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SOLID-STATE ^{13}C NMR AND X-RAY DIFFRACTION OF DERMATAN SULFATE

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SUMMARY Dermatan sulfate in the solid state has been studied by ^{13}C CP/MAS nmr and X-ray diffraction in order to establish the ring conformation of the L-iduronate moiety. The solid state nmr spectrum is similar to the solution spectrum obtained previously, indicating that a ring conformation at least approximating to $^{1}C_{4}$ predominates in the solid state. X-ray powder diffraction data from the same sample indicate the presence of the 8-fold helix form previously observed by fiber diffraction, and interpreted in terms of a $^{4}C_{1}$ ring form. A likely explanation of the results is that a distorted $^{1}C_{4}$ L-iduronate ring conformation, not considered in the initial X-ray analysis, may emerge to provide a satisfactory interpretation of all available physical-chemical data.

The conformation of the L-iduronate ring in dermatan sulfate has been the topic of considerable controversy. Small proton nmr vicinal coupling constants (1) around the ring indicate trans-diaxial hydroxyl groups as in the $^{1}C_{4}$ ring conformation (Figure 1) or a ring conformation very close to $^{1}C_{4}$. ^{13}C nmr data indicate the same (2). On the other hand, the large susceptibility of iduronate residues in dermatan sulfate to periodate oxidation (3) was interpreted as demonstrating a small dihedral angle between the O(2) and O(3) hydroxyl groups, as in the $^{4}C_{1}$ ring conformation (Figure 1). This aspect of the controversy has recently been resolved by invoking the Curtin-Hammett principle (4), according to which a reaction pathway for a molecule that exists as an equilibrium mix-

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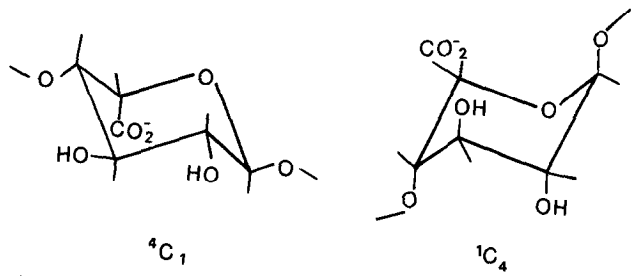

$^4C_1$ $^1C_4$

Fig. 1 Alternative ring conformations of iduronate linked (1-4) as in dermatan sulfate.

ture of two conformers is independent of their relative abundance if conformation interconversion is rapid in comparison with subsequent chemical reactions. Thus, a minor conformer ($^4C_1$) could determine the course of periodate oxidation, with the major conformer ($^1C_4$) acting solely as an unreactive reservoir. The reaction rate is explained by steric assistance in the transition state compensating for the low frequency of productive collisions expected when the minor conformer is the reactive species.

A second aspect of the controversy is that X-ray fiber diffraction evidence (5) is incompatible with standard $^1C_4$ geometry for the L-iduronate ring, and instead favors the alternative $^4C_1$ ring conformation. The postulation of a difference in ring conformation between the solid and solution states would explain these results, and is plausible in view of the small energy difference between the conformers (6). However, an experimental resolution of the question calls for a combination of techniques capable of detecting differences in the iduronate ring conformation of dermatan sulfate on going from solution, to amorphous solids, to oriented crystalline samples.

The CP/MAS nmr experiment permits the collection of high-resolution spectra from solids through the application of three different techniques. First, the use of high-power decoupling minimizes line broadening arising from static heteronuclear dipolar interactions (7). Secondly, spinning at the 'magic angle' of 54.7° with respect to the external field direction leads to a further sharpening through minimization of chemical shift anisotropy effects (8). Lastly, the long T_1's typical of carbon atoms in solids can be overcome by the crossed-polarization (CP) technique (7).

In the present work we have applied solid-state ^{13}C nmr, for direct comparison with solution nmr results, coupled with an X-ray powder diffraction study of the same solid state sample, for direct comparison with the X-ray diffraction pattern of oriented samples.

