A possible ring conformational change of iduronate residues detected by i.r. spectroscopy of aqueous solutions of lithium-heparin

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The i.r. spectra of iduronate residue-containing glycosaminoglycans may provide clues about S-O-C bond environments [1, 2]. A conversion of the ionized iduronate residue carboxyl groups in heparin to the unionized, free acid form (i.e. a conversion of COO\(^{-}\) to COOH) resulted in the displacement of the absorption band assigned to iduronate residue C2 sulphate groups from about 800 cm\(^{-1}\) to about 870 cm\(^{-1}\) [1]. A similar alteration occurred when the iduronate residue carboxyl groups were reduced to idose (i.e. a conversion of COO\(^{-}\) to \(\text{CH}_2\text{O}\)H) [2]. This change in absorption frequency is interpreted as reflecting a change in the orientation of the S-O-C bonds from an axial to an equatorial position. This, in turn, is consistent with a conformational change of the iduronate residue ring from a \(\text{C}_4\) (chair) to a \(\text{S}_0\) (skew-boat) form [3, 4]. It is apparent, then, that a reduction of the charge density at the C6 position of the iduronate residue ring, and/or an altered hydrogen-bonding capacity of OH groups in this part of the molecule may be a driving force for the sugar ring conformational change. Further elucidation of the possible factors influencing such conformational changes might be obtained by an examination of the i.r. spectra of particular metal cation-heparin complexes. In this communication, i.r. spectra of lithium-heparin complexes in solution are reported.

The source and properties of the heparin have been reported previously [5]. Traditional i.r. solution cell techniques were unsuitable for recording reliable spectra of heparin solutions because of the chemical reaction of the glycosaminoglycan with the salt- (e.g. BaF\(_2\)-) based i.r. cell windows. Reproducible spectra are obtainable by enclosing a weighed quantity of solution in a cuvette obtained by stretching a high-density polyethylene film of 2 \(\mu\)m thickness across a salt-disc sample-holder (Specac Ltd., Orpington, U.K., part no. 2304). A similar film, without sample, was placed in the spectrophotometer reference beam. Other details of i.r. spectroscopy are as described in Grant et al. [1].

The spectrum of a solid state, hydrated film of the lithium-heparin like those of several other metal cation-heparin complexes, includes an absorbance at about 800 cm\(^{-1}\) [5] assigned to the axially positioned iduronate residue 2-O-sulphate groups [1, 2]. This is consistent with the notion that the iduronate residue rings exist predominantly in a \(\text{C}_4\) chair conformation under these conditions. In contrast, the spectrum of a 4 mmol/cm\(^3\) aqueous solution of the lithium-heparin complex shows little absorbance at 800 cm\(^{-1}\), and substantial absorbance in the 862 cm\(^{-1}\) region (Fig. 1). As the concentration of the complex is increased, absorbance at 800 cm\(^{-1}\) is progressively established, and, at high concentration, the spectrum resembles that of hydrated films of the complex. Absorptions centred around 890 cm\(^{-1}\) (due to coupling of C-O-S and ring C-O-C), and at around 937 cm\(^{-1}\) (due to coupling of C-O-S and glycosidic C-O-C) also show concentration-dependent alterations in intensity. The absorption of the glucosamine residue 6-O-sulphate group remains at about 827 cm\(^{-1}\) as the concentration is increased. These results suggest that the \(\text{S}_0\) (skew-boat) iduronate residue ring conformer may predominate in solutions of lithium-heparin of low concentration, and that an increase in concentration results in the progressive production of the

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Fig. 1. I.r. spectra of aqueous solutions of lithium-heparin complexes

Concentrations of lithium-heparin complex (mmol/cm\(^3\)) were: (a) 4; (b) 8; (c) 17; (d) 22; (e) 45; (f) 80.
Iduronate residue ring conformations in heparan sulphates of normal and transformed fibroblasts

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Heparan sulphate-containing proteoglycans are widely distributed as pericellular components of animal tissues. Their extended molecular dimensions, structural complexity, polyanionic nature, and ability to self-associate and interact with several cell-surface proteins, have prompted suggestions that these molecules modulate interactions between cells and their immediate environment [1]. Heparan sulphate, present in increased amounts, has lower charge density in a range of transformed cells in culture [2-12], and in rat hepatoma tissue [13] and human hepatoma tissue [14]. These alterations may be due to reduced O-sulphation [7,9], particularly at position 6 of glucosamine residues [5]. It has been suggested that the biosynthetic epimerization at the polymer level of β-glucosamine to α-iduronic residues in heparin, heparan sulphates and dermatan sulphates permits versatility in ligand binding by these glycosaminoglycans, because of the ability of the iduronate residue ring to exist in more than one equienergetic conformational form [14]. Results from multiple specular reflectance i.r. spectroscopic studies have been interpreted to suggest: that the major conformer in hydrated films of heparin–metal cation complexes is 1'C, but that replacement of the metal cation by H⁺ makes the 3'S, conformer predominate [15]; that chemical reduction of iduronic acid residues to idose again makes the 1'S, conformer predominate [16]; and that the 3'S, conformer predominates in solutions of Li⁺-heparin of low concentration, but is progressively converted to the 1'C, conformer in solutions of higher concentration [17]. In this communication, i.r. spectra of heparan sulphates from surfaces of normal and transformed fibroblasts are compared.

Heparan sulphates were extracted from surfaces of cultures of BHK-21 (C13) and polyoma virus-transformed (PyY) counterpart cells by a procedure based on that of Underhill & Keller [2], and involving trypsin treatment of cells, and Pronase treatment, ion-exchange chromatography and chondroitin ABC lyase (EC 4.2.2.4) treatment of extracts. Examination of hydrated films of glycosaminoglycans by multiple specular reflectance i.r. spectroscopy was as described by Grant et al. [15].

1'C, form. Complexes of several other metal cations with heparin do not exhibit these concentration-dependent spectral changes (not shown). Of all cations, Li⁺ is most similar to H⁺ in size and in ability to form covalent bonds with oxygen atoms; it binds to heparin particularly strongly [6].

Like the changes seen when sodium-heparin is converted to heparinic acid [1] and when iduronate residues are chemically reduced to idose [2], the results reported here suggest that a reduction in anionic charge at C6 of the iduronate residue ring may stabilize the 3'S, conformational form of this sugar. In addition, Li⁺ evidently elicits (perhaps through [Li⁺]-dependent hydrogen-binding) a concentration-dependent alteration of the amounts of the different iduronate residue conformers present. These peculiarities may be clinically relevant: the rate of heparin-stimulated antithrombin III-thrombin interaction is apparently less in the presence of Li⁺ than in the presence of Na⁺ or K⁺ ions in solutions of similar ionic strength [7].

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Fig. 1. I.r. spectra of cell-surface heparan sulphate from (a) BHK and (b) PyY cells

In the Figure, spectra of normal and transformed cell-surface heparan sulphates extracted and examined under identical conditions of pH (7.5) and ionic strength (5%, w/v, NaCl) are compared. The absorbance at about 820 cm⁻¹, attributed to the glucosamine residue 6-O-sulphate group [15], is decreased in intensity in the sample obtained from the surfaces of transformed cells. This accords with information obtained by conventional chemical analyses [5]. An absorbance which centres at about 790 cm⁻¹ in the spectrum of the normal cell sample is less prominent in the spectrum of the transformed cell sample, in which increased absorption at about 880 cm⁻¹ is seen. In spectra of heparin films, an absorbance at about 800 cm⁻¹ is presumed to be that of the iduronate residue 2-O-sulphate group in an axial position (i.e. with the monomer ring in the 1'C, conformation), and an absorbance at about 870 cm⁻¹ that of that same group in an equatorial position (i.e. with the monomer ring in the 3'S, conformation) [15]. It is possible that the 790 cm⁻¹ to 800 cm⁻¹ shift seen in the spectrum of transformed cell heparan sulphates reflects a similar alteration in the conformation of the iduronate residue rings. N.m.r. spectroscopic studies of