On the Electrophoretic Separation of Acid Mucopolysaccharides on Cellulose Acetate Sheets

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The mucopolysaccharides were prepared from guinea pig skin using papain hydrolysis and studied by electrophoresis in "Oxoid" cellulose acetate sheets (barbiturate buffer, pH 8.6, ionic strength 0.125, voltage gradient 10—15 V/cm). Fig. 1—S shows the four fractions which stained with Alcian Blue (1 % aqueous solution) at pH 4 and 1. Chondroitin sulphate and hyaluronic acid were identified by comparison with purified substances. The small fraction moving behind the chondroitin sulphate is presumably heparitin sulfate.[1] The fourth fraction with slowest migration rate has not been described earlier. It stains metachromatically with toluidine blue (0.1 % aqueous solution), but it is P.A.S. negative.

After removal of the neutral mucopolysaccharides,[2] the acid mucopolysaccharides were fractionated in a cellulose column using cetylpyridinium chloride (CPC) according to the principle of Scott.[3] The elution was carried out consecutively with 0.1 N, 0.25 N and 1.5 N $MgCl_2$ solutions. Fig. 1 shows also the electrophoretic patterns of these fractions.

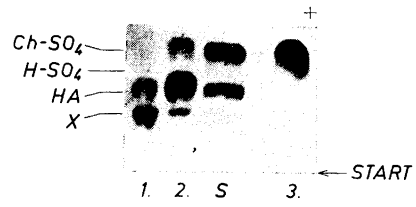

Fig. 1. The electrophoretic pattern of the acid mucopolysaccharides from guinea pig skin after fractionation by cetylpyridinium chloride (CPC), stained with Alcian Blue. 1. CPC-precipitate eluted with 0.1 N $MgCl_2$; 2. CPC-precipitate eluted consecutively with 0.25 N $MgCl_2$; 3. CPC-precipitate eluted consecutively with 1.5 N $MgCl_2$; S = the unfractionated sample; Ch-SO_4 = chondroitin sulfate; H-SO_4 = heparitin sulfate (faint); HA = hyaluronic acid; X = "0.1 N $MgCl_2$-fraction".

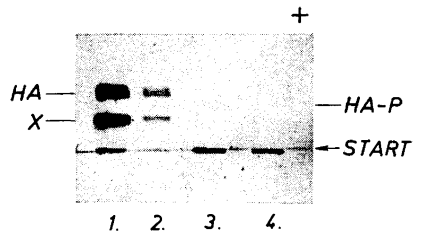

Fig. 2. Effect of papain-pretreatment on the liberation of the mucopolysaccharides from the vitreous body. 1 and 2 = mucopolysaccharides from papain-hydrolysed vitreous body material (in two concentrations); 3 and 4 = nontreated vitreous body material (in similar two concentrations as 1 and 2); HA = hyaluronic acid; X = "0.1 N $MgCl_2$ fraction"; HA-P = complex carbohydrate fraction of the unhydrolysed vitreous body.

We have found the unknown "0.1 N $MgCl_2$-fraction" in the acid mucopolysaccharides of, *e.g.*, umbilical cord, vitreous body, and tendon after papain hydrolysis. The amount of this fraction in tendon is quite small in relation to the chondroitin sulphate and the hyaluronic acid. The significance of the treatment with papain is shown in Fig. 2. Vitreous body mucopolysaccharides, prepared with ethanol precipitation from the papain hydrolysate, show in electrophoresis two bands, whereas from the native vitreous body material only one small fraction moves in the same conditions at all, but most of the matter remains at start (Fig. 2). Of the two fractions, which are liberated by papain, the faster migrates as hyaluronic acid. The slower fraction was shown to correspond to the mentioned "0.1 N $MgCl_2$-fraction".

Work is in progress for the further characterization of this hitherto unknown fraction.

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