MATERIALS AND METHODS

A 100 mg sample of dermatan sulfate (porcine skin, Na^+ salt, Sigma Lot 64F-0403) was used in the ^{13}C CP/MAS nmr experiments as received ("dry" sample) or after equilibration with distilled water for 24 hours prior to packing in the rotor ("wet" sample). In each case a small piece of NBS polyethylene standard reference material (No. 1475) was added as an internal reference. The nmr spectra were taken on a Bruker CXP200 spectrometer equipped with a Doty probe and operating at 50.3 MHz. The chemical shift of polyethylene relative to TMS in the solid state at this field strength is 32.9 ppm (9). The spectral window was 20000 Hz with 2K data points used in the acquisition of typically 9000 transients per spectrum. Spinning rates were 3-4 kHz and a contact time of 2 ms was employed. The acquisition time was 51.2 ms and the repetition rate was 6s.

The X-ray data were acquired on Osray M-3 (Agfa-Gevaert) film using Ni-filtered copper radiation from a Phillips fine focus tube operated at 35kV and 15mA. Samples of the humidified dermatan sulfate were removed from the nmr rotor immediately after completion of that experiment, sealed into short segments of quartz capillary tubes and the tubes were then dusted with a small amount of NaF (d = 0.2319 nm) as an external calibrant.

RESULTS

^{13}C CP/MAS spectra are shown in Figure 2. Table I contains a comparison of the chemical shifts observed in the solid with chemical shifts obtained in solution by Hamer and Perlin (2), by which the chemical shifts of the iduronate carbon atoms can be identified. The 174.7 ppm resonance is unambiguously assigned to the U-6 carbon atom [C(6) of the uronic acid moiety] and the 104.1 ppm resonance unambiguously assigned to the U-1 carbon atom. The broader carbonyl reso-

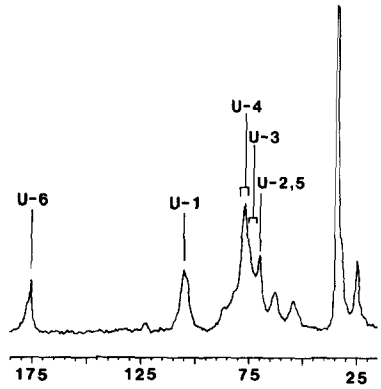

Fig. 2 ^{13}C CP/MAS nmr spectrum of dermatan sulfate, "dry" sample, ppm relative to TMS. NBS polyethylene standard reference is at 32.9 ppm. Assignments from Ref. 2.

Table I. ^{13}C CP/MAS chemical shifts (ppm) of dermatan sulfate in the solid state compared with solution data and assignments of Hamer and Perlin(2)

Assignment	Chemical Shift (ppm)		
	Solution (2)	"wet" sample	"dry" sample
A-Ac(C=O)	176.3	176.5 (broad component)*	176.7 (broad component)*
U-6	174.6	174.7	175.1
U-1	103.5	104.1	103.7
A-1	102.6	102.6	102.6
A-3	81.5	85.5 (broad) or 80.2	86.8 (broad)
A-4	76.7	72-79	72-79
U-4	76.7	72-79	72-79
A-5	75.8	72-79	72-79
U-3	72.0	72-79	72-79
U-2	70.4	68.8	68.6
U-5	70.4	68.8	68.6
A-6	62.5	61.6	62.7
A-2	53.4	52.9 (broad)*	52.8 (broad)*
A-CH_3	24.5	23.8	24.2

*Carbons alpha to nitrogens are expected to be broadened by quadrupolar influence.

nance centered at 176.7 ppm may be assigned to the amide carbon where quadrupolar effects of the adjacent nitrogen are expected to increase the linewidth. The resonance at 68.6 ppm is attributed to the U-2 and U-5 carbon atoms (2); the resonances of those carbon atoms are also not resolved in the solution spectrum. The U-4 carbon contributes to the large resonance which appears at 75.4 ppm; its chemical shift cannot be established any more precisely with the present data. On the basis of the solution data the U-3 resonance is expected to be located between the large resonance at 75.4 ppm (U-4) and the resonance at 68.6 ppm (U-2, U-5), and may correspond to the weak feature at 71.1 ppm.

