproteins) —

→ Made up of amino acids and some non protein part (Prosthetic group). Prosthetic group may be either a metal or a compound

Derived Protein

→ Derivatives of proteins resulting from the action of heat, enzymes or chemical reagents.

Histochemically all groups present in protein do not show demonstrations reactions There are few methods available for histochemical demonstration of only some of the groups of proteins and that are as follows —

group	Methods
Amino acid	(a) Ninhydrin Schiff
	(b) Diacetyl enzyme reaction.

Disulphide — (a) Performic acid oxidation
(b) DDT - reaction

Sulphydryl — (a) Mercury orange (RLR reaction)
(b) DDT - reaction

Guanidyl — Sakaguchi method

Indol — D.M.A.B. Method

Phenol — Millon Method.

Ninhydrin schiff's Method

It was introduced by Yasuma and Itchikawa in 1953.

Principle

The nin-hydrin at neutral pH reacts with α-amino groups and yeild an ~~aldehyde~~ aldehyde and this aldehyde react with schiff's reagent and gives specific colouration.

$-NH_2$ + Ninhydrin neutral pH — CHO

— CHO + schiff's reagent — colour.

Reagent required

i) Ninhydrin - 0.5%.
ii) Absolute alcohol
iii) schiff's reagent

Preparation of sol^n

Ninhydrin 0.5% solution
(ie, Ninhydrin - 500mg and alcohol - 100ml)

schiff's reagent

By de Tomali 1936, Method —

i) Dissolve 1 gm of basic fuschin in 200 ml of distilled water and shake the solution for 5 minutes and allow to cool.
ii) When the temp is down to 50°c filter and to the filtrate add 20 ml of N-HCl.
iii) Cool to 25°c and add 1 gm of Sodium meta bisulphate.
iv) Add 2 gm of activated charcoal and shake for one minute. Filter and store the filtrate in dark bottle at 4°c.

Sections

Freeze dried
Paraffin Section
Cryostate unfixed
Cryostat prefixed.

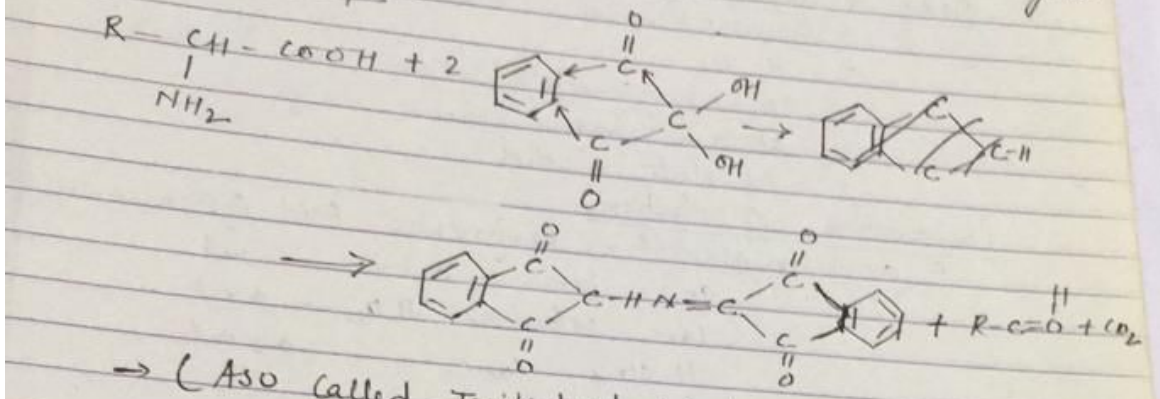
Method of Staining

i) Bring all sections down to 70% alcohol.
ii) Treat with Solution 1 (Ninhydrin) at 37°c overnight.
iii) Wash in running tap water.
iv) Put sections in schiff's reagent for 45 minutes.
v) Wash well in running tap water.
vi) Stain in haematoxylin if required
vii) Wash in tap water.
viii) Dehydrate through graded alcohols to xylene and mount in DPX.

Result = α amino acid → Pink to red

Remark = 1% alloxan, also in absolute alcohol may be used instead of 0.5% ninhydrin.

Under principle



→ (Also called Triketo hydrindene hydrate ~~test~~ Test)
or
Ninhydrin test.

This test is +ve for all amino acids containing free amino and carboxylic group. Hence it is +ve. for protein, Peptones and peptides.

→ It is also +ve with other primary amino ~~and~~ including ammonia.

. The triketohydrindene hydrate forms a complex with the amino or carboxylic group of the amino acids, or other primary amino developing a blue colour on heating.

Short Notes.

Performic Acid Alcian Blue Method

It was introduced by ADAMS & SLOPER (1955-56)

Reagent required

i) Formic acid
ii) H_2O_2
iii) H_2SO_4 (Conc)
iv) Alcian Blue
v) Absolute alcohol

Preparation of Solution

i) **oxidising Solⁿ** → Performic Acid (Pearse-1957)
98% formic acid – 40 ml
100 volume H_2O_2 – 4 ml
H_2SO_4 Conc 0.5 ml

ii) **Staining Solution**
Alcian blue = 1 gm
98% H_2SO_4 — 2.7 ml
Distilled water = 47.2 ml

Sections

Freeze dried
Paraffin
Cryostat

Staining Method

i) All sections put in water.
ii) Remove excess water by boiling
iii) Place sections in oxidising Solⁿ for 5 minutes
iv) Wash in tap water for 10 minutes
v) Dry sections by gentle heating at 60°C
vi) Rinse in tap water
vii) Stain the section in Alcian blue Solⁿ for one hour.

viii) Wash in running tap water
ix) Counter stain if required
x) Wash in tap water
xi) Dehydrate through graded (alcohols to xylene and mount in DPX).

Result → Disulphide → Dark blue
If in small amount → light blue.