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Molecular Conformation of Sodium Heparan Sulphate in the Condensed Phase

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By using the X-ray-diffraction results reported previously for sodium heparan sulphate, a twofold helical conformation with an axially projected disaccharide repeat (h) equal to 0.93nm has been examined in detail. On the basis of a repeating sequence of 1,4-α-D-glucosamine and 1,4-β-D-glucuronic acid, trial and stereochemically feasible molecular models were computer-generated. An optimum twofold helical conformation is proposed, incorporating stabilizing intra-chain hydrogen bonds across both glycosidic linkages.

Heparan sulphate is a glycosaminoglycan which was first isolated by Jorpes & Gardell (1948) from lung and liver. Dietrich & Monte de Oca (1970) have presented evidence for the widespread occurrence of this polymer among various cells, and in particular it has been reported that heparan sulphate is attached to the cell surface membrane of many cultured cells (Kraemer, 1971*a,b*; Chiarugi *et al.*, 1974). The polysaccharide chains are covalently linked to a protein (Jansson & Lindahl, 1970), and heparan sulphate proteoglycans have been described (Kraemer & Smith, 1974; Öbrink *et al.*, 1975).

Heparan sulphate is related structurally to the blood anticoagulant heparin, and consists of repeating disaccharide units of glucosamine and uronic acid. The uronic acid may be either D-glucuronic acid or its C-5 epimer L-iduronic acid. The relative uronic acid composition varies according to the source material, but for the particular sample used in these investigations the ratio of D-glucuronic acid to L-iduronic acid was nearly 4:1 (Höök *et al.*, 1974). The glucosamine is partly *N*-sulphated and partly *N*-acetylated and also contains some sulphate ester groups. The D-glucosamine and L-iduronic acid residues have the α-1,4 configuration, whereas recent chemical evidence favours the β-1,4 configuration for the D-glucuronic acid (Helting & Lindahl, 1971; M. Höök, U. Lindahl & P. H. Iverius, unpublished work; Höök, 1974).

X-ray diffractions of ordered molecular conformations in the condensed phase for sodium heparan sulphate were reported by Atkins & Laurent (1973). Their results favoured an alternating sequence of α-1,4-D-glucosamine and β-1,4-D-glucuronic acid, both residues existing in the energetically favourable $^4C_1$ chair. The X-ray fibre-diffraction pattern has a layer-line spacing of 1.86nm with meridional reflexions on even layer lines, giving a value for the axially projected disaccharide repeat (*h*) of 0.93nm (Nieduszynski & Atkins, 1975; Atkins & Nieduszynski, 1975). In addition, a meridional streak is observed on the first layer line (Atkins & Laurent, 1973) which could be accounted for by an irregular distribution of sulphate appendages on the glucosamine residues, the backbone remaining a regular twofold helix.

It might be thought that the 20% iduronate residues are also distributed along the polymeric backbone, replacing glucuronate residues in some irregular manner and making the idealized covalent repeat even more difficult to define. However, recent X-ray evidence argues in favour of a concentrated domain consisting of iduronate residues alternating with glucosamine (Atkins & Nieduszynski, 1976*a,b*). A summary of these results is included here. On formation of the calcium salt of the same heparan sulphate preparation as discussed above, a new X-ray fibre pattern emerges, identical with that obtained from calcium heparin. These results suggest that this heparan sulphate preparation (Höök *et al.*, 1974) also contains polydisaccharide regions formally similar to heparin. Independent evidence from chemical degradation methods also supports the presence of a trisulphated disaccharide that is apparently identical with the repeating sequence of heparin (Hovingh & Linker, 1974). It would appear that the preparation is a mixture of a 'heparan sulphate-like' phase and a 'heparin-like' phase and that the former crystallizes preferentially in the sodium-salt form.

In generating possible helical models consistent with the X-ray-diffraction results for sodium heparan sulphate, we feel justified in needing to examine only polysaccharide models based on the covalent repeat shown in Fig. 1(*a*). Other models with β-D-glucuron-

ate in the alternate $^1C_4$ chair or incorporating α-D-glucuronate (Arnott *et al.*, 1975) will not be considered.

Chemical Structure and Geometry at the Glycosidic Linkages

The three-dimensional arrangement and dimensions of the basic disaccharide repeat unit of heparan sulphate are illustrated in Fig. 1. Such a conformation gives rise to an alternating sequence of 1 eq → 4 eq* and 1 ax→4 eq glycosidic linkages.

