Histochemical Identification of Proteins

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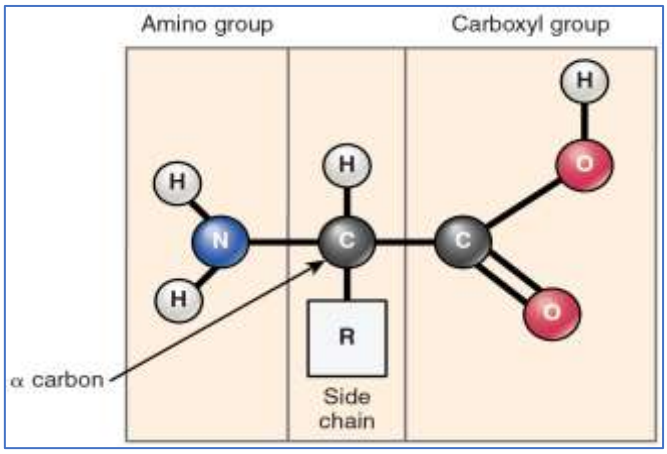
The histochemical identification of proteins are based on two basic techniques. The first technique detects proteins based on their specific characteristics e.g. molecular weight, solubility *etc*. The second method detects proteins based on specific amino acids present in the proteins which can react with certain stains or reagents and produce coloured products. The second technique is widely employed to visualize proteins in histological sections and is described in further detail.

The histochemical staining of proteins relies on two factors. First, there is presence of functional groups in amino acids in proteins, and second, formation of functional groups which takes place on the side chains of amino acids. The functional groups will react with stains and reagents to form coloured or chromogenic products. The basic structure of amino acids with amino and carboxyl functional groups is shown in figure 1.



https://upload.wikimedia.org/wikipedia/commons/3/3d/223_Structure_of_an_Amino_Acid-01.jpg

Figure 1: Structure of Amino Acid

1. Histological Stains of Proteins

1.1. Mercury-Bromophenol Blue (Hg-BPB)

Hg-BPB is mainly used for the detection of total proteins. Hg-BPB has following advantages over other histological stains of proteins (Mazia et at, 1953; Bhandari, 1997).

- Very low amount of protein can easily be detected in tissues using Hg-BPB stain.
- Basic proteins can bind to BPB dye (Figure 2) even in absence of Hg. Non-basic proteins will bind to BPB dye by coupling through Hg.
- Dye bound is proportional to the amount of protein.

1.1.1. Preparation of Hg-BPB

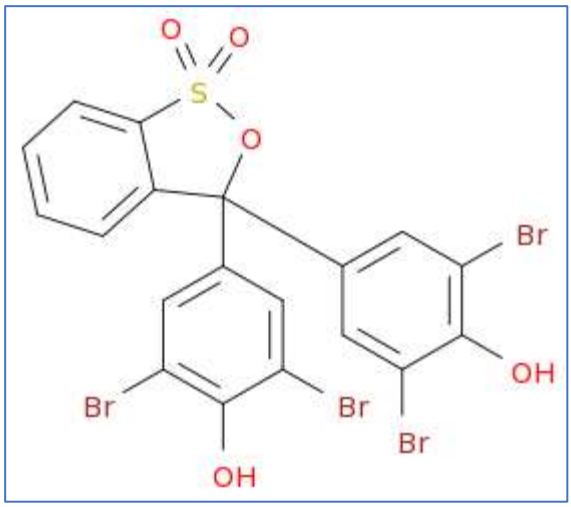
Dissolve 10 mg of BPB in 100 ml of 10% mercuric chloride solution in 95% alcohol. Alternatively, dissolve 1 g mercuric chloride and 0.05 g BPB in 100 ml of 2% aqueous acetic acid (Naik et al. 2002).

1.1.2. Principle

Hg ions of BPB solution react with acidic, sulphydryl and aromatic residues in amino acids of protein to produce blue colour.

1.1.3. Staining procedure

a. De-paraffinized tissue sections are dehydrated in absolute alcohol and incubated for 15 minutes in Hg-BPB at room temperature.
b. Tissue sections are then rinsed with 0.5% acetic acid to remove excess stain for 5-10 minutes and washed in tap water until the sections become blue.
c. The sections are then air dried, cleared in butanol and xylol, and finally mounted with DPX mounting media (Naik et al. 2002).
d. Colour development: Blue
e. Conditions: Room temperature, 15 min



Mol-Instincts Chemical Database

Figure 2: Chemical structure of Bromophenol Blue dye

1.2. Ninhydrin-Schiff

Ninhydrin-Schiff staining is mainly used for the detection of protein-bound amino ($–NH_2$) groups. This is a modification of older ninhydrin reaction in which tissue is heated with ninhydrin solution. In this method, the production of transient unstable blue-violet colour indicates presence of proteins in tissue.

Yasuma and Ichikawa (1953) found that a stable non-diffusible aldehyde is produced when ninhydrin reacts with the alpha-amino groups of proteins (Figure 3). This aldehyde is allowed to react with leuco-basic fuchsin or Schiff's reagent to produce the magenta or purple colour (Figure 4).

Ninhydrin-Schiff Method for amino groups is an exception to side chain functional group histochemistry; this method demonstrates all protein bound-amino groups by the reaction of ninhydrin with the amino group to produce aldehydes which are then made visible by the Schiff reaction.

