APPLICATION OF PEROXIDASE-LABELED ANTIBODY TO IMMUNOCYTOCHEMISTRY

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Enzyme and antibody conjugated with bifunctional reagents retains enzymatic and immunologic activity and serves excellently for light and electron microscopic localization of antigens. This system is highly sensitive because of an amplifying effect. Enzyme is not consumed in the reaction with the substrate and each molecule of enzyme-labeled antibody bound to the antigenic site deposits many molecules of reaction product.

Horseradish peroxidase was chosen as a marker enzyme since it was available from commercial firms in relatively pure form, and the histochemical reactions for light and electron microscopic studies had been developed. Moreover, the peroxidase has a relatively small molecular weight of 40,000 and the endogenous localization of a peroxidase is limited to certain well defined sites. The conjugates of peroxidase and antibody are prepared using p,p'-difluoro-m,m'-dinitrodiphenyl sulfone as a bifunctional reagent. The indirect immunohistochemical method was usually employed. Tissues were fixed briefly, reacted with rabbit antisera, and followed with reaction with peroxidase-labeled sheep anti-rabbit gamma globulin. The peroxidase was localized histochemically, thus marking the site of antigens.

At the light microscopic level, two or three different antigens may be localized in a single histologic section. For this, the first antigen was localized by the indirect method and the antisera removed from the section by elution, leaving the colored reaction product identifying the antigenic sites. The second and subsequent antigens were localized similarly, using substrate that developed reaction products of a different color.

At the ultrastructural level, three different methods have been used to localize antigens:

1. Thin slices of tissue were fixed briefly, washed in phosphate buffered saline (PBS) with 10% DMSO and frozen sectioned 20–30 µ in thickness. DMSO protected the tissues from damage caused by freezing and improved preservation. Sections were reacted with rabbit antisera, washed in PBS, reacted with peroxidase-labeled sheep anti-rabbit gamma globulin and washed. The sections were postfixed in 5% glutaraldehyde, washed, and histochemically stained for peroxidase using diaminobenzidine (DBA) and H2O2 as substrates. The stained sections were osmicated, dehydrated, embedded in Epon, and processed for electron microscopy.

2. Localization of some tissue antigens on ultrathin sections: Tissues were fixed, dehydrated, and embedded in either methacrylate or Epon. Ultrathin sections on the tissues were exposed to xylene saturated water when they were
embedded in methacrylate and to 10% hydrogen peroxide when they were embed-
ded in Epon. The ultrathin sections were placed on a droplet of specific rabbit
antisera, washed, and exposed to peroxidase-labeled antirabbit gamma globulin.
The sections were stained histochemically using 4-Cl-1-naphthol and H2O2 as
substrates and were examined with an electron microscope. The antigenic sites
were marked with electron dense reaction product of peroxidase.

3. Localization of tissues embedded in polyethylene glycol. The tissues were
fixed and dehydrated either in polyethylene glycol or alcohol. Tissue sections,
5–8 μ in thickness, were either mounted on a glass slide using either 0.1 to 1%
gelatin, air-dried, and dipped in 1% gelatin to insure their adhesion, or were floated
on 5% glycerol and mounted on glass slides. The sections mounted with gelatin
were then dipped in 0.1% trypsin in order to remove some gelatin so that antisera
would gain access to the antigenic sites. The sections were reacted with rabbit
antisera and followed with a reaction with peroxidase-labeled anti-rabbit gamma
globulin. The sites of peroxidase were localized using DAB and H2O2. The
stained sections were examined briefly with the light microscope, photographed if
needed, and when desired areas were present, they were osmicated and dehydrated
in a graded ethanol series. A gelatin capsule filled with Epon was inverted on top
of the stained section, and the Epon was polymerized. The slides attached to
capsules were then dipped into liquid nitrogen and the capsule bearing the section
was separated from the slide. The Epon block was trimmed to the desired area,
ultrathin sections were obtained and examined with an electron microscope.

Using these methods, various hormones in the anterior pituitary gland and islets
of pancreas, basement membrane in parietal yolk sac and kidney, and immuno-
globulin and the complements in human kidney biopsies were localized.

REFERENCES


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