EVALUATIONS OF DIGESTION METHODS WITH PROTEOGLYCAN-DEGRADING ENZYMES
IN LIGHT MICROSCOPY
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It is known that a series of factors have various effects on both the precision and reproducibility of the results obtained by enzyme digestion procedures in histochemical systems. These factors involve, for instance, the conditions of enzyme digestion, the methods of tissue preparation and the sensitivity of staining methods for detecting the enzyme substrates. The sensitized diamine methods recently established by us for the precise detection of acidic glycoconjugates have not necessarily been very successful, when these were used in combination with digestion procedures using proteoglycan-degrading enzymes. In the present study, attempts have been made to disclose the most favorable conditions for some enzyme digestions followed by sensitive staining methods.
MATERIALS AND METHODS
Buffered formalin-fixed mandibular and knee-joint regions from adult male SD rats (13 weeks) were routinely decalcified, dehydrated, cleared and paraffin-embedded. Dewaxed and hydrated sections (4 μm thick) were subjected to the high iron diamine (HID)-potassium trichloro(ethylene) platinum (KTP)-sodium borohydride (BH)-physical development (PD) staining sequence reported previously. Prior to the staining, hydrated sections were digested with proteoglycan-degrading enzymes as follows: 1) Chondroitinase (Chase) ABC (protease free) digestion [0.5 or 2.0 U/ml of 0.1 M Tris-HCl buffer (pH 8.0) containing 0.03 M sodium acetate at 37 °C for 6 hr or twice for 3 hr each time]. 2) Chase B digestion [0.5 U/ml of 0.1 M Tris-HCl buffer (pH 8.0) at 30 °C for 3 hr] followed by Chase ACII digestion [2.0 U/ml of 0.1 M Tris-HCl buffer (pH 7.3) containing protease inhibitors at 37 °C for 3 hr]. 3) Chase ACII digestion followed by Chase B digestion [the reverse sequence of 2]. 4) Chase B digestion followed by testicular hyaluronidase (T-Hylase) digestion [1 mg/ml of 0.1 M phosphate buffer (pH 5.5) at 37 °C for 3 hr]. 5) T-Hylase digestion followed by Chase B digestion [the reverse sequence of 4].
RESULTS AND DISCUSSION
1) Digestion with Chase ABC: In the connective tissues densely packed with isomeric chondroitin sulfates (ChS), the effects of the single digestion with
Hirabayashi Y et al: Studies on enzyme digestions
the high enzyme concentration (2.0 U/ml) upon the stainings were nearly comparable to those of two digestions. In similar tissues, the single digestion with the low enzyme concentration (0.5 U/ml) apparently had less effect upon the stainings than the two digestions with the same enzyme concentration (Figs. 1-3). The use of higher concentrations of enzymes is to be recommended so as to obtain the true effects of the digestions upon the stainings in such tissues as examined here.

2) Combined digestion with Chase B and Chase ACII: In ChS-B rich tissues, the digestion with the Chase B-Chase ACII sequence was much more effective than that with the Chase ACII-Chase B sequence in terms of the suppression of the sensitized diamine stainings (Figs. 4-6). Since this finding indicates that ChS-B inhibits Chase ACII activity, the sequence Chase B-Chase ACII is to be preferred to the reverse sequence of the digestions.

3) Combined digestion with Chase B and T-Hylase: In ChS-B rich tissues, the digestion sequence Chase B-T-Hylase did not have significantly different suppressive effects upon the stainings from T-Hylase-Chase B. In view of this, T-Hylase is a better counterpart of Chase B than Chase ACII, in case such combined digestion procedures are to be performed.

Figs. 1-3: Epiphyseal cartilage (x200). Buffer control (Fig. 1), digestion with 0.5 U/ml of Chase ABC (6 hr: Fig 2, twice for 3 hr: Fig. 3). Figs. 4-6: Oral mucosa (x 400). Buffer control (Fig. 4), Chase B-Chase ACII digestion (Fig. 5), Chase ACII-Chase B Digestion (Fig. 6).

REFERENCES