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Preliminary communication

Resolution-enhanced ^{1}H-n.m.r. spectra of dermatan sulfate and chondroitin sulfates: conformation of the uronic acid residues

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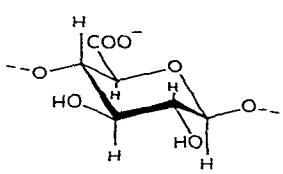
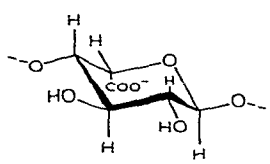
The uronic acid residues are thought to play an important role in controlling the biological function of glycosaminoglycans, and their conformation has been the object of many studies and speculations[1]. It is generally accepted that the β-D-glucopyranosyluronate residues of chondroitin sulfates are in the $^4C_1$ conformation, with the carboxylate group oriented equatorially[2]. The conformation of the L-idopyranosyluronate residues of dermatan sulfate (DeS) is also most commonly referred to as being $^4C_1$. This conformation involving in this case an axial carboxylate group, appeared to account for the differences in pK_a and binding properties of dermatan sulfate as compared with the "isomeric" chondroitin sulfates[3]. $^4C_1$(L)-Rings, with *trans*-diequatorial OH groups at C-2 and C-3 (as opposed to the *trans*-diaxial orientation in the alternative $^1C_4$ conformation), would also explain why DeS reacts with periodate[4,5]. These conclusions are supported by X-ray diffraction data on oriented films of DeS, which suggest the presence of "extended" disaccharide repeat-units built up with both L-iduronate and 2-amino-2-deoxy-D-galactose in $^4C_1$ conformation (equatorial glycosidic bonds)[6]. On the other hand, the coupling characteristics of the anomeric ^{1}H- and ^{13}C-n m.r. signals[7,8] pointed to the alternative $^1C_4$ conformation of the L-iduronic acid residues.

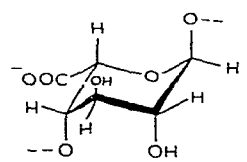
To throw light on the above discrepancies, completely resolved ^{1}H-n m r. spectra of DeS, with unequivocal assignment of all the signals of the uronate residues, were re-



D-*gluco* $^4C_1$



L-*ido* $^4C_1$



L-*ido* $^1C_4$

quired. Severe overlap of signals in the "normal" ^{1}H-n.m.r. spectrum of DeS, even at fields as high as 270 MHz, prevented a complete analysis of the coupling pattern and the assessment of the conformation of the L-iduronic acid residues. We now report the complete resolution of the proton resonances of DeS, as achieved by the "convolution-difference" technique successfully used for resolution-enhancement of the spectra of proteins[9] and, more recently, of heparin[10]. The signals of the uronic acid residues were assigned by spin-decoupling, and the interproton coupling constants measured by first-order analysis. Satisfactory spectra were also obtained from chondroitin sulfate A (Ch-4S) and chondroitin sulfate C (Ch-6S).

Fig 1 compares the "convolution-difference" 270-MHz ^{1}H-n.m.r. spectra of DeS and Ch-4S. (DeS was a sample obtained[11] from urine of a patient affected by Maroteaux-Lamy mucopolysaccharidosis; Ch-4S, from sturgeon notocord, was a standard preparation from Drs. M. B. Mathews and J. A. Cifonelli, University of Chicago.) The chemical shifts

