

Principles of enzyme histochemistry

Enzyme histochemistry (also called catalytic histochemistry) is a method used to detect the activity of enzymes in the cells or tissues of an organism. Currently, about 150 enzymes have been demonstrated in situ, i.e. about 1/10 of the total number of enzymes. The very principle of catalytic activity consists of two steps.

The first step is the *histochemical reaction itself: tissue with enzyme + substrate = product*.

The second step is the "**visualization reaction**": a colored and insoluble compound is formed from the tested product of the first reaction.

In order for both of the above reactions to take place, certain conditions must be met.

- Preservation of enzyme activity
- Preservation of cell and tissue structure - cryostat sections (chemical fixation almost always reduces enzyme activity)
- pH environment
- Substrate in excess

We ensure these optimal conditions using an incubation environment that has the following components:

1. Substrate: it should be soluble in water at a pH optimal for the given enzyme and should have the ability to create a colored final product, and at the same time penetrate quickly enough into the physiological localization of the enzyme. If the reaction product is colorless, a reagent used to make it visible is added to the IP.
2. Activators: i.e. substances that increase enzyme activity
3. Inhibitors: i.e. substances that reduce enzyme activity
4. Suitable buffer: ensures the necessary pH optimum (phosphate, citrate, tris-maleate, acetate, etc.)

First, we offer the enzyme a suitable substrate:

Enzyme	Function
oxidoreductases	they catalyze oxidation or reduction reactions
transferases	they transfer chemical groups from one mlk to another
hydrolases	cleaves ester, glycosidic and peptide bonds in the presence of water
phosphatases	various phosphates <ul style="list-style-type: none">■ glycerol phosphate (obsolete)■ α-Naphthyl phosphate (or substituted)■ BCIP
dehydrogenases	hydrogen splitting oxidizable substrate
peroxidases	hydrogen peroxide
esterases	hydrolyzable ester
glycosidases	glycosidic bond (sugars, glycoproteins, glycolipids)
sulfutases	sulfoesters
nucleotidsaes	nucleotide chain
proteases	protein or peptide
ligases	they catalyze synthetic reactions with the simultaneous splitting of ATP.

Then we make the reaction product visible by reacting with a chromogen - **visualization reaction** :

Precipitation with metal cations	the formation of a colored insoluble salt Pb, Co
Simultaneous azocopulation	the product (naphthol) is converted to an azo dye
Indigogenic reaction	cleavage to indoxyl and its oxidation to indigo
Tetrazolium method	reduction of tetrazolium salt to colored formazan
Peroxidase reaction	oxidation of DAB (diaminobenzidine)

The execution itself

- The test is performed on free sections (on a glass slide or in tiny Petri dishes in an incubation solution) or on sections of adhesions stuck to a glass slide.
- Incubate at 37°C or room temperature. The length of incubation is from a few minutes to two hours

(depending on the amount of enzyme in the tissue and the sensitivity of the method)

- Fixation:
 - fresh cryostat sections from unfixed tissue
 - sections from fixed specimens
 - sections prepared by lyophilization
- Each histochemical certificate must be supplemented with a control experiment and inhibition tests.

Control experiment : serves to verify the results and is performed simply by omitting the substrate in the incubation solution.

Inhibition tests : are indicated in cases where the same substrate is attacked by several enzymes simultaneously.

Links

References

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