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Conformational analysis of heparin epoxide in aqueous solution. An NMR relaxation study

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Abstract

^{1}H and ^{13}C NMR relaxation measurements at various magnetic fields have been used to characterize the nature of overall and internal motions in heparin epoxide in aqueous solution. A two-dimensional homonuclear NOESY experiment showed a considerable number of cross-relaxing protons in the molecule. The inter-proton distances calculated from NOE data were compared with those obtained by molecular mechanics calculations. Several discrepancies between the experimental and the theoretical inter-proton distances as well as the variations in ^{13}C spin-lattice relaxation times, measured at two magnetic fields, indicated that the polysaccharide tumbles anisotropically in solution. The rates of overall and internal motions as well as the order parameters have been calculated using a model-free spectral density function. The numerical values indicate that the correlation times which characterize overall molecular motion are outside the extreme narrowing limit ($\tau_{\parallel} = 8 \times 10^{-10}$ s and $\tau_{\perp} = 4.2 \times 10^{-8}$ s) and that internal motion correlation time is on a picosecond timescale.

Keywords: Conformation; NMR relaxation; Heparin epoxide; Glycosaminoglycan

1. Introduction

Glycosaminoglycan heparin is a negatively charged polysaccharide found in animal tissues and it is well known for its anticoagulant and antithrombotic properties. Heparin, as well as its various derivatives obtained by chemical modifications, have been

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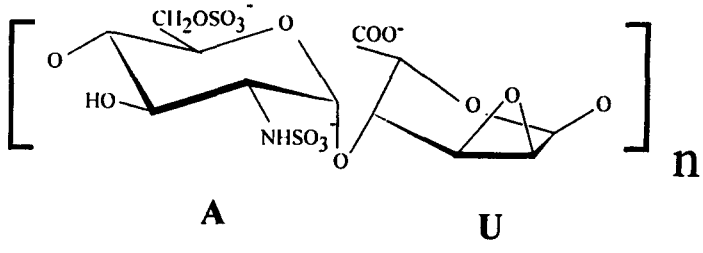

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intensively studied biochemically and structurally in order to understand their biological activity at the molecular level [1]. Recently, a new derivative of heparin — heparin epoxide (**1**) has been prepared by stereospecific epoxidation in alkaline conditions [2,3]. Preliminary biological tests indicate that the desulfation changes antiproliferative and anticoagulant activities [4]. It was found, however, that the decrease of activities is affected not only by the extent of desulfation, but probably by other structural factors as well. It has been suggested [4] that conformational behavior might also influence the interaction with membrane proteins. To test this assumption, an analysis of the conformation and dynamics of this compound in aqueous solution has to be carried out.

In the first part of our study we report the experimental results of the analysis of motional behavior of heparin epoxide in aqueous solution. ^{1}H and ^{13}C NMR relaxation data, collected at various magnetic field strengths, are interpreted with a symmetric top model and the rates of overall and internal motions are estimated.

2. Experimental

Heparin epoxide was synthetized as described previously [2,3]. The compound (M_w ~ 12,000) was purified by ion exchange, lyophilized twice from D_2O (99%) and then dissolved in D_2O (99.996 atom % D_2O, Cambridge Isotope Labs) giving approximately 10 mM solution; pH was adjusted to 7. The solution was bubbled with argon to remove dissolved oxygen and the tube was then sealed.

NMR spectra were measured at 500 MHz on a Bruker AMX 500 spectrometer, at 400 MHz on a Bruker AM 400, and at 300 MHz on a Bruker AC 300. All measurements were performed at 308 K. Two-dimensional (2D) double-quantum-filtered COSY and 2D NOESY [5] were carried out in phase sensitive mode for quadrature detection in F1 dimension using time proportional phase incrementation [6]. The data matrix was 512 × 2K (F1 × F2) and the spectral width 2500 Hz. The spectra were recorded with presaturation of the residual HDO signal. In the NOESY experiments, a 6 s relaxation delay was followed by 2 s of presaturation, and 32 transients were accumulated per fid. The experiments were performed with three different mixing times, 15, 30, and 45 ms at 500.1 MHz. The data matrix was zero-filled to 2K × 2K, and a shifted ($\pi/3$) sine-bell-squared function was applied in both dimensions prior to Fourier transformation. The cross-peak volumes were measured using the UNIXNMR software package running on a

Bruker X32 computer and they were referenced to the cross-peak volume between (A-6) and (A-6′). The inter-proton distances were calculated from data using the extrapolation of the distance to zero mixing time. 2D SNOESY [7] spectra were collected with the same spectral size as 2D NOESY. A 4.8 ms long gaussian pulse was used to invert the resonances of interest, the length of the spin-lock pulse was 22 ms. The total of three cycles were used to create the 198 ms long mixing time. Four data sets were collected applying the semiselective gaussian pulses at different regions in the spectrum.