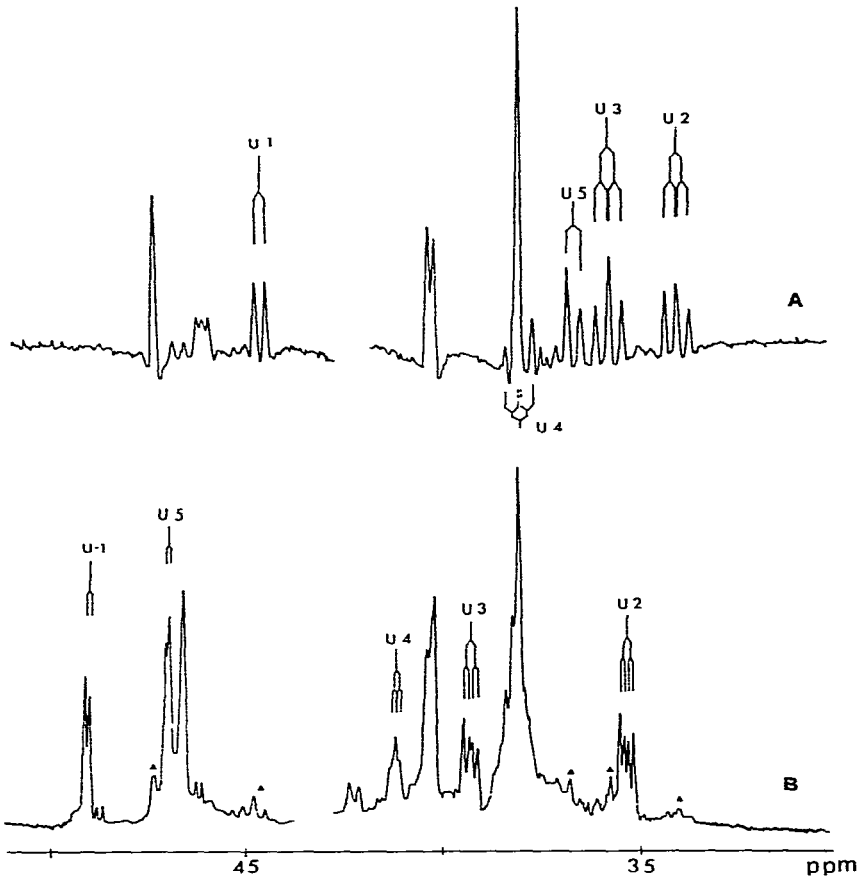


Fig. 1. "Convolution-difference" ^{1}H-n m.r. spectra of chondroitin 4-sulfate (A) and dermatan sulfate (B) in D_2O (8% w/v); 270 MHz; 70°. Signals labelled ▲ refer to "chondroitin-like" structures in DeS. U-1–U-5 are the signals for the protons of the uronic acid residues

and the interproton coupling constants for the protons of the uronic acid (U) residues of Ch-4S and DeS are compared in Table I with the corresponding (computer-refined) values for heparin (Hep).

A relevant feature of the above data is the striking difference of the coupling patterns for Ch-4S and DeS, and the close similarity of these patterns for DeS and Hep. The ^3J values for Ch-4S are all large (> 7 Hz) and therefore consistent with all *trans*-diaxial ring-protons, as expected for the $^4C_1$ conformation of the D-glucuronic acid residues

On the contrary, the corresponding values for DeS (and Hep) are all small ($\leqslant 6$ Hz), therefore ruling out the $^4C_1$ conformation Another possible candidate, namely, the skewed $^1S_3$ conformation[12], is also ruled out by the present data This conformation implies trans-diaxial 2,3 and 3,4 hydrogen atoms, and would therefore produce large couplings. The $^1C_4$ conformation, or a closely related form, is clearly indicated by the coupling values. As discussed for heparin[10], variations within the coupling range for gauche protons[13] can be largely accounted for by the nature (oxygen, or carbon) of atoms antiperiplanar to the considered protons. It is also worth noting that the large chemical-shift difference observed for H-2 in DeS relative to Hep is explicable by the deshielding effect of the *O*-sulfate group at C-2 in Hep. The smaller differences for H-1 and H-3 of the uronic acid residues in the two glycosaminoglycans are similarly explicable.

Other preparations of DeS (from pig skin and pig-intestinal mucosa) gave essentially the same spectra as the urinary sample, except for a lower resolution attributable to higher molecular weight and solution viscosity. As expected from the present knowledge[2] of the structure of DeS and also apparent from the ^{13}C-n.m.r. spectra[8], these preparations contained from 10 to 20% of "chondroitin-like" structures (minor signals labelled with a triangle in Fig. 1B).

