Pure & Appl. Chem., Vol. 49, pp. 1135 - 1149. Pergamon Press, 1977. Printed in Great Britain.

CONFORMATIONS OF URONIC ACID CONTAINING POLYSACCHARIDES

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Abstract - Analyses of X-ray diffraction patterns obtained from ordered specimens of a variety of polyuronides have provided the opportunity to examine their molecular geometry in the condensed phase. A brief review is given of the simpler homopolysaccharide components of alginic acid, followed by the connective tissue polydisaccharides: hyaluronic acid, the chondroitin sulphates, dermatan sulphate, heparan sulphate and the blood anti-coagulant heparin. The interaction of these molecules with proteins and their molecular architecture in cartilage is mentioned. In addition some examples of the bacterial capsular polysaccharides are examined. In particular the three serotypes K5, K57 and K8 from Klebsiella.

INTRODUCTION

During the past five years notable advances have been reported concerning the molecular geometry of the uronic acid containing polysaccharides (polyuronides). The results obtained encompass the plant, animal and microbial domains with corresponding increase in the complexity of the repeating chemical sequences. Progress in this area has emanated from the development of crystallization techniques, involving stress fields and annealing (Ref. 1, 2 & 3), to provide oriented, para-crystalline samples suitable for the application of X-ray diffraction.

X-ray diffraction methods are the central stereobate for the elucidation of the three-dimensional shape and structure of molecules. The most detailed information is obtained when the material is crystalline and in the case of long chain polymeric substances with essentially regular repeating chemical sequences, such as the polysaccharides discussed below, the best that can be usually achieved is to induce the molecular chains to orient in approximately parallel register and then to encourage disciplined molecular inter-connections between neighbouring chains (Ref. 1 & 2). Such an array is analogous to the molecular morphology of many natural and synthetic fibres. Indeed the study of the X-ray diffraction of fibres and in particular the polysaccharides is almost as old as the X-ray diffraction of crystals. It is with some considerable historical interest to recall, especially with regard to the venue of this Symposium, that as early as 1913, in Tokyo, Nishikawa and Ono (4) passed X-rays through the fibrous cellulosic substance asa - a kind of hemp.

Figures 1, 6, 8, 15, 17 & 27 are examples of X-ray diffraction patterns obtained from polysaccharide fibres and oriented films. All the patterns exhibit diffracted intensity confined to (approximately) horizontal layer lines which reflects the periodic nature along the chain direction. Thus measurement of the layer line spacing gives the pitch of the helix. Further, for a helix with n units in one turn (i. e. per pitch (p)), diffracted intensity on the meridian (the vertical bisector of the pattern) only occurs on layer lines whose integer index l is a multiple of n. The quotient p/n yields the axial projection (h) which corresponds to the chemical repeat. Models of molecular helices incorporating the known stereochemistry of the various saccharide units, together with the glycosidic linkage geometries, can be computer generated while preserving the helix symmetry and dimensions as defined by the values of n and h. Additional information about the molecular structure involves the use of the full intensity data in a high resolution X-ray diffraction pattern together with more sophisticated chain packing procedures.

In this contribution a brief review is given of the progress to date of the plant polysaccharide alginates, the uronic acid containing connective tissue polysaccharides and some examples of microbial capsular polysaccharides from the Klebsiella serotypes.

PLANT POLYURONIDES

Alginates

Alginic acid (Ref. 5) is a polysaccharide preparation which can be obtained from brown algae and some bacteria. It is a copolymer and contains two building units, β-D-mannuronic acid (M) and α-L-guluronic acid (G), both of which are 1→4 linked and occur in domains or blocks of $(-M-)_n$, $(-G-)_n$, and $(-MG-)_n$ (Ref. 6). Their block composition is not constant but depends on the location in the plant and also varies widely from species to species according to such factors as season and geographical location. For example, Haug, Larsen and Baardseth (7) were able to show that alginic acid samples could be prepared with compositions ranging from 97% mannuronic acid, in the intercellular regions of Fucus and Ascophyllum species, to 27% mannuronic acid in the stripes of Laminaria hyperborea. The X-ray diffraction pattern will therefore be expected to depend on the composition of the particular alginic acid preparation, a fact first noticed by Frei and Preston (8) who also correlated the X-ray powder photographs of certain preparations with polymannuronic acid (M blocks) and polyguluronic acid (G blocks). Their observations explained how Astbury (9)(before guluronic acid was known to be a constituent) had been misled in trying to fit the structure of polymannuronic acid to the X-ray diffraction pattern of polyguluronic acid. More recently, Atkins, Mackie and Smolko (10) obtained X-ray diffraction patterns from oriented fibres of alginic acid of known composition and these enabled them to distinguish clearly the two extreme types of crystalline polymer sequence.

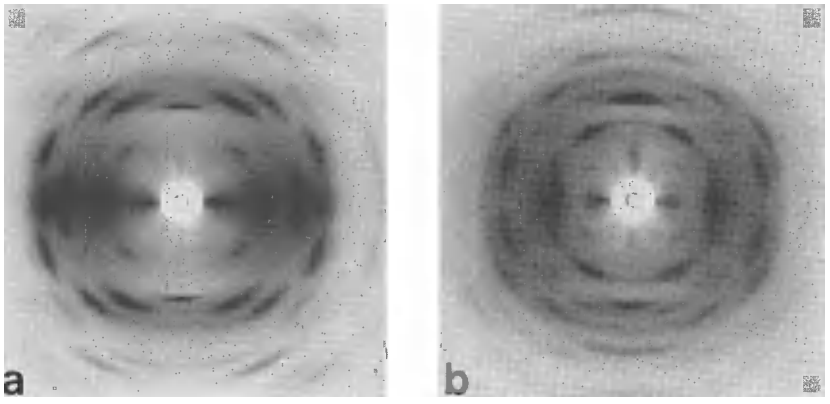


Fig. 1. X-ray diffraction patterns obtained from oriented fibres of (a) polymannuronic acid, and (b) sodium polymannuronate.

