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# X-Ray-Diffraction Patterns from Chondroitin 4-Sulphate, Dermatan Sulphate and Heparan Sulphate

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Ordered conformations from the sodium salts of chondroitin 4-sulphate, dermatan sulphate and heparan sulphate were observed by X-ray diffraction. Chondroitin 4-sulphate shows similar threefold helical character to that previously reported for chondroitin 6-sulphate and hyaluronates. Dermatan sulphate forms an eightfold helix with an axial rise per disaccharide of 0.93 nm, which favours the L-iduronic acid moiety in the normal C1 chair form. The layer-line spacing and axial projection in heparan sulphate of 1.86 nm favours a tetrasaccharide repeat with glycosidic linkages alternating β-D-(1→4) and α-D-(1→4).

We have obtained X-ray fibre-diffraction patterns from the sodium salts of chondroitin 4-sulphate, dermatan sulphate and heparan sulphate. The results, together with those obtained previously for chondroitin 6-sulphate (Atkins *et al.*, 1972*a*) and the hyaluronates (Atkins *et al.*, 1972*b*), have enabled us to make an initial comparison between molecular conformations.

Oriented films of all three polysaccharide preparations were obtained by drying down aqueous solutions of the sodium salts on clean glass slides. The films were crystallized in a stress field at high relative humidity in a manner described in some detail by Atkins *et al.* (1972*b*).

## *Materials*

The chondroitin 4-sulphate was a preparation from bovine nasal septa with a $\bar{M}_w$ of 25300 and a $\bar{M}_n$ of 20800 (Wasteson, 1969). It contained one sulphate group per disaccharide unit. Heparan sulphate and dermatan sulphate were the preparations described by Iverius (1971) as HS II and DS III. Preparation DS III had a $\bar{M}_w$ of 41500, a $\bar{M}_n$ of 32600 and 1.1 sulphate groups per disaccharide unit. Preparation HS II had a $\bar{M}_w$ of 58300, a $\bar{M}_n$ of 40500 and contained 0.46 sulphate group per disaccharide unit. Recent analyses on preparation HS II (M. Höök, U. Lindahl & P. H. Iverius, unpublished work) have shown that L-iduronic acid constitutes 19% of the total uronic acid content of the preparation, the remainder being D-glucuronic acid. The latter units occur largely in block structures, containing *N*-acetylated D-glucosamine residues and essentially no ester sulphate groups. A major portion of the L-iduronic acid is confined to sections containing both *N*-sulphate and ester sulphate residues.

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## *Results and discussion*

*Chondroitin 4-sulphate.* The X-ray pattern shown in Plate 1(*a*) shows discrete layer lines with a spacing of 2.85±0.03 nm with meridional reflections occurring on layer lines 3, 6 and 9. These reflexions, similar to those found for the sodium salt of chondroitin 6-sulphate (Atkins *et al.*, 1972*a*) and sodium hyaluronate (Atkins & Sheehan, 1972), may be interpreted as orders of the projected disaccharide repeat of 0.95 nm. These X-ray results are consistent with three disaccharide units per complete turn in the 2.85 nm repeat. Bettelheim (1964) obtained partially oriented films for calcium chondroitin 4-sulphate and observed meridional arcs at spacings 0.98 and 0.327 nm, which were assigned as first and third orders of the identity period. The first rho line occurs at a spacing of 1.19 nm, with further, more diffuse, rho lines at wider angles. The quality of the X-ray photograph is not sufficiently good to establish the precise packing of the chains. The pattern is critically sensitive to humidity, with indications of better order on increasing the relative humidity.

*Dermatan sulphate.* The X-ray pattern obtained from dermatan sulphate is shown in Plate 1(*b*) and is different in general features from that for chondroitin 4-sulphate. The layer-line spacings are orders of 7.44±0.08 nm, with meridional reflexions on layer lines 4, 8, 12 and 16, with the meridionals on layer lines 8 and 16 being noticeably more intense. The simplest interpretation of these results is an eightfold helix with an axially projected disaccharide repeat of 0.93 nm (eighth order of 7.44 nm). The fourfold pseudo character, which gives rise to the weak meridional reflexions on layer lines 4 and 12, is probably due to the packing of the chains.

Dermatan sulphate differs from chondroitin 4-sulphate in containing L-iduronic acid rather than D-glucuronic acid (for recent review see Fransson, 1970). The L-iduronic acid moiety is the C-5 epimer

of D-glucuronic acid, so that in the normal C1 chair form the carboxyl group at C-5 is axially positioned. It is of interest to consider in which of the two chair forms the L-iduronic acid exists. It could be in the alternative 1C chair form so that the carboxyl group is positioned equatorially and the glycosidic linkages become diaxial. Fransson (1970) has reviewed the evidence for and against particular chair forms of L-iduronic acid.

