Short Communications

Crystallization of Macromolecular Heparin

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X-ray fibre-diffraction photographs were obtained from oriented films of the sodium salt of macromolecular heparin (molecular weight approx. 10^6) prepared from rat skin. Two distinct molecular chain conformations corresponding to two different crystal lattices were observed as a function of relative humidity. The first conformation, obtained at 78% relative humidity, has a layer-line spacing of 1.73 nm, which can be interpreted as an approximate twofold helix. On increasing the relative humidity to 84% a second phase with a layer-line repeat of 1.65 nm is obtained with the reflexions indexing on a triclinic unit cell similar to that obtained previously (Nieduszynski & Atkins, 1973) for pig mucosal heparin.

We have obtained X-ray fibre-type diffraction photographs from samples of macromolecular heparin isolated from rat skin. This material has a molecular weight of approx. 10^6 (Horner, 1971), which is much higher than other published values for heparin, which are usually approx. 10^4 . The macromolecular heparin is thought to consist of heparin-like chains attached to, and distributed along, a common core. This core is a polysaccharide, but as yet its chemical structure is ill-defined (Horner, 1971). The macromolecular material has similar sulphate ester content and anti-coagulant activity (Horner, 1971) to that of heparin isolated from pig intestinal mucosa, which is the usual commercial source.

The heparin molecule is thought to consist of alternating units of α -D-glucosamine and uronic acid residues joined together through (1 $\rightarrow$ 4)-glycosidic linkages. Originally the uronic acid residues were considered to be α -D-glucuronic acid, but more recent evidence from nuclear magnetic resonance, chemical degradation and X-ray diffraction favours α -L-iduronic acid as the major acid present in heparin (Perlin *et al.*, 1970; Lindahl & Axelsson, 1971; Lindahl, 1974; Nieduszynski & Atkins, 1974). Further, Lindahl *et al.* (1972) and Höök *et al.* (1974) have suggested that α -L-iduronic acid is produced by epimerization of β -D-glucuronic acid residues, at the polymer level, and that this would account for the observation of some β -D-glucuronidic linkages in heparin (Helting & Lindahl, 1971). In addition, Lindahl & Axelsson (1971) have suggested that sulphated L-iduronic acid is the major sulphated uronic acid present.

X-ray-diffraction data provided by Nieduszynski & Atkins (1973) for pig intestinal mucosa heparin give a periodicity along the molecular chain axis that is compatible with a tetrasaccharide repeat. Taking into

consideration the probable three-dimensional shapes of the sugar residues, they proposed three molecular chain conformations that differ by the chair form adopted by the uronic acid residues. The detailed conformation depends on the relative α -L-iduronic acid/ β -D-glucuronic acid composition and the proportion of L-iduronic acid sulphated. It is considered likely that a twofold helical model with a covalent repeat approximating to a disaccharide would fit the overall general features of the diffraction pattern (Atkins & Nieduszynski, 1974; Nieduszynski & Atkins, 1974).

In the present communication we report the crystallization of macromolecular heparin from rat skin, comment on some of the structural features and make comparisons with both pig intestinal mucosa heparin and heparan sulphate (Atkins & Laurent, 1973). Films of the material were obtained by drying down aqueous solutions of the sodium salt form on to glass slides. The films were oriented and crystallized in a stress field at high relative humidity as described in detail by Atkins *et al.* (1972).

The sodium salt of macromolecular heparin was prepared from rat skin by the published method (Horner, 1971), except that Bio-Gel A-50m agarose-gel granules (100–200 mesh) (supplied by Bio-Rad Laboratories, Richmond, Calif., U.S.A.) were used for gel filtration, instead of Sagavac 4F.

When films of rat skin heparin were stretched at 78% relative humidity and maintained at this humidity the diffraction pattern shown in Plate 1(a) was obtained. This pattern may be indexed on an orthorhombic unit cell with a layer-line spacing of 1.73 nm.