The pure-absorption HMQC experiment with BIRD sequence [8] was used for the assignment of the carbon signals. The data matrix was $512 \times 2K$ with spectral widths 2500 Hz (F2) and 10 kHz (F1), respectively. A shifted sine-bell-squared function was applied in F2 with exponential filtering in the F1 dimension before Fourier transformation. Spin-lattice and spin–spin relaxation times were measured in a 10 mm probe on non-spinning samples at 75.8 MHz, the T_1 data were also measured at 100.6 MHz. For measurements of longitudinal relaxation times the inversion–recovery method was used with 15 τ values. A total of 1280 scans were accumulated for each τ value; the recycle time was 1.5 s. For determination of spin–spin relaxation times the Carr–Purcell–Meiboom–Gill (CPMG) [9] sequence was used with eight different lengths of the CPMG pulse train. The exponential filtering and zero-filling were used before Fourier transformation. T_1 and T_2 relaxation times were calculated with three-parameter fit to the experimental data for T_1 and with two-parameter fit for T_2 using Bruker software. The standard deviations of the fit were less than 1% for T_1 and 1–4% for T_2. The analytical expressions relating the spectral density functions to the relaxation times are given by [10]

$$1/T_1 = (1/10)(\mu_o/4\pi)^2\gamma_H^2\gamma_C^2(h/2\pi)^2 < r_{C-H}^{-6} > \left[J^0(\omega_H - \omega_C) + 3J^1(\omega_C) + 6J^2(\omega_H + \omega_C)\right], \quad (1)$$

$$1/T_2 = (1/20)(\mu_o/4\pi)^2\gamma_H^2\gamma_C^2(h/2\pi)^2 < r_{C-H}^{-6} > \times \left[4J^0(0) + J^0(\omega_H - \omega_C) + 3J^1(\omega_C) + 6J^2(\omega_H + \omega_C) + 6J^0(\omega_H)\right]. \quad (2)$$

The homonuclear cross-relaxation rate is given by [11]

$$\sigma_{ij} = (1/10)(\mu_o/4\pi)^2\gamma_H^4(h/2\pi)^2 < r_{H-H}^{-6} > \left[6J^2(\omega_H) - J^0(\omega_H)\right]. \quad (3)$$

The relaxation data were analyzed within the model free approach [12]. In this case, for an isotropically tumbling molecule the spectral density function has the form

$$J_{IS}(\omega) = \frac{S^2\tau_o}{1 + (\tau_o\omega)^2} + (1 - S^2)\frac{\tau}{1 + (\tau\omega)^2}, \quad (4)$$

where τ_o is the tumbling time of the molecule, $1/\tau = 1/\tau_o + 1/\tau_e$ and τ_e is the effective correlation time. S^2 is the generalized order parameter which characterizes the