The X-ray fibre diffraction pattern from polymannuronic acid is shown in Fig. 1a. The layer line spacing is measured to be 1.04 nm and meridional reflections occur on layer lines with index $l = 2n$. The value for the axially projected repeat of 1.04/2 = 0.52 nm correlates with that expected for the β-D-mannuronic acid in its energetically favourable 4C1 chair and diequatorially linked (Fig. 2a). Highly extended two-fold helical models can readily be constructed and stabilized by an intra-molecular hydrogen bond O(5)--O(3), giving rise to a ribbon-like structure (Fig. 3a) similar to the three other β, 1→4 linked hexosans, cellulose (Ref. 11), chitin (Ref. 12) and mannan (Ref. 13). However the

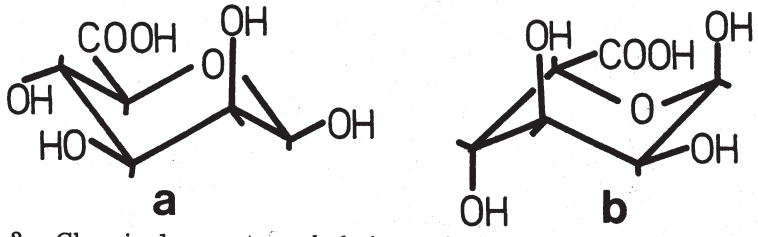


Fig. 2. Chemical repeats and chair conformations for (a) β-D-mannuronic acid, and (b) α-L-guluronic acid.

charges attendant on ionization form an electrostatic environment which modulates the backbone conformation. Thus on formation of the univalent salt the conformation becomes a three fold left-handed helix (Fig. 3b) similar to β, 1→4 xylan (Ref. 14). Since the glycosidic bonds are close to and nearly parallel with the helix axis, the angular distribution of side groups may be varied with little change in the axially projected monomer repeat. Thus although the fibre repeat alters drastically (Fig. 3b) to 1.51 nm the spacing of the meridional reflection 1.51/3 = 0.50 nm hardly changes (Fig. 1b). This is a feature that will also be noticeable for the connective tissue polysaccharides.

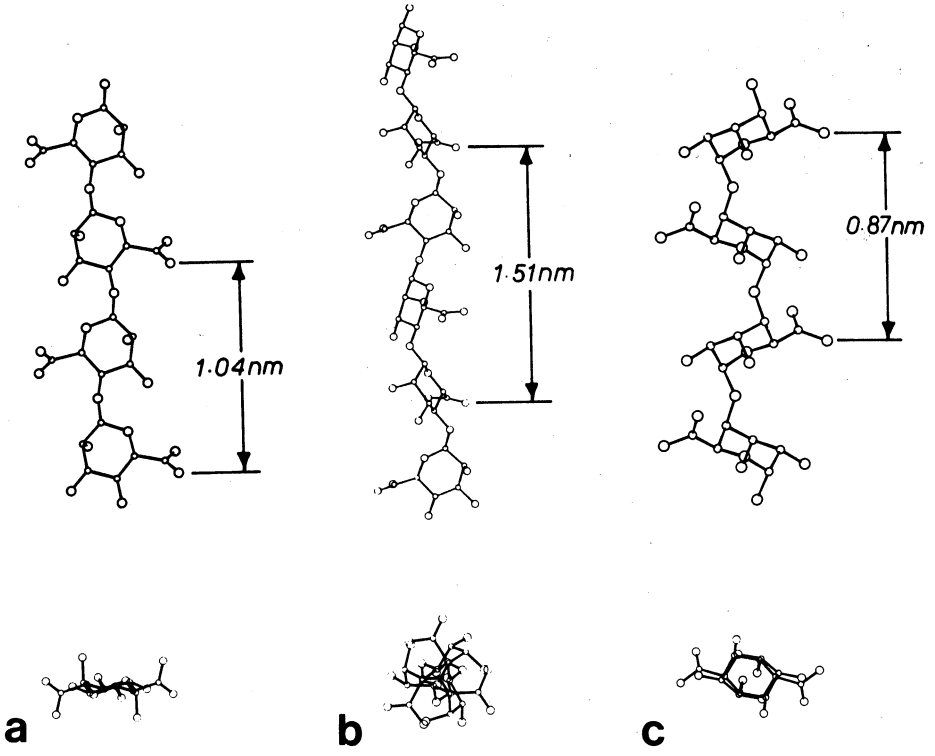


Fig. 3. Computer generated chain conformation of: (a) the two-fold polymannuronic acid helix, in directions perpendicular and parallel to the chain axis; and (b) corresponding projections for the three-fold sodium salt form. (c) Similar projections for the two-fold polyguluronic acid helix.

The X-ray fibre diffraction pattern of polyguluronic acid exhibits a layer line spacing of 0.87 nm with meridional reflections on even layer lines only. These dimensions and helical symmetry can be obtained by placing the α-L-guluronic acid residue in the alternate 1C4 chair conformation (Fig. 2b) so that we have a 1→4 diaxially linked polymer similar to sodium pectate (Ref. 15). The resulting polymeric shape (Fig. 3c) is substantially altered having a pronounced zig-zag molecular geometry. L-guluronic acid is obtained by C(5) epimerization of D-mannuronic acid and there is good evidence to suggest that this occurs at the polymer level (Ref. 16). The block-like structure in alginate enables the G-blocks to associate and provide junction zones for the well known gelation behaviour (Ref. 17). Further details of the crystalline structure of the alginate components have been reported previously (Ref. 18).

CONNECTIVE TISSUE POLYURONIDES

Hyaluronate, the chondroitin sulphates and dermatan sulphate are essentially poly-disaccharides with alternating 1→3 and 1→4 glycosidic linkages. Heparan sulphate and the blood anti-coagulant heparin also have a polydisaccharide backbone but the glycosidic linkages for these biopolymers are all 1→4.