When the same film was raised to 84% relative humidity an intermediate photograph with two crystalline phases was in evidence (see Plate 1b).

After a further 24h the pattern in Plate 1(c), which corresponds to a single phase, was obtained. Decreasing the humidity did not allow recovery of the former phase.

The pattern shown in Plate 1(c) proves to be a more highly oriented version of that obtained by Nieduszyński & Atkins (1973) for pig intestinal mucosa heparin. The quality of the X-ray photograph is sufficient to allow the layer-line spacing to be measured directly and equals 1.65 nm, rather than 1.59 nm as computed previously. (In the poorly oriented diffraction pattern from pig intestinal mucosa heparin it was not possible to measure layer-line spacing directly and with accuracy, so that the 21 measured reflexion spacings were fitted to six reciprocal unit-cell parameters by a least-squares method. With the higher-quality data now available one of the six reciprocal unit-cell parameters needs to be changed. However, we believe that both sets of data relate to the same crystalline lattice. The changes in the unit-cell parameters are such as to strengthen the validity of our previous conclusions.) The observed layer-line periodicities and lattice parameters of both patterns (Plate 1a and 1c) are sensitive to variation with humidity.

Several noteworthy features emerge from these results. First, both of the crystalline modifications involve a tetrasaccharide crystallographic repeat.

Secondly, the line broadening of the 001 reflexion is less than that observed for pig intestinal mucosa heparin (Nieduszyński & Atkins, 1973). The evidence now presented for macromolecular heparin favours a much longer coherent scattering length (than the 16 nm found previously) and therefore a larger molecular weight of the individual polysaccharide chains. This is consistent with the observation that the heparin chains separated from the higher-molecular-weight core by gel filtration, after digestion of rat skin heparin with a depolymerase preparation from rat small-intestinal mucosa, are appreciably longer than pig intestinal mucosa heparin (Horner, 1972).

Thirdly, macromolecular heparin is capable of adopting two distinct conformations of periodicity 1.73 nm and 1.65 nm. The triclinic unit cell corresponding to the conformation with 1.65 nm periodicity is characteristic of both rat skin and pig intestinal mucosa heparin.

Although it is too early to specify the conformations adopted, it is noteworthy that the difference between the two tetrasaccharide extensions (0.08 nm) is similar to the difference between the extension of a C1 and a 1C chair of L-iduronic acid, suggesting that the heavily sulphated macromolecular heparin molecule has conformational flexibility. Perhaps most noteworthy is the fact that pig intestinal

mucosa and rat skin heparin, which have differences in their precise chemical composition, are capable of crystallizing in the same lattice.

Horner (1972) has shown that macromolecular rat skin heparin inhibits lipoprotein lipase activity, whereas pig intestinal mucosa heparin and an enzymically depolymerized rat skin heparin fraction of similar size to pig intestinal mucosa heparin activate the same enzyme. As macromolecular rat skin heparin can adopt two distinct conformations with different periodicities, whereas pig intestinal mucosa heparin shows only 1.65 nm periodicity, it is possible that conformational changes due to depolymerization could account for the marked difference between the biological activities of intact and degraded rat skin heparin. Further findings by Horner (1974) suggest that macromolecular heparin, which also occurs in monkey tissues, probably represents a biological precursor of normal-molecular-weight heparin.

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EXPLANATION OF PLATE I

X-ray fibre-type diffraction photographs obtained from the sodium salt of macromolecular heparin

(a) Pattern obtained on initial crystallization of the sample at 78% relative humidity. The layer-line spacing is 1.73 nm and the reflexions can be indexed on an orthorhombic unit cell. (b) On increasing the relative humidity to 84% a gradual transition is observed giving rise to a new crystalline phase. The pattern shown is a mixture of the two phases. (c) Final phase obtained from (b) after 24 h at 84% relative humidity. The reflexions index on a triclinic unit cell similar to that obtained from pig mucosa heparin with a layer-line repeat of 1.65 nm.