1.2.1. Principle

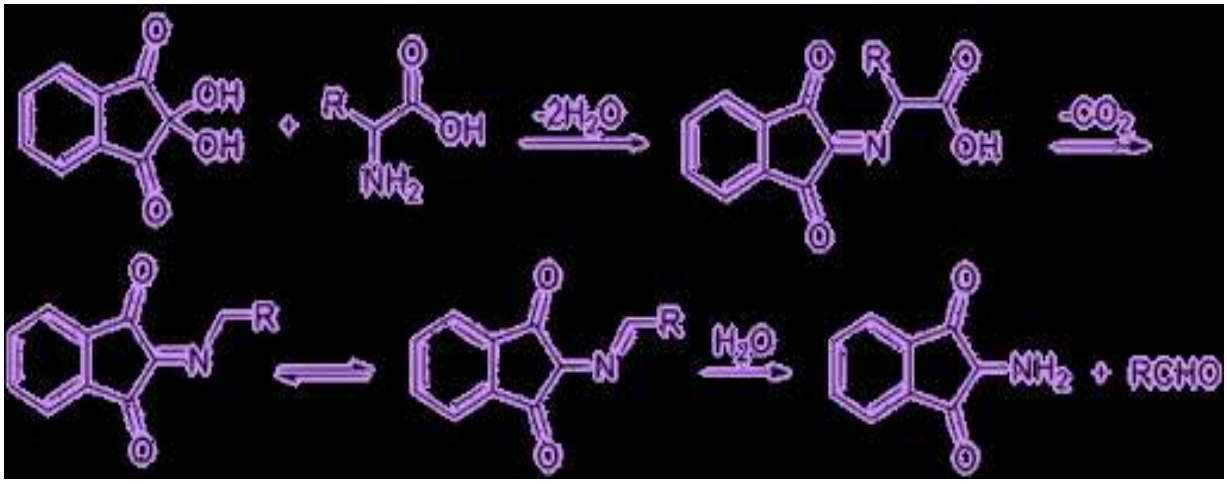
In the course of oxidative deamination with ninhydrin, stable tissue aldehydes are produced. These are demonstrated with Schiff's reagent.

1.2.2. Composition

Dissolve 0.5 g ninhydrin in 100 ml of absolute alcohol to obtain 0.5% Ninhydrin. For Schiff's reagent, dissolve 1 g basic fuchsin in 200 ml boiling distilled water. Shake for 5 minutes and cool to 50°C. Add 20 ml of 1 N hydrochloric acid to the filtrate, cool to 25°C and add 1 g sodium meta-bisulphite. Keep in dark for 14-24 hours in fridge. Add 2 g activated charcoal and shake for 1 minute. Keep the filtrate in the dark at 0-4°C. Allow to reach room temperature before use (Subramoniam, T., 1982)

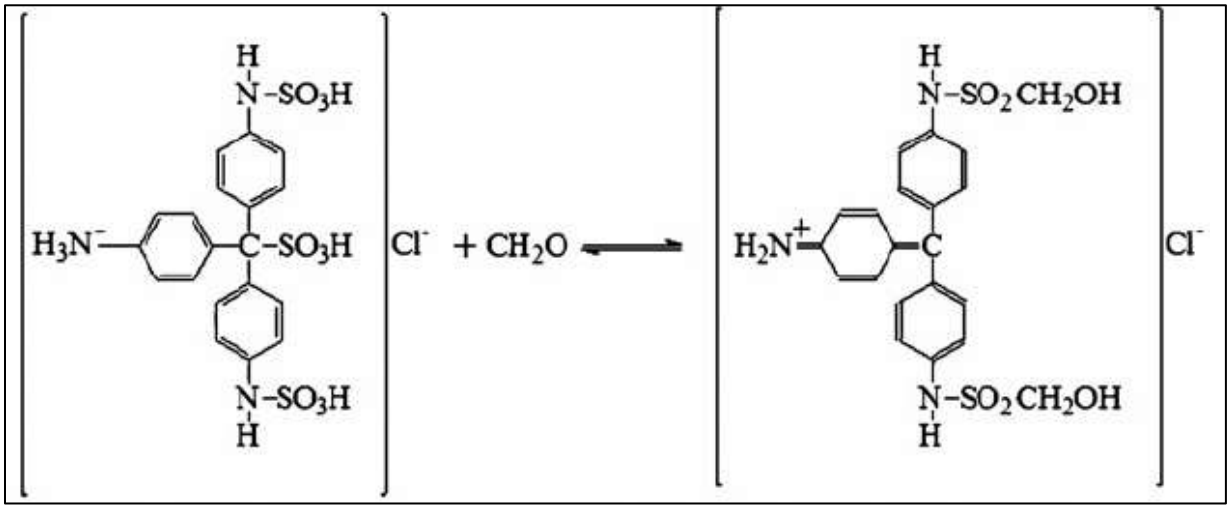
1.2.3. Staining Procedure

a. Tissue sections are de-paraffinized and dehydrated in 70% or absolute (100%) alcohol.
b. These are immersed for 20-24 hours or overnight in 0.5% ninhydrin in absolute alcohol at 37°C.
c. The tissue is rinsed twice in absolute alcohol, then in distilled water.
d. The tissue is treated with leuco basic fuchsin or Schiff's reagent for 15 minutes.
e. Rinsed in running water.
f. Counterstained with hematoxylin.
g. Washed in tap water, dehydrated in alcohol, cleared in xylene and finally mounted in xylene miscible mounting media.
h. Colour development: Magenta or Pinkish-Purple



http://www.chm.bris.ac.uk/motm/ninhydrin/ninhydrinh.htm

Figure 3: Chemical reaction of ninhydrin with alpha amino acids. Initially, Ninhydrin is dehydrated and reacts with alpha amino acid to form a Schiff base. This is decarboxylated to release a carbon dioxide molecule. Then, it reacts with water and the side chain alkyl (R group) forms an aldehyde and diketohydrindamine as products.



(Pielichowska, K. 2012. DOI: 10.1007/s10965-011-9788-y)

Figure 4: Reaction of Schiff with formaldehyde to produce pink-purple compound.

References

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