Hyaluronates

Hyaluronic acid is a high molecular weight glycosaminoglycan (about 1,000,000 Daltons) found in soft connective tissues such as umbilical cord, synovial fluid and vitreous humour. The chemical and physical properties of hyaluronate have been discussed by Laurent (19). X-ray diffraction patterns obtained from oriented hyaluronate films have been analysed and molecular models constructed (Ref. 20-25). The first X-ray pattern of sodium hyaluronate suggested a highly extended three-fold helix with an axially projected repeat of 0.95 nm (Ref. 20) which correlates with the disaccharide repeat (Fig. 4) and a computer generated structure is illustrated in Fig. 5a. Recently a detailed structure refinement has been reported (Ref. 25). By changing the experimental conditions such as pH, ionic strength, relative humidity, etc. other distinct conformations have been observed. For example, on lowering the pH to below 2.5 a two-fold conformation is observed with a slight increase in the projected axial disaccharide repeat ($\underline{h}$) from 0.95 nm to 0.98 nm (Ref. 20). Also a highly extended four-fold helix with $\underline{h}$ = 0.93 nm has been reported (Ref. 22, 26 & 27), a model of which is shown in Fig. 5b. A further contracted form with a value of $\underline{h}$ = 0.84 nm (Ref. 21 & 22) was originally thought to be a double-helix (Ref. 21) but more detailed calculations have favoured a contracted single stranded conformation (Ref. 24). Typical X-ray diffraction patterns obtained from oriented hyaluronate films are illustrated in Fig. 6.

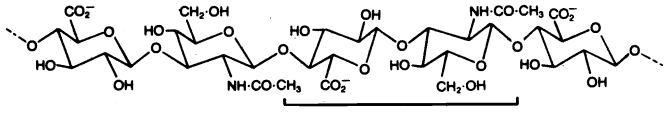


Fig. 4. Repeating sequence of hyaluronic acid.

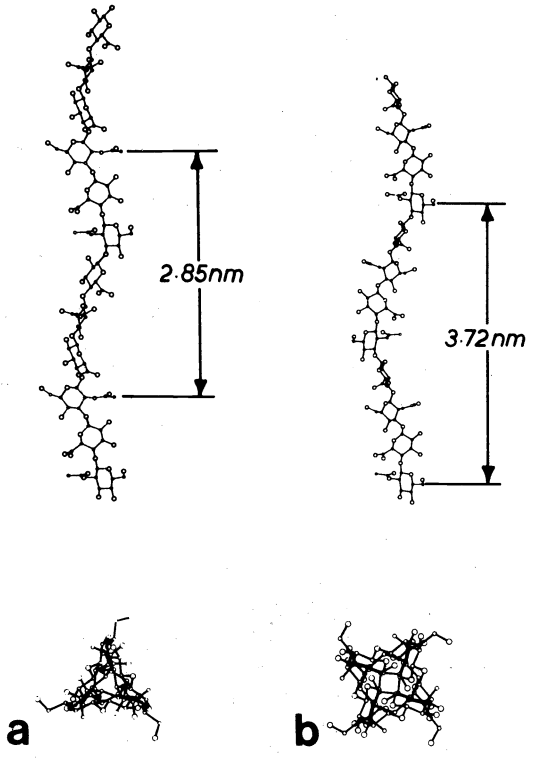


Fig. 5. Projections of hyaluronate conformations (a) a three-fold helix, (b) a four-fold helix.

Thus hyaluronate exhibits a variety for molecular conformations depending on the local environment during crystallization. Such results offer scope for monitoring the conformation in the condensed phase as a function of ionic strength, cationic species etc.

Chondroitin sulphate

The two types of chondroitin, i.e. chondroitin 4-sulphate and 6-sulphate differ from hyaluronic acid by replacement of N-acetylglucosamine with sulphated

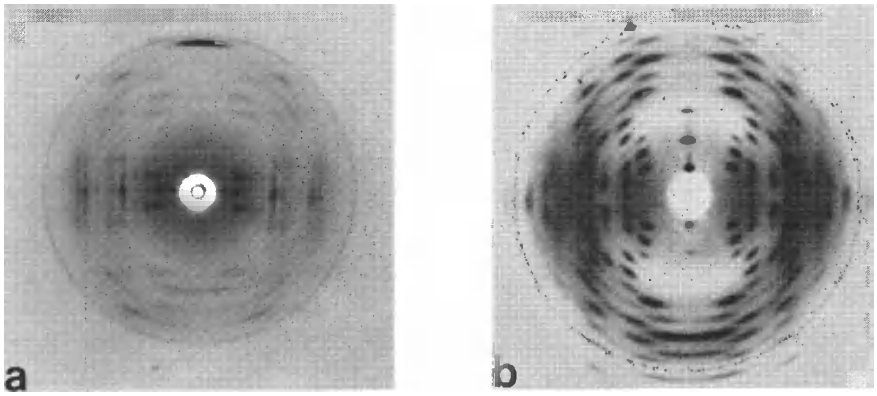


Fig. 6. X-ray diffraction patterns obtained from oriented hyaluronate films. (a) is interpretated as a three-fold helix and (b) a four-fold helix.

N-acetylgalactosamine as shown in Fig. 7. Chondroitin 4-sulphate is extremely sensitive to humidity and both three-fold ($\underline{h}$ = 0.96 nm) and two-fold ($\underline{h}$ = 0.98 nm) helices have been reported (Ref. 28). A typical X-ray diffraction pattern and a computer generated projection are illustrated in Fig. 8 and Fig. 9a respectively. Note the axially positioned sulphate groups lie on the periphery of the molecule. Chondroitin 6-sulphate also exhibits similar properties (Ref. 29) positioning the sulphate groups even further from the helix axis (Fig. 9b). In addition yet another distinct pattern interpreted as an eight-fold helix has been reported (Ref. 30).

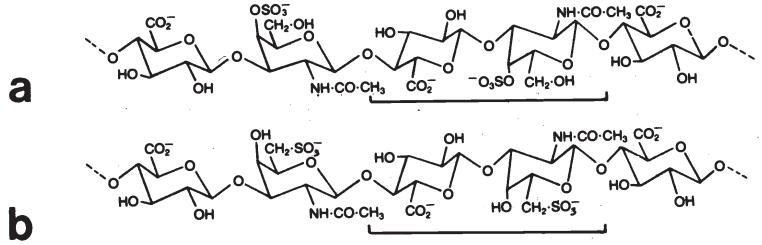


Fig. 7. Repeating sequencies of: (a) chondroitin 4-sulphate and (b) chondroitin 6-sulphate.