Linkage (1): *β-D-GlcA-(1→4)-α-D-GlcNAc*

At this linkage only one hydrogen bond, the GlcA-$O_{(5)}\cdots$H-$O_{(3)}$-GlcNAc with $O_{(3)}$ acting as donor, is possible in the conformationally allowed region (Fig. 2*a*). There is a similarity between this linkage in heparan sulphate and the 1→4 di-equatorial linkage in cellulose. It is noteworthy that in the latter there is evidence from infra-red experiments (Mann & Marrinan, 1958; Liang & Marchessault, 1959) for intra-molecular hydrogen bonds across these linkages between the $O_{(5)}$ and H$\cdots O_{(3)}$ atoms of adjacent residues.

Linkage (2): *α-D-GlcNAc-(1→4)-β-D-GlcA*

At this linkage, again, only one hydrogen bond is possible in the conformationally allowed region (Fig. 2*b*). This is GlcNAc-N-H$\cdots O_{(3)}$-GlcA, with the N acting as donor. To form a linear hydrogen bond it is necessary to rotate the amide group by about 40° in a clockwise direction, when viewed along the $C_{(2)}$–N bond, from the normal *trans* planar configuration (Johnson, 1966); the departure from linearity is then 7°. It is noteworthy that there is a similar rotation of the amide group in the fully extended threefold helical model of hyaluronic acid proposed by Winter *et al.* (1975).

There is a similarity between this linkage in heparan sulphate and the (1 ax→4 eq) linkage in amylose. Again it is noteworthy that there is evidence for a probable hydrogen bond between $O_{(2)}$ and $O_{(3)}$ atoms of two consecutive sugar residues in amylose (Sathyanarayana & Rao, 1972). In heparan sulphate, the $O_{(2)}$ oxygen atoms are replaced by nitrogen atoms in the hexosamine residues.

* eq, means the glycosidic bond is directed equatorially; ax, that it is axially directed.

Results and Discussion

A starting model for a conformational least-squares refinement was set up with backbone coordinates based on those of Arnott & Scott (1972) and side-group coordinates calculated by using bond lengths and angles from Ramakrishnan & Prasad

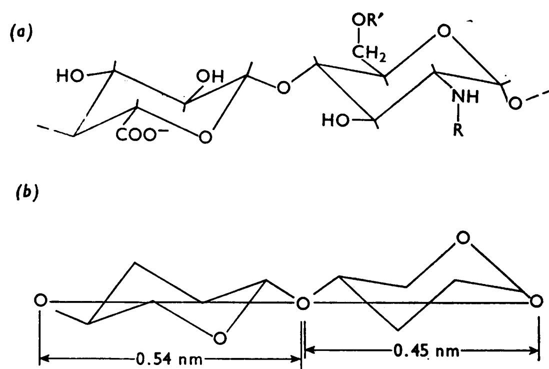

Fig. 1. *The disaccharide repeat for heparan sulphate used for the model-building trials*
(*a*) The three-dimensional arrangement: R is either $COCH_3$ or SO_3^-. R′ is usually H, but is sometimes SO_3^-; (*b*) The dimensions of this disaccharide repeat.

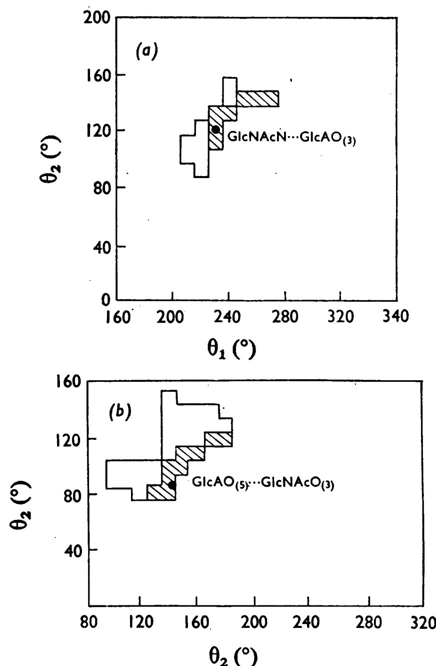

Fig. 2. *Conformational maps of the glycosidic linkages of heparan sulphate showing the stereochemically allowed regions and possible hydrogen bonds*
In each case the final position of our proposed model is marked (●). (*a*) α-D-GlcNAc-β(1-4)-D-GlcA linkage: $\theta_1 = C_{(2)}$-$C_{(1)}$-O-$C_{(4)}$; $\theta_2 = C_{(1)}$-O-$C_{(4)}$-$C_{(3)}$. (*b*) β-D-GlcA-α(1-4)-D-GlcNAc linkage: $\theta_1 = C_{(2)}$-$C_{(1)}$-O-$C_{(4)}$; $\theta_2 = C_{(1)}$-O-$C_{(4)}$-$C_{(3)}$.