Chemical shifts for the acetamido residue in the solid state are also similar to those in solution. Furthermore, the ^{13}C chemical shifts of the iduronate moiety in dermatan sulfate are similar to those of the iduronate moiety in heparin, after taking into account the presence of a sulfate group at U-2 in heparin but not in dermatan (2). Independent data on ^{1}H chemical shifts,

Table II. Comparison of observed X-ray spacings for dermatan sulfate with those of Mitra et al.(5)

This Work		Mitra et al.(5)								
		8_3			3_2			2_1		
d(nm)	I_o[a]	d(nm)[b]	hkl	I_o[c]	d(nm)[b]	hkl	I_o[c]	d(nm)[b]	hkl	I_o[c]
1.141	M	1.126	103	M	1.154	011	M	NONE		
0.965	MW	0.960	105	M	0.942	102	W	0.981 0.939	101 002	W VS
0.863- 0.829	VVS	0.896 0.889 0.881 0.871 0.842	110 111 106 112 113	VVS	NONE			0.927	011	W
0.603	MW	0.613 0.613 0.604	103 00.12 119	M	0.617 0.616	210 104	W	0.601	112	S
0.428	M	0.432 0.432 0.430 0.429	21.11 10.16 11.15 225	M	0.426	123	W	0.430 0.430	122 014	M

[a] I_o for this work is visually estimated from the diffraction pattern. W, weak; M, medium; S, strong; V, very.

[b] d values for Mitra et al. were calculated from their lattic constants. NONE indicates no reflections are predicted in the vicinity of our observed d.

[c] I_o for Mitra et al. 8_3 and 3_2 conformers was obtained by forming a composite based on their published structure amplitudes. I_o for the 2_1 conformer of Mitra et al. was visually estimated from the published diffraction pattern.

proton vicinal coupling constants, carbon-proton coupling constants, and the effect of Gd^{3+} on ^{13}C relaxation, all lead to the conclusion that the iduronate ring approximates to the $^1C_4$ (L) conformation in heparin and in dermatan sulfate solution(2). The absence of any significant change in chemical shifts for dermatan sulfate on moving from the aqueous to the solid phase leads us to conclude that this same conformer must also predominate in the solid state.

Our X-ray powder diffraction study of the solid material indicates that the sample is significantly crystalline, with six readily measurable maxima

(Table II). The most intense maximum corresponds to interplanar spacings in the range 0.81-0.87 nm. From the published lattice constants of Mitra et al. (5), it is possible to generate all of the possible spacings for each of the three crystalline polymorphs; i.e., the 8_3, 3_2 and 2_1 conformers packed in their respective lattices. Only the 8-fold form has any diffraction maxima with spacings in the range 0.87-0.82 nm. Similarly, all of the other observed maxima correspond (Table II) to expected values for the 8-fold form.

DISCUSSION

The X-ray diffraction pattern observed in the present experiment is consistent with an 8_3 helix as observed in oriented fibers (5). Thus, the structure present in the unoriented solid state is the same as the structure in the oriented solid state; i.e., the X-ray fiber pattern is not induced by the orientation process. Using the same unoriented solid state sample, we have obtained a ^{13}C nmr spectrum the same as that observed in solution (2), indicating the presence of the $^1C_4$ iduronate ring conformation, or a somewhat distorted variant of it (10), in both solution and the solid state. [The same conclusion was recently drawn from a vacuum ultraviolet circular dichroism study of dermatan sulfate in solution and in solid films (11).]

One might rationalize these results by implying a biphasic system in the solid state, consisting of crystalline domains of the type described previously and amorphous domains in which iduronate moieties assume a distorted form of the alternative $^1C_4$ chair conformation. The X-ray results, however, indicate a substantial degree of crystallinity, and the CP/MAS experiment shows no evidence for two separate domains.

We are therefore left with a preference for the conclusion that the ring conformation is a distorted version of the $^1C_4$ family. The X-ray and modeling studies (5) involved the construction of helices where the individual pyranose rings assume "standard" geometries (12). Whether a distorted $^1C_4$ structure might assume 8_3 or 8_5 fold helices with a fiber repeat of 7.353 nm, while relieving the steric compression of the strictly $^1C_4$ model, has yet to be addressed. We are

informed, however (S. Arnott, personal communication), that a more detailed analysis is currently in progress.

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