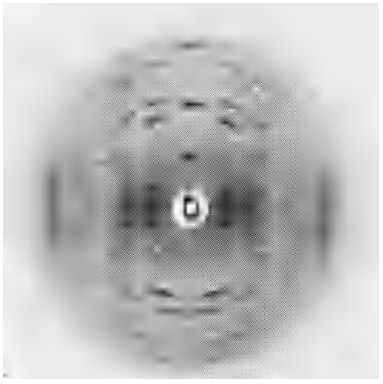


Fig. 8. X-ray diffraction pattern obtained from an oriented film of chondroitin 4-sulphate at 95% relative humidity.

Dermatan sulphate

Of those connective tissue polyuronides based on the hyaluronic acid repeat, the molecular shape of dermatan sulphate has probably aroused most discussion. The chemical repeat is characteristically distinguished by replacement of the usual D-glucuronic acid by its C(5) epimer L-iduronic acid. A schematic representation of the shape and disposition of side groups for both uronic acids in the normal 4C1 chair form are shown in Fig. 10a. Such a

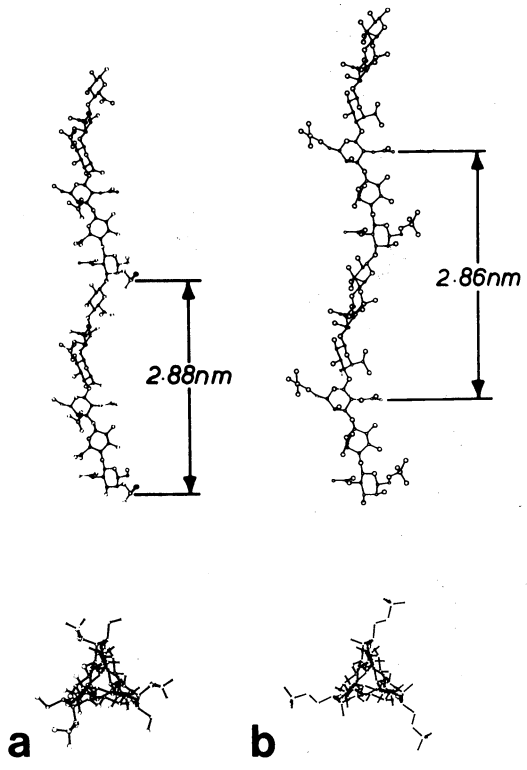


Fig. 9. Projections of the 3_2 helices for: (a) chondroitin 4-sulphate, and (b) chondroitin 6-sulphate.

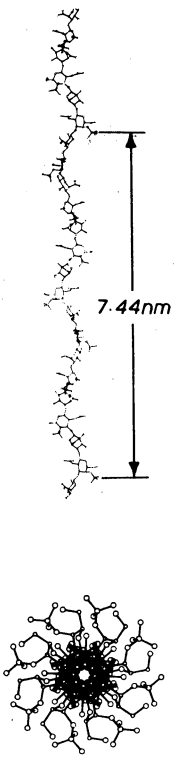


Fig. 11. Projections of the 8_5 helix for dermatan sulphate.

chair conformation for D-glucuronic acid appears quite satisfactory with all the side appendages favourably positioned equatorially. On the other hand, L-iduronic acid has a large carboxyl group axially positioned (Fig. 10b) and serious consideration must be given to the alternate 1C4 chair conformation. This bears a resemblance to the situation found in the alginates discussed above. The effect of converting the L-iduronic acid residue to the alternate 1C4 chair conformation would introduce a diaxially linked unit substantially altering the shape of the backbone. X-ray diffraction patterns obtained from dermatan sulphate (Ref. 31 & 32) yield values of h = 0.93 nm (8_5 helix), 0.95 nm (3_2 helix) and 0.98 nm (2_1 helix). These spacings are not compatible with the L-iduronic acid residue in the alternate 1C4 chair conformation (Ref. 32). Computer projections of the 8_5 helix are shown in Fig. 11.

Proteoglycans

Unlike hyaluronic acid, the chondroitin and dermatan sulphate chains are of lower molecular weight (about 50,000 Daltons) and occur *in vivo* covalently linked to a protein core. The resulting proteoglycan is similar to a laboratory test tube brush. Recent biochemical studies (Ref. 33) have established a specific interaction between hyaluronic acid and the proteoglycans in the intercellular matrix in cartilage. The essential features of the proposed model (Fig. 12) are that many proteoglycans are able to bind along the entire length of a hyaluronic acid chain and that at saturation there is one proteoglycan bound to each region of hyaluronic acid of about twenty disaccharide units long (about 20nm). Thus for a hyaluronic acid chain of molecular weight 500,000 Daltons there are about forty proteoglycans regularly distributed. Each proteoglycan can bind to only one hyaluronic acid chain so the system does not readily form a network or gel by an interaction of the form HA-PG-HA. These proteoglycan aggregates containing hyaluronic acid interact electrostatically through the charges on the polysaccharide side chains with collagen to form the molecular organization in cartilage (Fig. 13). X-ray diffraction patterns from such proteoglycan aggregates have been reported (Ref. 34).

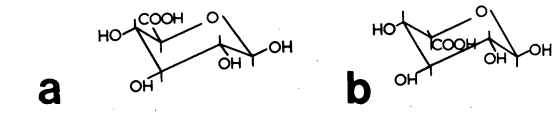


Fig. 10. Schematic representation of: (a) β-D-glucuronic acid, and (b) α-L-iduronic acid.

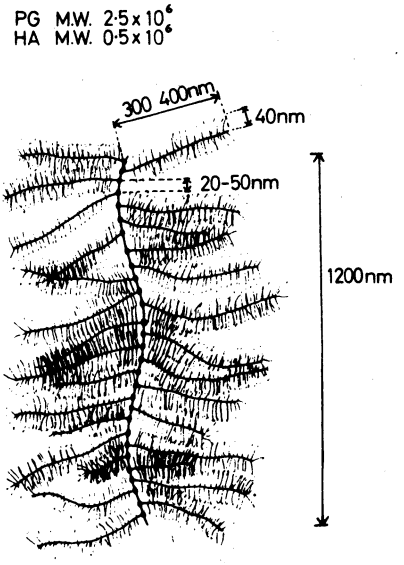


Fig. 12. Proteoglycan-hyaluronic acid complex.

