Histochemical methods, principles and application

Histochemical methods rely on distinct chemical reactions that yield insoluble colored or electron-dense reaction products. These techniques are employed to detect different chemical substances or to assess enzyme activity within tissue sections.

Detection of Chemical substances:

1. Lipids - The presence of lipid-rich structures is most effectively highlighted using lipid-soluble dyes to bypass steps in slide preparation that typically remove lipids, such as heating, treatment with xylene, or embedding in paraffin. Typically, frozen sections are stained in alcohol solutions saturated with a lipophilic dye such as Sudan Black, which imparts a black stain to lipids. Lipids existing as fats exhibit an affinity for Sudan Dyes or Oil Red. Baker's fluid (a mixture of formalin, water, and $CaCl_2$) is recommended for fixation, and frozen sections are utilized. The staining method Fast Luxol Blue MBS is used to stain phospholipids, particularly useful for staining the myelin sheath of nerve fibers.

2. Polysaccharides - Glycogen, glycoproteins, glycolipids, and mucopolysaccharides (comprising the ground substance of the extracellular matrix in mucus) can be visualized through the PAS reaction (Periodic Acid Schiff's reagent). This method is predicated on the ability of Schiff's reagent to react with aldehyde groups, producing a distinctive purple color. Alcian Blue is a specialized dye used for staining acid mucopolysaccharides (GAG) in mucous and goblet cells.

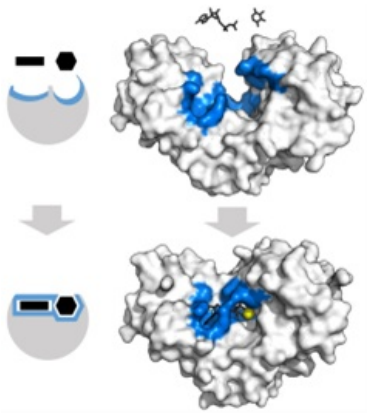
3. DNA - Feulgen's reaction is a histochemical technique utilized for detecting DNA, often employed for determining sex chromatin. This method relies on the acid hydrolysis of DNA.

4. RNA - Methylene blue and toluidine blue are commonly used dyes for visualizing RNA.

Enzyme:

Enzymes function as large biological catalysts, accelerating chemical reactions within organisms. At the onset of a process, molecules termed substrates interact with enzymes, which then convert them into distinct molecules known as products. Before catalyzing any chemical reaction, enzymes must first bind to their substrates. Typically, enzymes exhibit high specificity regarding the substrates they bind and the subsequent chemical reactions they catalyze.

Histochemistry refers to techniques employed to pinpoint cellular structures within tissue sections based on the distinct enzymatic activity inherent to these structures. To maintain the integrity of these enzymes, histochemical methods are typically applied to unfixed or lightly fixed tissue, often sectioned using a cryostat to mitigate the potentially detrimental impact of heat and paraffin on enzymatic activity.



Enzyme histochemistry:

1. Tissue sections are submerged in a solution containing the substrate specific to the enzyme being targeted for localization.

2. The enzyme is permitted to catalyze the reaction with its substrate.

3. At this juncture or subsequently, the section comes into contact with a marker compound.

4. This compound interacts with a molecule generated as a result of the enzymatic activity on the substrate.

5. The resultant reaction products, which must be insoluble and visible under light or electron microscopy only if they are colored or electron-dense, precipitate around the area containing the enzyme.

For this technique to be effectively utilized, proper fixation and precise incubation of the sample under controlled conditions (including time, temperature, and pH) are essential. Examples of enzymes that can be identified histochemically include phosphatases, dehydrogenases, and peroxidase.

Alkaline phosphatase is a characteristic enzyme found in the brush border of the absorptive epithelium of proximal tubules in the kidney. Utilizing the Azo-coupling method, we can assess alkaline phosphatase activity in the kidney.

References

- JUNQUIERA, Anthony – MESCHER,. *Junqueira's Basic Histology.* 16. edition. McGraw Hill LLC, 2001. 576 pp. ISBN 1260462978.