It is important to establish if a preliminary examination of the X-ray results is sufficient to distinguish between C1 and 1C chair forms, at least in these solid-state preparations. The maximum projected disaccharide repeat that is theoretically possible for dermatan sulphate with L-iduronic acid in the 1C chair form is 0.92nm. Consideration of stereochemically feasible molecular models decreases this value to approx. 0.90nm. This distance is less than the experimentally observed value of 0.93nm, even allowing for errors in measurement, and is therefore sufficient to rule out the 1C chair form for L-iduronic acid in these preparations.

*Heparan sulphate.* The detailed structure of heparan sulphate has not been established with the same degree of certainty as those of the galactosaminoglycuronans, but has been thought to involve D-glucuronic acid and D-glucosamine units joined by α-(1→4)-linkages. A more complex structure was suggested by the finding of Cifonelli & Dorfman (1962) that heparan sulphate contains L-iduronic acid in addition to D-glucuronic acid. Recent studies on the related polysaccharide heparin have shown that most, and possibly all, of the glucuronic acid residues have the β-anomeric configuration (Helting & Lindahl, 1971). Results of a preliminary qualitative study have also indicated the presence of β-D-glucuronic acid residues in heparan sulphate (M. Höök, U. Lindahl & P. H. Iverius, unpublished work).

The heparan sulphate preparation (HS II) used in the present investigation has few sulphate groups, which are unevenly distributed along the polysaccharide chain (see also Cifonelli, 1968). Since the presence of L-iduronic acid units is restricted to the sulphated portions of the polymer (see above), it is apparent that sections composed of alternating D-glucuronic acid and *N*-acetyl-D-glucosamine units occupy a major portion of the molecule.

The X-ray pattern obtained from heparan sulphate is shown in Plate 1(*c*). The layer-line spacing is 1.86nm, with a meridional streak on the first layer line. The maximum theoretical extension of a tetrasaccharide repeat corresponding to D-glucuronic acid and *N*-acetyl-D-glucosamine units having all α-D-(1→4)-glycosidic linkages is $4 \times 0.45 = 1.80$nm, and a stereochemically feasible molecular model is somewhat less than this value. Thus if we interpret the 1.86nm meridional reflexion to be the axial projection of a tetrasaccharide repeat, then it cannot be accounted for by all α-D-(1→4)-glycosidic linkages. A more plausible model that we tentatively offer is one with α-(1→4)(1*ax*→4*eq*)-D-glucosaminidic and β-(1→4)(1*eq*→4*eq*)-D-glucuronidic linkages respectively. We can hopefully expect that a structural refinement of the X-ray data will enable the correct model to be established.

The crystallization of these connective-tissue polysaccharides means that all the common connective-tissue polyuronides have now been crystallized. We expect that a more detailed study of the molecular architecture of these biopolymers will reveal much information both relative and pertinent to biological function and properties of this important group of substances.

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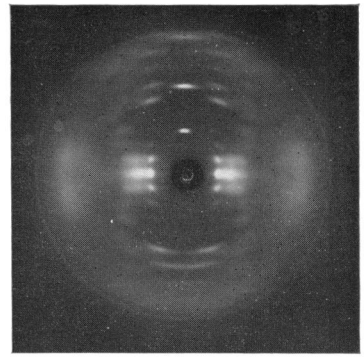
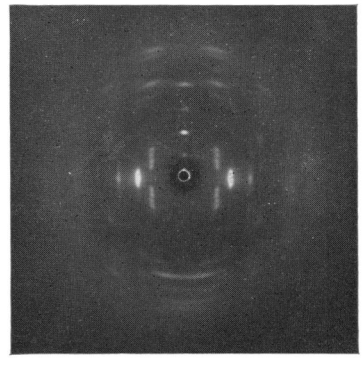
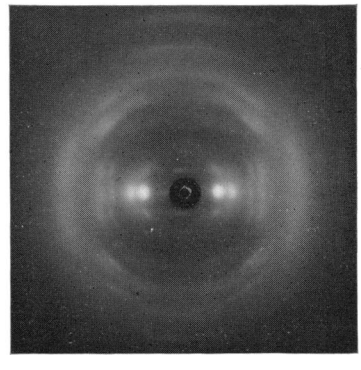
(*a*)



(*b*)



(*c*)



EXPLANATION OF PLATE 1

*X-ray fibre-diffraction patterns obtained from the polyuronides*

(*a*) Chondroitin 4-sulphate; (*b*) dermatan sulphate; (*c*) heparan sulphate. The molecular chain axis is vertical in each case.