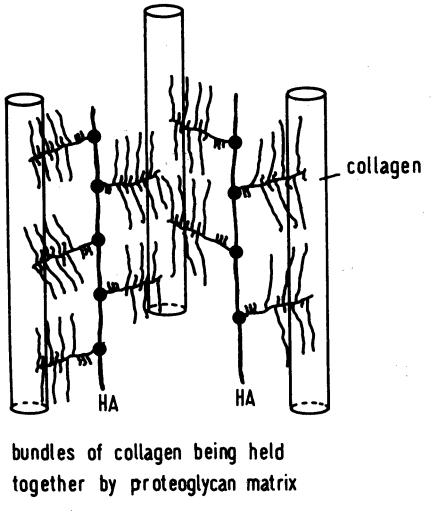


Fig. 13. Model for connective tissue.

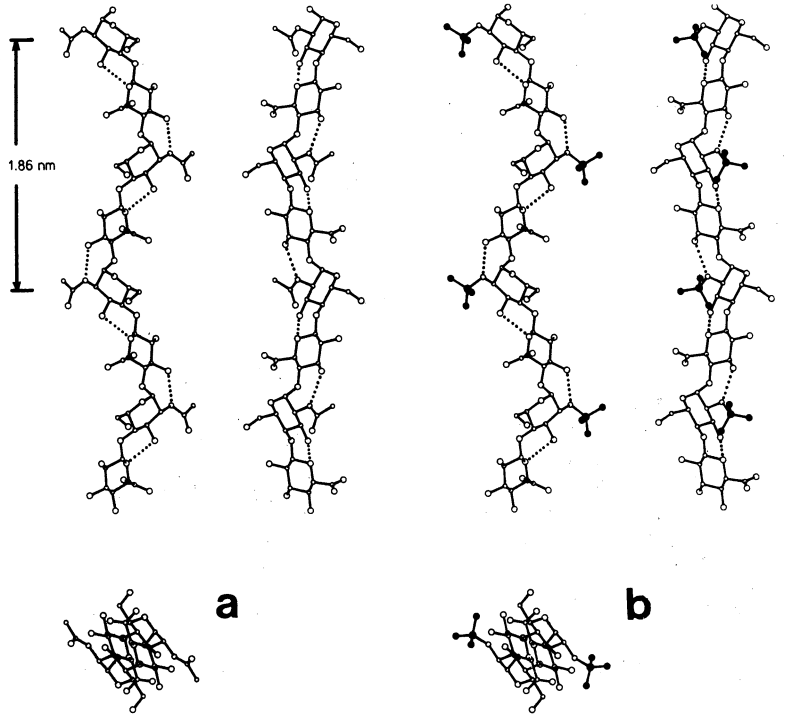


Fig. 14. Projections of the two-fold helix for heparan sulphate. (a) with no sulphate groups included, (b) with NSO_3^- groups on the glucosamine residues (black).

Heparan sulphate

Heparan sulphate consists of repeating disaccharide units of glucosamine and uronic acid. The uronic acid may either be D-glucuronic acid or its C(5) epimer L-iduronic acid. 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 while recent chemical evidence favours the β-1,4 configuration for D-glucuronic acid (Ref. 35). Such a repeat is shown in Fig. 16a.

The first X-ray diffraction patterns obtained from ordered conformations in the condensed phase for sodium heparan sulphate were reported by Atkins and Laurent (34). The X-ray fibre diffraction pattern has a layer line spacing of 1.86 nm with meridional reflections on even layer lines yielding a value for h = 0.93 nm (Ref. 36 - 38). These results are consistent with an extended two-fold helical structure as shown in Fig. 14.

On formation of the calcium salt of the same preparation of heparan sulphate a new X-ray fibre pattern emerges (Fig. 15) identical to that obtained from calcium heparin (Ref. 39). These results suggest that heparan sulphate preparations can also contain regions formally similar to heparin. Independent evidence from chemical degradation methods also supports the presence of a trisulphated disaccharide that is apparently identical to the repeating sequence of heparin (Ref. 40). It would appear that heparan sulphate is a block copolymer containing a "heparan sulphate-like" phase and a "heparin-like" phase and that the former crystallises preferentially in the sodium salt form.

Heparin

Structural investigations of the blood anti-coagulent heparin using chemical degradation methods (Ref. 41) currently favour a linear polydisaccharide of alternating 1,4 -linked 2-deoxy-2sulphamino- α-D-glucose-6-sulphate and 1, 4-linked 2-sulphated- α-L-idopyranosyluronic acid to represent the major portion of the macromolecule. The covalent repeat (Fig.16b) is supported by nuclear magnetic resonance spectroscopy (Ref. 42 & 43) and is consistent with the X-ray fibre diffraction results (Ref. 44) and electron microscopical observations (Ref. 45). A typical X-ray diffraction pattern is shown Fig. 17, which together with other X-ray results favour a two-fold helical structure (Ref. 46-48) as shown Fig. 18.

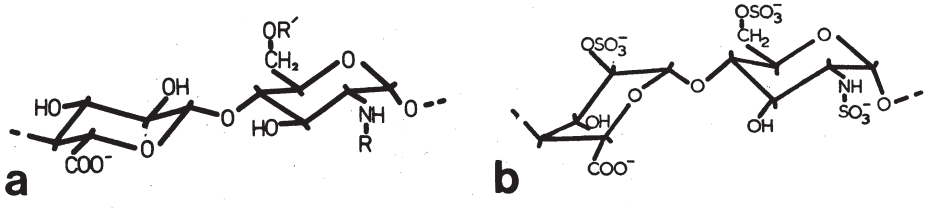


Fig. 16. Repeating sequences of: (a) heparan sulphate, and (b) heparin. R is either $COCH_3$ or SO_3^- and R is usually H but sometimes SO_3^-.

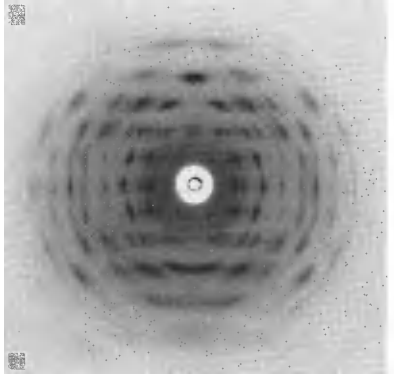


Fig. 15. X-ray diffraction pattern of calcium heparan sulphate.