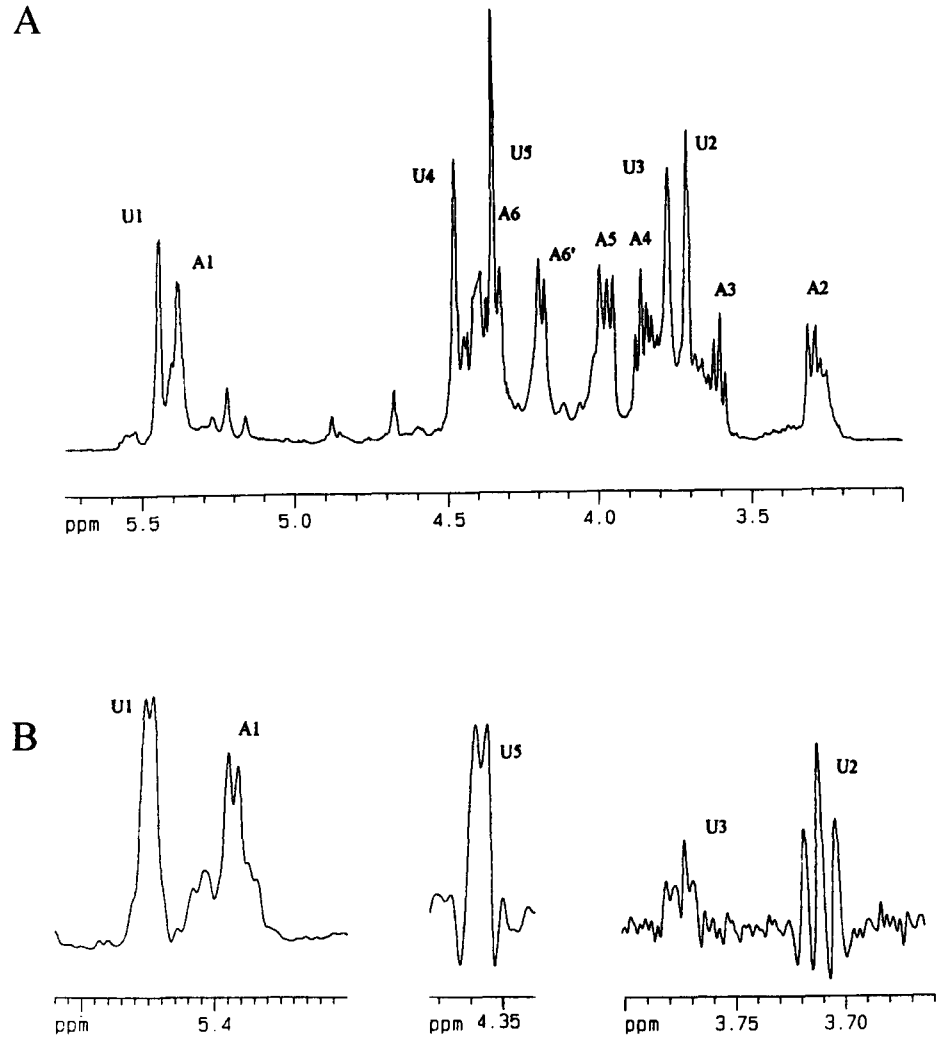

Fig. 1. (A) ^{1}H-500 MHz NMR spectrum of the heparin epoxide in aqueous solution at 313 K. Chemical shifts are referenced to external DSS. (B) Expanded regions with signals of the uronic acid ("epoxide") residue. Lorenzian-to-Gaussian filtering functions were used prior to Fourier transformation.

spatial restriction of internal motions. If the molecule is tumbling anisotropically and it can be approximated with the symmetric top model [13], the resulting spectral density $J_{IS}(\omega)$ can be approximated in the form [14]

$$J_{IS}(\omega) = S^2 J_{an}(\omega) + (1 - S^2)\frac{\tau}{1 + (\tau\omega)^2}, \quad (5)$$

where

$$J_{an}(\omega) = 0.25(3\cos^2\theta - 1)^2 \tau_a / \left[1 + (\omega\tau_a)^2\right] + (3\sin^2\theta\cos^2\theta)\tau_b / \left[1 + (\omega\tau_b)^2\right], + 0.75(\sin^4\theta)\tau_c / \left[1 + (\omega\tau_c)^2\right]$$

and

$$\tau_a = \tau_\perp$$
$$1/\tau_b = 5/(6\tau_\perp) + 1/(6\tau_\parallel),$$
$$1/\tau_c = 1/(3\tau_\perp) + 2/(3\tau_\parallel)$$

where $\tau_\parallel$ and $\tau_\perp$ are the correlation times about the long and short axis, respectively, and θ is the angle between the C–H relaxation vector and the symmetry axis.

Computed data have been obtained by molecular mechanics calculations with an updated force field (see accompanying paper by Ferro et al.).

3. Results and discussion

The ^{1}H NMR spectrum of heparin epoxide in aqueous solution is shown in Fig. 1A; the expanded regions with signals from the uronic acid ("epoxide") residue of the resolution enhanced spectrum are presented in Fig. 1B. ^{1}H Chemical shifts and the three-bond proton–proton coupling constants are given in Table 1. Proton chemical shifts agree with those published [2] and the $^3J_{H-H}$ between the H-2 and H-3 (~ 3.6 Hz) in the epoxide unit agrees with the *cis* arrangement between the oxirane and the α glycosidic linkage of the pyranose ring [15]. This conformation is also supported by the 3 Hz coupling constant between the H-1 and the H-2; in the *trans* conformation the value of $^3J_{(H\text{-}1)\text{-}(H\text{-}2)}$ can be expected to be significantly higher since the H-1 and the H-2 protons would be approximately antiperiplanar. The three-bond coupling constants also indicate that the pyranose ring of the uronic acid departs from $^4C_1$ conformation, and the magnitudes agree with the assumption of half-chair conformation [15]. The measured

Table 1
^{1}H chemical shifts (δ, ppm, referenced to external DSS) and three-bond proton–proton coupling constants (J, values in Hz, ± 0.3 Hz) of **1** in aqueous solution (pH 7) at 308 K

	δ(H-1)	δ(H-2)	δ(H-3)	δ(H-4)	δ(H-5)	δ(H-6)	δ(H-6′)
U	5.45	3.71	3.78	4.48	4.35		
A	5.39	3.30	3.61	3.86	3.96	4.19	4.34
	J_{12}	J_{23}	J_{34}	J_{45}	J_{56}	$J_{56'}$	$J_{66'}$
U	3.1	3.6	2.0	2.0			
A	3.8	10.2	8.8	9.6	a	4.2	12.4

[a] Not resolved.

