EFFICIENT HISTOCHEMICAL METHODS FOR THE DETECTION OF ACIDIC GLYCOCONJUGATES BY MEANS OF SENSITIZED DIAMINE PROCEDURES

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Diamine methods\(^1\) are known to be one of the most sensitive histochemical staining methods for the detection of acidic glycoconjugates in light microscopy. With such methods, however, it is difficult to visualize histological structures containing extremely small amounts of acidic glycoconjugates such as the basement membrane and intracellular organelles. Davies et al.\(^2\) developed electron microscopical Gram stainings by means of combined potassium trichloro(ethylene) platinum (KTPt) and crystal violet. KTPt is known as an anionic compound containing platinum and to form water-insoluble complexes in combination with basic dyes\(^2\). If KTPt containing nuclei available for physical development is used for sensitizing the diamine techniques, more sensitive histochemical staining methods can be developed for the detection of acidic glycoconjugates. In the present study, we succeeded in establishing such sensitive staining methods.

MATERIALS AND METHODS

A series of organs such as the salivary glands, stomach, colon, kidney, lung and trachea were dissected out from adult male Wistar rats. Blocks of these organs were fixed in either Carnoy's fluid or 25 % acetic acid in ethanol at 4 °C for 18-24 hr, dehydrated in 100 % ethanol, cleared in xylene and embedded in paraffin wax. Sections were cut at a thickness of 4 μm and affixed to glass slides. Dewaxed and hydrated sections were stained by the high (HID) or low (LID) diamine method\(^1\) at 37 °C for 1-2 hr, incubated in boric acid (Clark-Lubs) buffered (pH 8.0) 0.5 mM KTPt solution at room temperature for 1 hr, immersed in 0.5 % sodium borohydride solution at room temperature for 1 min and physically developed by means of a modified method of Nakamura et al.\(^3\) (PD) at 20 °C for 4-8 min. Prior to staining, some hydrated sections were subjected to either digestions with enzymes (chondroitinase ABC, testicular hyaluronidase or neuraminidase)\(^4\) or chemical modification (nitrous acid degradation)\(^5\).

RESULTS AND DISCUSSION

When tissue sections were stained by the present methods, acidic glycoconjugate-containing structures such as mast cell granules, acinous mucins in
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sublingual and broncho-tracheal glands, surface and glandular mucins in
gastro-intestinal tract (Fig. 3) and trachea, basement membranes in renal
glomerulus (Fig. 7) and other tissues, lamina propria connective tissues in
gastro-intestinal tract and trachea and septal connective tissues in salivary
glands were stained distinctly in black or brown shades. According to the
results obtained by the sensitized diamine methods in combination with enzyme
digestions (Fig. 4) or chemical modification (Fig. 8), the specificity,
reliability and utility of the present methods were sufficient.

Figs.1-4: Stomach, X 50, (Fig.1 LID stained, Fig.2: KTPt-PD stained, Fig.3: LID-KTPt-PD stained, Fig.4: neuraminidase digestion-LID-KTPt-PD stained).
Figs.5-8: Kidney, X 100, (Fig.5: HID stained, Fig.6: KTPt-PD stained, Fig.7: HID-KTPt-PD stained, Fig.8: nitrous acid degradation-HID-KTPt-PD stained).

REFERENCES