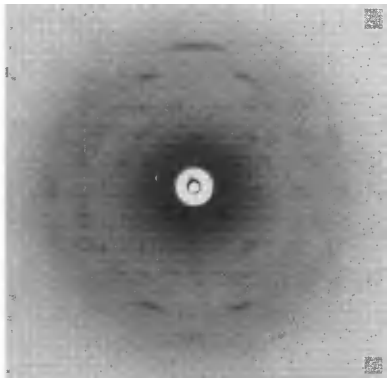
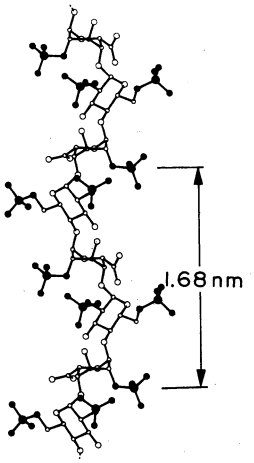


Fig. 17. X-ray diffraction pattern of sodium heparin.



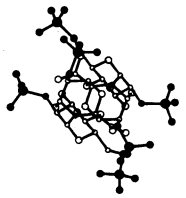


Fig. 18. Projections of a two-fold helical conformation of heparin.

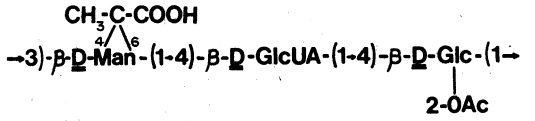


Fig. 19. Chemical sequence of serotype K5.

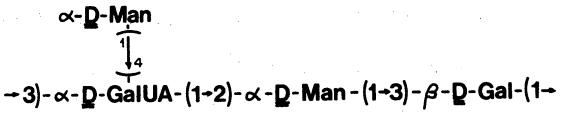


Fig. 22. Chemical sequence of serotype K57

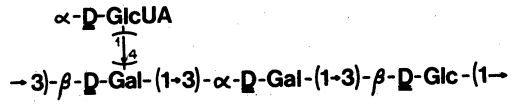


Fig. 25. Chemical sequence of serotype K8.

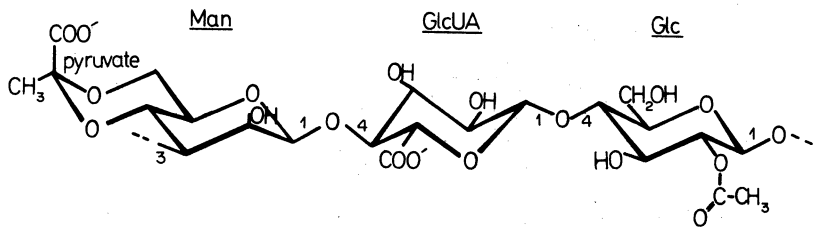


Fig. 20. Schematic representation for repeat of K5.

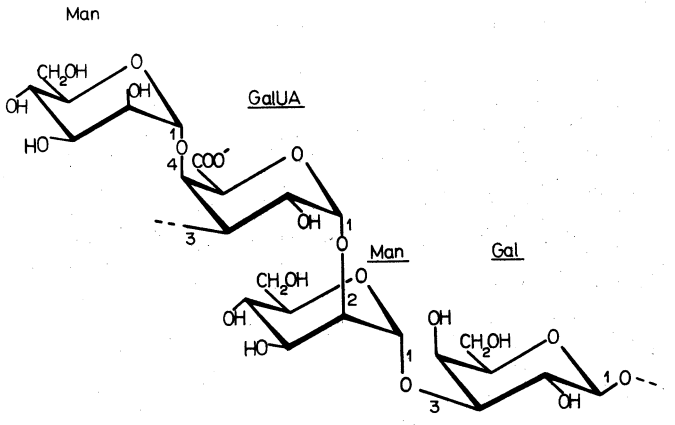


Fig. 23. Schematic representation for repeat of K57.

MICROBIAL POLYURONIDES

In this section some examples have been taken from the capsular polysaccharides of the genus Klebsiella. They have been chosen in order of increasing complexity and an overall model building procedure has been adopted described as follows.

Molecular models were generated using a procedure similar to the linked-atom description (Ref. 49&50). Bond lengths, bond angles and sugar residue conformations were held constant. Only the torsion angles at the various backbone glycosidic linkages were allowed to vary. Pendant groups were placed in stereochemically reasonable positions after the chain conformation had been determined. A conformation was considered possible when it simultaneously met two criteria: adherence to the appropriate helix pitch and symmetry while having no unacceptable non-bonded contacts. The presence (or absence) of unacceptable interatomic contacts was defined using steric criteria (Ref. 51). In cases where alternative models were possible the model which contained the maximum number of intramolecular hydrogen bonds were favoured. To aid in following the course of the model building, and to assess the possibility of hydrogen bonding across each of the glycosidic linkages, steric maps were calculated for each glycosidic linkage.

Klebsiella serotypes

The genus Klebsiella belongs to the family of Enterobacteriacae and are found in the intestines of man and animals. In general Klebsiella bacteria produce extracellular polysaccharides which surround the bacterium as an additional outer layer. These capsules act as a visoelastic barrier against mechanical damage and protect the cells against desiccation and phagocytosis. The capsular materials also exhibit specific antigenic properties, and the serological classification of Klebsiella is based on immunological reactions of these capsular or K antigens (Ref. 52). To date about eighty serologically different strains have been recognized, the variety of which presumably has evolved due to the host defence mechanisms. In all cases known the isolated capsular material consists of heteropolysaccharides with a regular repeating unit of three to six saccharides, which are chemically different for each strain. The repeating unit is often branched being a mono or disaccharide as a side appendage. The presence of a uronic acid, pyruvate or both seems to be a characteristic feature of these glycans (Ref. 53).

A number of these serotypes have been induced to form oriented, crystalline films suitable for X-ray diffraction analysis. The three serotypes K5, K57 and K8 have been chosen from our collection because they are all polyuronides and they are arranged in order of increasing complexity.