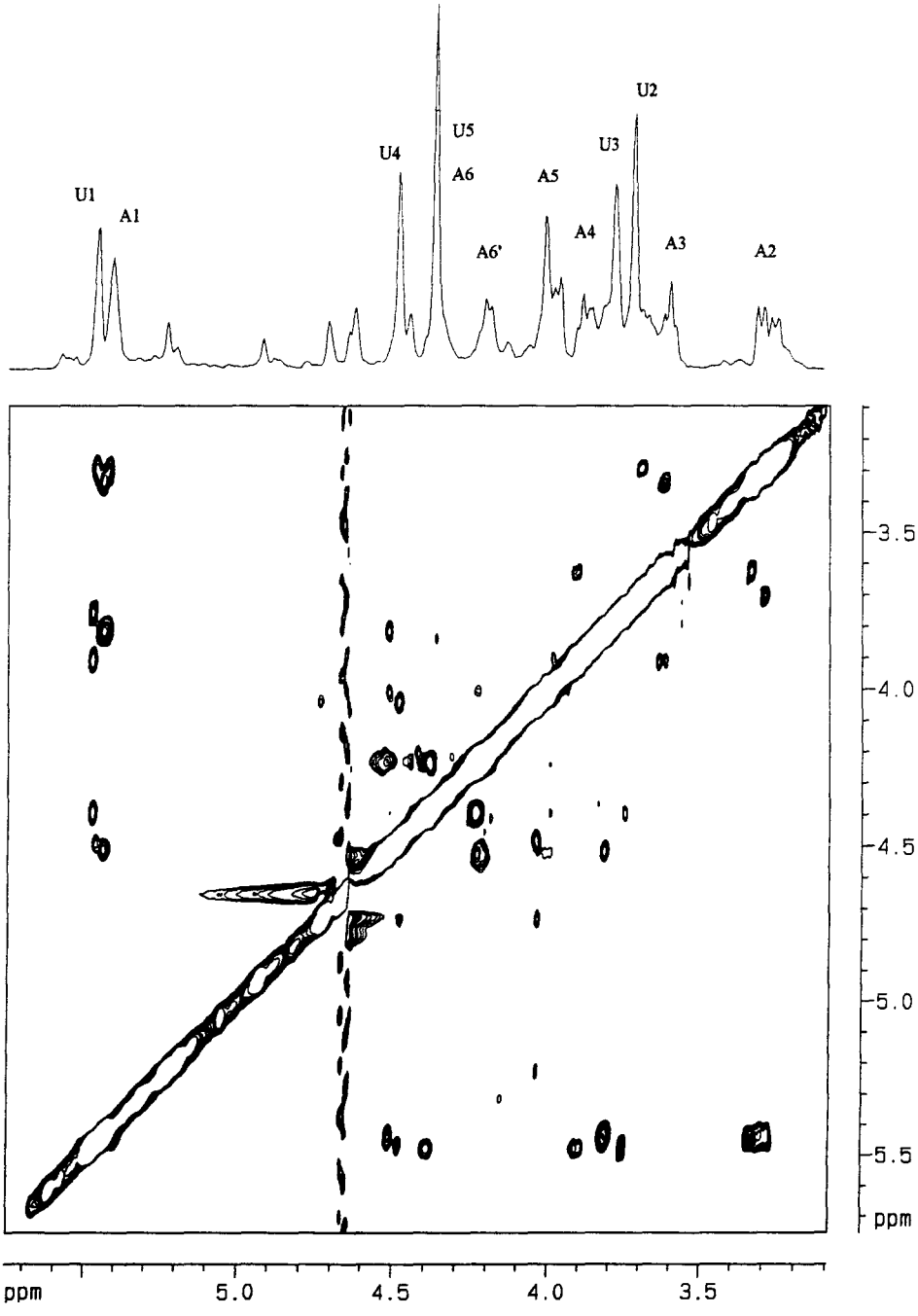

Fig. 2. 500 MHz phase sensitive 2D NOESY spectrum of heparin epoxide in aqueous solution collected with presaturation of the residual HOD during relaxation delay. The spectrum was measured with 15 ms mixing time.