Although the present spectrum of DeS was obtained at 70° in order to decrease the solution viscosity and obtain a better resolution, essentially the same conclusions about the coupling patterns can be drawn from the spectrum at 37°. A noticeable temperature-dependence of the DeS spectrum may be explicable in terms of changes in the chain conformation. Work is in progress to clarify this point

TABLE I

N.M.R. DATA[a] FOR PROTONS OF THE URONIC ACID RESIDUES OF CHONDROITIN A (Ch-4S), DERMATAN SULFATE (DeS), AND HEPARIN (Hep) IN SOLUTION IN D_2O

	H-1	($J_{1,2}$)	*H*-2	($J_{2,3}$)	*H*-3	($J_{3,4}$)	*H*-4	($J_{4,5}$)	*H*-5
Ch-4S	4.46	(8.0)	3.40	(8.5)	3.58	(9.0)	3.81	(9.0)	3.66
DeS	4.91	(3.0)	3.54	(6.0)	3.93	(3.5)	4.13	(3.3)	4.69
Hep[10]	5.22	(2.64)	4.35	(5.90)	4.20	(3.44)	4.11	(3.09)	4.82

[a] Chemical shifts: δ, p.p.m. from internal TSP; interproton coupling constants: J, Hz

With respect to the conformation of the D-glucuronic acid residues of Ch-6S, essentially the same conclusions can be drawn as for Ch-4S, in spite of the complexity of the spectrum of the former due to heterogeneity (up to 30% of Ch-4S-like components in the Ch-6S).

The present conclusions on DeS are clearly at variance with those from X-ray[6] and periodate-oxidation[4] studies. The discrepancy relative to the X-ray data could simply mean that the conformation of DeS in solution is different from that in the solid state. Alternatively, as already suggested[14], the X-ray pattern observed for DeS could arise from the chondroitin-like (glucuronic acid-containing) segments rather than from the major constituents of this heterogeneous polymer.

A third possibility, which cannot be ruled out by the present data, is suggested by the fact that the value of ~6 Hz for $J_{2,3}$ both in Hep and in DeS is slightly larger than the largest value (5.5 Hz) predicted[13] for gauche protons antiperiplanar to carbon. As observed[10] for Hep, this slight discrepancy might well be only apparent, when "normal" ranges for gauche couplings are available for carbohydrates bearing charged groups. Alternatively, a $J_{2,3}$ value larger than 5.5 Hz could be accounted for by admitting a dihedral angle between H-2 and H-3 somewhat lower than 60° or larger than 120°. The first possibility is highly improbable because of severe steric interactions between the diaxial 1,3 and 2,4 carbon–oxygen bonds. The second possibility is more reasonable, since it implies a relief of the latter interactions. Moreover, this arrangement (dihedral angle between O-2 and O-3 of ~120°) is much more favourable for complexing with periodate ions than the undistorted $^1C_4$ form. The compatibility with X-ray diffraction data of such a distorted chair is being checked by computer model-building (E. D. T. Atkins, personal communication).

Admittedly, the present work has to be extended to solutions of lower concentrations and higher ionic strength before attempting to explain the susceptibility of DeS to periodate oxidation in terms of the conformation of the uronic residues. Consideration could also be given to the possibility that intermolecular (interchain) interactions control the availability of reactive sites of these polysaccharides[1]. On the other hand, Scott and Tigwell made the challenging hypothesis that *intra*molecular hydrogen bonds involving an OH group of the uronic residues (in addition to H-bonds between acetamido N–H groups and carboxylate groups on adjacent residues) could be a major factor controlling the rate of periodate oxidation of glycosaminoglycans[5]. Inspection of molecular models of DeS with $^1C_4$ (or closely related) L-idopyranosyluronate rings shows that the axial orientation of their hydroxyl groups is unfavourable for formation of hydrogen bonds with acceptor groups on adjacent residues. On the contrary, these H-bonds appear to be easily formed in models of hyaluronic acid and chondroitin sulfates[5].

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