Serotype K5

Of the three Klebsiella serotypes to be discussed the K5 polysaccharide has the least complicated chemical covalent repeat. It is a linear polytrisaccharide of the form $(\text{-A-B-C-})_n$, and the detailed chemical constitution has been reported by Dutton and Mo-Tai Yang (54) as shown schematically in Fig. 19. The essential backbone structure consists of two neutral sugars, a 1, 3-linked-β-D-mannose and a 1, 4-linked β-D-glucose, sandwiching a 1, 4-linked β-D-glucuronic acid residue. There are two appurtenances worth mentioning: pyruvic acid is linked to the D-mannopyranose as a 4, 6 acetal, and an O-acetate group is attached to the 2-position of the glucopyranose ring. Thus the repeating sequence contains two charged carboxylate groups and the glycosidic linkage geometry is illustrated in Fig. 20. All three saccharide units would be expected to exist in the normal 4Cl chair conformation resulting in a pair of $1 \rightarrow 4$ diequatorial glycosidic linkages together with a single $1 \rightarrow 3$ diequatorial linkage. Both these linkage geometries are common to the simpler plant and animal polyuronides discussed above. If the vectors between each glycosidic oxygen atom were to align precisely the maximum theoretical extension per chemical repeat would be 1.56 nm. Of course it is extremely unlikely that a stereochemical acceptable model could exist with such a repeat but at least it indicates an upper limit. For the simpler polyuronides such as the alginates and connective tissue polyuronides the reported axially projected repeats in the condensed phase are all within 18% of the theoretical limit and typically highly extended conformations gave repeats centred around value 10% less than the maximum.

The X-ray diffraction results from the sodium salt of K5 (Ref. 55) yielded a layer line spacing of 2.70 nm and with meridional reflections only occurring on even layer lines suggesting a two-fold helical conformation of the molecule. The value for h of 2.70/2 =

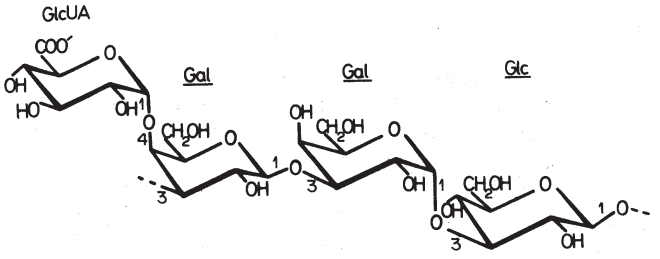


Fig. 26. Schematic representation for repeat of K8.

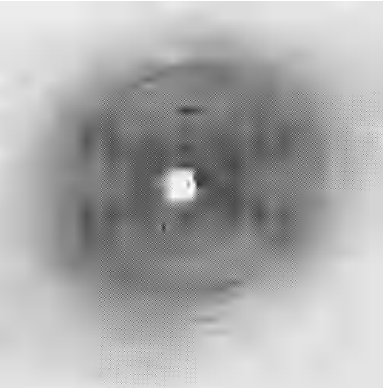


Fig. 27. X-ray diffraction pattern from serotype K8.

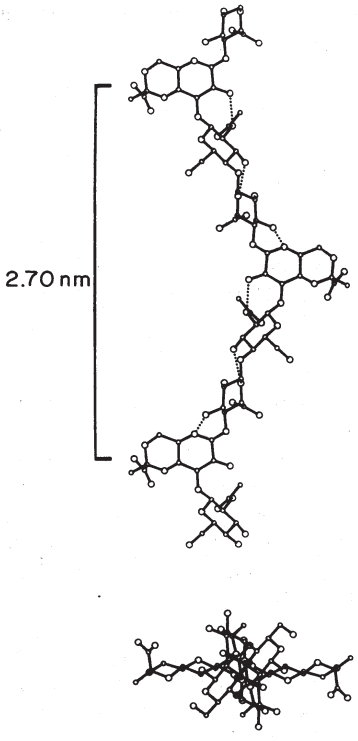


Fig. 21. Two-fold helix for K5.

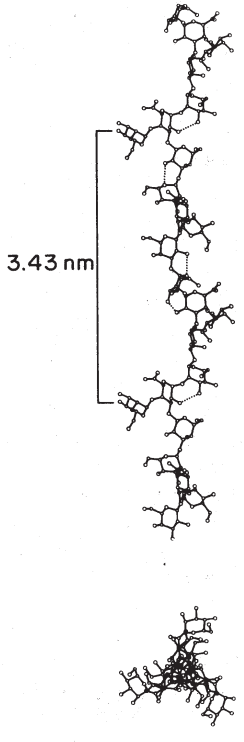


Fig. 24. Three-fold for K57.

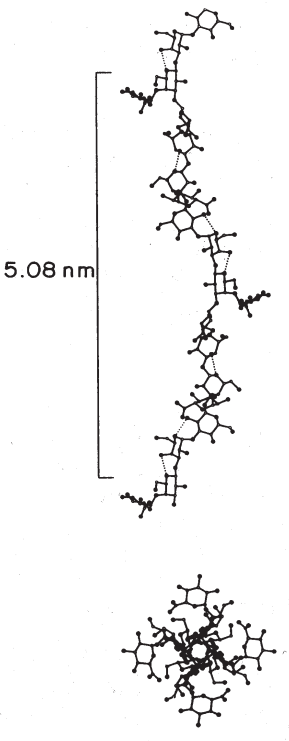


Fig. 28. Four-fold helix for K8.

1.35 nm correlates well with the trisaccharide repeat and is only 14% below the theoretical limit suggesting an extended chain conformation.

Stereochemical acceptable structures with the required helical parameters have been computer generated and it has been found possible to form intra-chain hydrogen bonds across all three glycosidic linkages and fit the experimentally recorded helical symmetry and dimensions. Computer drawn projections are shown in Fig. 21. The structure incorporates an O(5)...H-O(3) hydrogen bond at both the β-D-Man-(1→4)-β-D-GlcUA and the β-D-GlcUA-(1→4) - β-D-Glc glycosidic linkages. This hydrogen bond is present in many homopolysaccharides such as cellulose (Ref. 11) and the connective tissue polydisaccharide structures (Ref. 24). It is gratifying to find it also appearing in this more complex structure. At the third glycosidic linkage (β-D-Glc-(1→3)-β-D-Man) there is an O(2)...O(2) hydrogen bond.