magnitudes of the $^{3}J_{(H-3)-(H-4)}$ and the $^{3}J_{(H-4)-(H-5)}$ (both ~ 2.0 Hz) support the prevalence of $^{5}H_{0}$ conformer. The higher stability of the $^{5}H_{0}$ conformer has been recently suggested [16] based on molecular mechanics calculations and this was also confirmed in our study. The values of coupling constants in the glucosamine residue agree with $^{4}C_{1}$ conformation of the pyranose ring. It was not possible to determine $^{3}J_{(H-5)-(H-6)}$ with sufficient precision due to the linewidth and the spectral overlap, and consequently, the conformation on the (C-5)–(C-6) linkage could not be fully analyzed. The slightly longer N T_1 value for C-6, observed at both magnetic field strengths, compared with the other ring carbons (see later analysis) might suggest some degree of motional freedom in this linkage, however, more quantitative conclusions, i.e. an estimation of the relative abundances of gg, gt and tg conformers, cannot be drawn from the present experimental data.

The 2D NOESY spectrum of the epoxide derivative, collected with 15 ms mixing time, is shown in Fig. 2. A considerable number of intra- and inter-residue NOEs have been detected in the spectrum even at very short mixing times. In addition to cross-relaxation of the anomeric protons and those on the glycosidic linkage, i.e. (U-1)–(A-4) and (A-1)–(U-4), we also observed dipolar interactions among the protons on the glycosidic bond and other ring hydrogens. For example, quite strong interactions were detected between the U-3 and the A-1 protons, the U-4 and the A-5 and also between U-1 and A-5, A-6, and A-6′. A strong dipolar interaction was also observed between U-4 and A-6′. Since the latter interaction was rather unexpected and could not be interpreted by molecular mechanics calculations the origin of this cross-peak was checked by an independent experiment. Very short mixing times in 2D NOESY experiments, i.e. 15, 30 and 45 ms were used in order to minimize the effect of indirect magnetization transfer. However, to support the assumption that spin diffusion did not

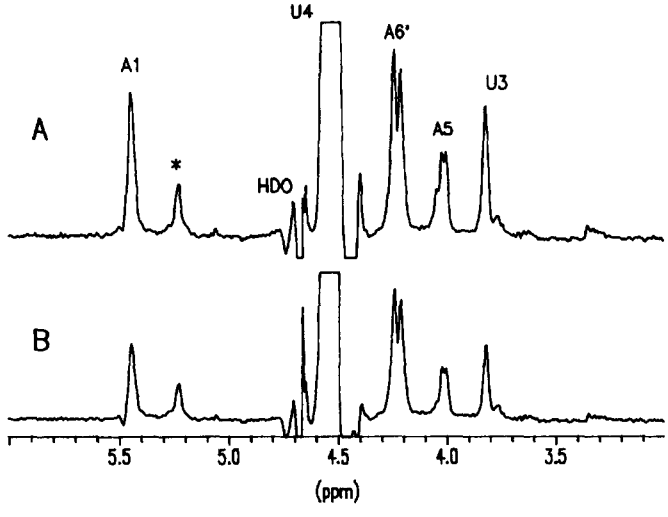

Fig. 3. Cross-sections, parallel to ω_2, through the U-4 signal from 500 MHz 2D NOESY (A) and 2D SNOESY (B) spectra. The resonance depicted with asterisk originates from impurity.

Table 2
Time-averaged distances (in Å) between protons in heparin epoxide based on the 2D NOESY spectra, collected with three different mixing times at 308 K in aqueous solution at 500.1 MHz. The spectral density function for isotropic motion was used in the calculation of the distances [a]

	A-1	A-2	A-3	A-4	A-5	A-6	A-6′	U-1	U-2	U-3	U-4	U-5
A-1												
A-2	2.5 [b]											
A-3												
A-4		2.5										
A-5			3.0									
A-6					2.4							
A-6′					2.3	1.85						
U-1				2.3	3.0	2.4	3.3					
U-2						3.0		2.2				
U-3	2.0											
U-4	2.3				2.7		2.3			2.4		
U-5												

[a] Reference distance A-6–A-6′: 1.85 Å.
[b] Affected by impurities.

affect the cross-peak intensities in the spectra, NOE spectra were also recorded using the pulse sequence which allowed us to eliminate these effects [7]. Thus, we ran a set of 2D SNOESY experiments where protons in different regions were selectively inverted by semiselective 180° pulses. For example, when inverting the region close to the U-4 signal the 2D spectrum was detected whose cross-section, parallel to ω_2 (through the U-4 signal), is illustrated in Fig. 3B. