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Preliminary communication

A previously unreported, mammalian, sulphated glycosaminoglycan

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Keratan sulphates (KSI and KSII) differ from the other classes of sulphated glycosaminoglycans (GAGs) in that they contain no uronic acid and are also associated, rather specifically, with corneal and cartilaginous tissues, respectively. The occurrence of a uronic acid-free GAG (which seems to be related structurally to the keratans) in material derived from intestinal mucosa was therefore not anticipated.

A batch of mixed GAGs rich in heparan sulphate (HeS), a by-product from the production of porcine mucosal heparin and having a low content of peptide and nucleic acid, has been used for the isolation of HeS fractions[1]; in separating the least-sulphated components, a previously unreported species of GAG has been isolated. Heparin (Hep), most of the HeS, and some dermatan sulphate (DeS) had been precipitated as barium salts by 2-propanol (45%), some condroitin 4-sulphate (C4S) was then precipitated as the calcium salt with 18% ethanol, and the residual GAG was incubated with testicular hyaluronidase[2] and chondroitinase ABC[3] until there was no further decrease in non-dialysable 2-amino-2-deoxygalactose. The product was adsorbed on to a column of IRA-904 (Cl^-) resin and eluted successively with 0.1, 1.0, and 2.0M NaCl. The bulk of the material was eluted with M salt and contained nearly equal amounts of 2-amino-2-deoxyglucose and 2-amino-2-deoxygalactose[4,5].

The presence of 2-amino-2-deoxygalactose-containing polysaccharide in the eluate that was resistant to chondroitinase ABC was unexpected. There was some evidence for heterogeneity during electrophoresis on cellulose acetate, but no clear separation with the buffer systems then employed[6]. The results suggested that an unknown GAG related to DeS might be present, and an alkaline copper precipitation[7] (generally regarded as specific for DeS) gave the expected kind of gelatinous precipitate, leaving some HeS in the supernatant solution. However, the precipitate still contained considerable amounts of 2-amino-2-deoxyglucose. When the precipitation was repeated, the ratio of 2-amino-2-deoxyglucose to 2-amino-2-deoxygalactose in the product remained unaltered. The polysaccharide was converted into the sodium salt, $[\alpha]_D^{24}$ $-4°$ (water), for further investigation.

The circumstances of the isolation of this polysaccharide indicated it to be a previously unrecognised species of GAG, and in electrophoresis on cellulose acetate it migrated as a single band in five different buffer systems[6]; the results for two buffer systems are shown in Fig. 1. Occasionally, there was a little "tailing", which is not unexpected for polymers of high molecular weight in this medium. Fig. 2 shows a two-dimensional

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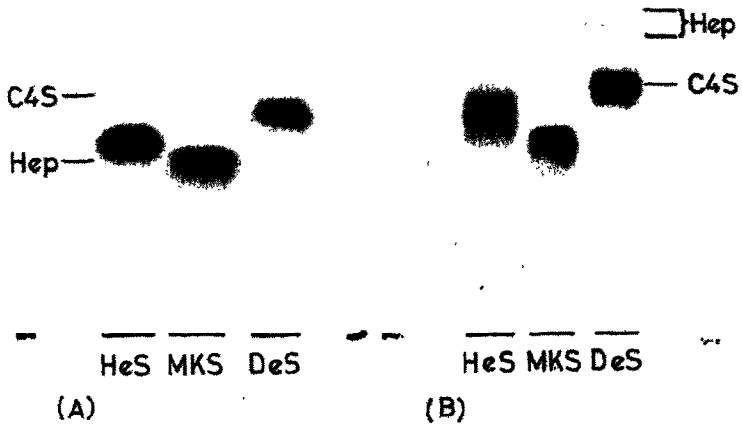

Fig. 1. Electrophoresis on cellulose acetate of GAGs isolated from porcine, intestinal, mucosal polysaccharides in A, 0.05M barium acetate (pH 5); B, 0.12M sodium phosphate (pH 2). The HeS is the low-sulphated material which accompanied the mucokeratan sulphate (MKS) up to the stage of precipitation of the copper salt. The migration distances of other GAGs under the same conditions are indicated; most heparin samples give double bands in buffer B.

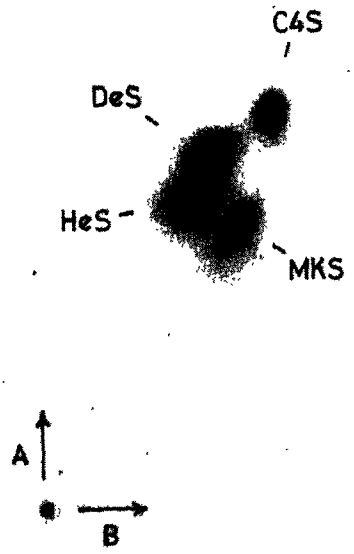

Fig. 2. Two-dimensional electrophoresis on cellulose acetate of a mixture of the GAGs in Fig. 1 plus C4S; A, 0.05M barium acetate (pH 5); B, 0.08M chromic acetate + 0.03M dipropylenetriamine acetate (pH 5).

electrophoretic pattern, using for the second run a buffer which is the best so far found for separating this GAG from its accompanying HeS. Gel-permeation chromatography on Sepharose 4B (elution with 0.3M NaCl) gave a broad distribution centred at K_{av} 0.57, which corresponds to a mean molecular weight of ~100,000, with that of some material approaching 1,000,000. Conductimetric titration[8] gave a sulphate content of 1.1 mequiv./g, and also demonstrated the absence of carboxylate groups; the content[9] of uronic acid was <0.02 mequiv./g. The hexosamine content[10] was 1.87 mequiv./g, of which 60% was 2-amino-2-deoxygalactose and 40% 2-amino-2-deoxyglucose. The principal neutral sugar was galactose, and some fucose[11] was present. Glucose and mannose appeared to be absent, and there was little if any xylose. The total content of amino acid appeared to be <3%; interference from the products of breakdown of hexosamine makes the determination of small amounts of amino acids in GAGs difficult[12].

The i.r. spectrum (KBr pressing, 650–1800 cm^{-1}) of the new GAG differed significantly from those of the sulphated glycosaminoglycuronans, notably in the region 1300–1500 cm^{-1}, and also in the absence of carboxylate absorption (near 1600 cm^{-1}) between the two prominent amide bands. However, a close resemblance, in the region 800–970 cm^{-1}, to the spectrum of chondroitin 6-sulfate suggested some of the hexosamine to be 6-sulphated[5] (perhaps the 2-amino-2-deoxygalactose, which is present in an amount approximately equivalent to that of the sulphate). The analytical and preparative procedures have been discussed elsewhere[5].

Many of the characteristics of the new GAG point to an affinity with the keratan sulphates, but the high content of 2-amino-2-deoxygalactose and molecular weight contrast strongly with those of KSI and KSII. 2-Amino-2-deoxygalactose occurs only in KSII (in the protein linkage sequence), and both keratans, even in the undegraded forms, have mean molecular weights nearly an order of magnitude lower. The new GAG (mucokeratan sulphate*) appeared to constitute ~1% of the GAG mixture as supplied to us; the proportion of the total mucosal GAGs and the cellular origin of mucokeratan sulphate are unknown.

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*Mucokeratan sulphate is distinct from both KSI and KSII by electrophoresis and by i.r.