Serotype K57

K57 is a polytetrasaccharide consisting of three saccharide units in the backbone and with a single saccharide unit (α-D- mannose) in the side chain. The detailed chemical repeat has been established by Kamerling, Lindberg, Lonngren and Nimmich (56) and is given in Fig. 22. As in the case of the K5 serotype the backbone consists of two neutral sugars and a uronic acid residue. This 1, 3 linked α-D-galacturonic acid residue is attached to a 1, 2 linked α-D-mannose residue followed by a 1, 3 linked β-D-galactose residue. The side appendage is attached to the uronic acid residue. Again it would be anticipated that all saccharide units exist in the normal 4C1 chair conformation resulting in one 1→3 diequatorial glycosidic linkage, a 1→2 diaxial linkage and one lax→3eq linkage (Fig. 23). In addition the mannopyranose side chain is 1→4 diaxially attached. This structure introduces some novel glycosidic linkage geometries together with the added complication of a side chain. The maximum theoretical extension for the chemical repeat, following the method described previously, is 1.27 nm.

The X-ray diffraction patterns show layer lines with a measured spacing of 3.43 nm with meridional reflections present only on layer lines with index $l = 3n$ (Ref. 57). The simplest interpretation of this diffraction pattern is that the polysaccharide backbone forms a three-fold helix with the value of $h = 1.143$ nm. This value, 10% less than the maximum permissible, correlates with the chemical repeat and indicates a highly extended conformation.

Trial models have been generated conforming to the helical symmetry and dimensions. Both left-handed and right-handed models have been generated using the techniques and criteria outlined above. Attempts were made to form the maximum number of intra-chain hydrogen bonds. It was found that no model could be constructed that included hydrogen bonds across all three backbone glycosidic linkages. Only a left-handed helix allowed the formation of two intra-chain hydrogen bonds in the backbone: α-D-GalUA-0(2)...0(3)-α-D-Man and α-D-Man-0(5)...H-0(2)-β-D-Gal. Computer drawn projections of this conformation are illustrated in Fig. 24.

Serotype K8

The covalent chemical repeating sequence has been established by Sutherland (58) and is given in Fig. 25. It is similar to the K57 polysaccharide having a polytrisaccharide backbone and a single saccharide unit for the side chain. Both structures have a uronic acid in the repeat, however, in K57 the uronic acid is incorporated in the backbone and the side chain is a neutral sugar. The converse is true in serotype K8 which has an uncharged backbone and an α-D-glucuronic acid side chain. All four saccharide units are expected to exist in the normal 4C1 chair conformation and a schematic diagram representing the glycosidic linkage geometry is shown in Fig. 26.

The theoretical maximum extension for the chemical repeat is 1.38 nm falling partway between the values for K5 and K57. The X-ray diffraction pattern for the sodium salt of the K8 polysaccharide is shown in Fig. 27. The material is highly oriented and crystalline and from the systematic absences of odd order meridional reflections it can be seen that the molecule is a 2_1 helix. However the layer line spacing of 5.078 nm is far too large for a repeat of two asymmetric units. In fact it is very close to the theoretical maximum extension for four complete covalent repeats, i.e. $4 \times 1.38 = 5.52$nm. Thus the observed repeat is only some 10% less than the maximum permissible extension.

From preliminary model building it appears reasonable to assume that the structure of the isolated model is a perfect four-fold helix with an axial advance per covalent repeat (h) = 1.27 nm. Perturbations from an idealized four-fold helix would be expected to result in a lower symmetry and consequently the packing of the molecular chains in an orthorhombic rather than a tetragonal unit cell (Ref. 59). The phenomenon has also been observed in hyaluronic acid (Ref. 24).

Stereochemically feasible models were constructed to fit a four-fold helical symmetry and incorporating the maximum number of intra molecular hydrogen bonds. It was found impossible to construct a model which incorporated hydrogen bonds at all three backbone linkages. Only a left-handed helix allowed the formation of two hydrogen bonds in the backbone. Projections of this structure are shown in Fig. 28. The conformation contains hydrogen bonds O(5)...O(4) and O(5)...O(2) at the β-D-Gal-(1→3)-α-D-Gal and α-D-Gal-(1→3)-β-D-Glc glycosidic linkages respectively. It was found not possible to form a four-fold helix (with the observed extension) which contained a hydrogen bond at the β-D-Glc-(1→3)-β-D-Gal glycosidic linkage. This linkage is adjacent to the attachment site of the uronic acid side chain.

Lacking any information to define the position of the side chain in the isolated molecule, other than that it must be in a stereochemically allowed position, it was necessary to use X-ray intensity information to determine the orientation of the pendant residue (Ref. 60).

DISCUSSION

Examples have been briefly reviewed covering the simpler plant homopolyuronides, the uronic acid containing connective tissue polydisaccharides and the more complex bacterial capsular polyuronides from Klebsiella. Conformations have been derived from the X-ray diffraction evidence in an attempt to provide a general outlook on the behaviour of these macromolecules in the condensed phase. This has recently become possible with the crystallization of these macromolecules in a form suitable for X-ray diffraction studies. It is only through these X-ray studies that the helical parameters necessary for meaningful model building can be obtained. One noticeable feature of the results presented is that all the conformations are highly extended, all that is except for the contracted four-fold of hyaluronic acid (Ref. 24). No simple explanation appears available to explain this puzzling phenomenon.

We have witnessed an avalanche of molecular information relevant to the uronic acid containing polysaccharides which will naturally take time to be completely digested and fully understood, yet we have probably only scratched the surface. Much work is still to be done in refining the molecular conformations outlined in this report. However, one instinctively feels that this is an exciting period since not only has the crystallization of these complex substances been achieved, but a whole variety of molecular conformations are evident as a function of the chemical and thermodynamical environment. The ability to monitor detailed molecular geometries, even in the condensed phase, will greatly facilitate our general understanding of their properties and molecular biology.

Acknowledgements - I am indebted to my colleagues Drs. K.H. Gardner, D.H. Isaac, W. Mackie, I.A. Nieduszynski and J.K. Sheehan, and Ms. H.F. Elloway, for many valuable discussions and for allowing me to reproduce some of their results. My thanks also to the many collaborators who have generously supplied material which made much of this work possible. I am grateful to Professors F.C. Frank, FRS and A. Keller FRS for their constant support and encouragement.

This work was financed by the Science Research Council and in part by the Arthritis and Rheumatism Research Council.

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