(1972). This model was then refined to give a conformation that was consistent with the observed helical parameters, as obtained from the X-ray-diffraction pattern, and that allowed the formation of stabilizing hydrogen bonds across each of the two different glycosidic linkages. Before refining, model-building was used to assess whether the hydrogen bonds possible in the conformationally allowed regions were capable of forming linear hydrogen bonds, and in the refinement we used the criteria that the O···O distance should be between 0.26 and 0.29 nm. It was not found necessary to alter any of the backbone or side-group angles or lengths to obtain a satisfactory model, the only variables being the torsional and glycosidic-bond angles. Since we are using six conformational variables governing the backbone conformation, which are adjusted against only four constraints, it is not possible to generate a unique conformation, only an optimum one. An initial value of 116.5° was used for each glycosidic bond angle and the refinement changes this value to 117.8° (1eq→4eq) linkage and 117.6° at (1ax→4eq) linkage. The values of the torsional angles at each glycosidic linkage for this refined model are marked on the conformational maps of Fig. 2, and computer-drawn projections of the proposed helical structures are shown in Fig. 3. These drawings show model structures either totally *N*-acetylated (Fig. 3*a*) or totally *N*-sulphated (Fig. 3*b*).

As discussed above, the true structure is intermediate, with an irregular distribution of sulphate appendages. In addition, some ester sulphate groups will be present. These perturbations could account for the meridional streak on the first layer-line in the X-ray-diffraction pattern.

This resulting conformation contains no contacts which are less than the allowed inter-atomic contact distances as discussed by Ramachandran & Sasisekharan (1968). It is noteworthy that the values of the conformational angles at both the (1eq→4eq) and the (1ax→4eq) linkages are very similar to those found for cellulose (Gardner & Blackwell, 1974) and amylose (Sarko, 1975) respectively, adding support to the stability of the proposed conformation.

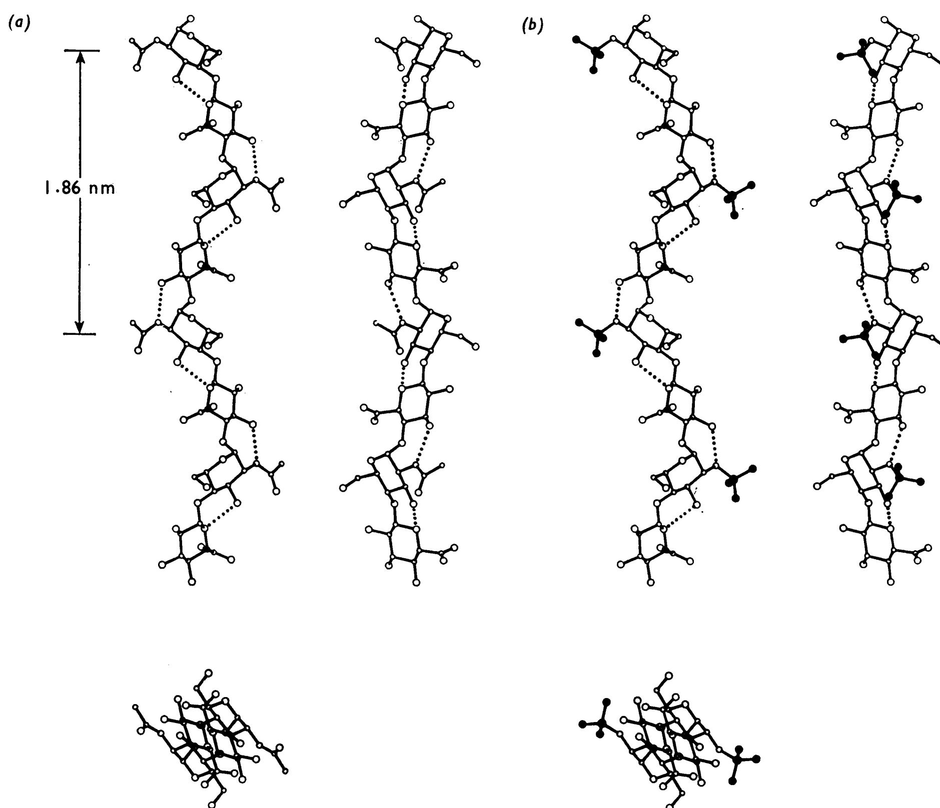

Fig. 3. *Computer-drawn projections perpendicular and parallel to the chain axis of the proposed model for sodium heparan sulphate*

(*a*) With no sulphate groups included; (*b*) with NSO_3^- groups on the glucosamine residues. Hydrogen bonds, both 0.275 nm, are indicated by dotted lines.

It must be remembered that this conformation only relates to that fraction of the molecule that crystallizes as the sodium-salt form. The measured helical repeat of 1.86nm is only 6% less than the theoretical maximum extension (Fig. 1*b*). The highly extended nature of the chain is in common with that observed for a series of other connective-tissue polyuronides (Atkins *et al.*, 1974) and microbial polysaccharides of the *Klebsiella* serotypes (Atkins *et al.*, 1977.)

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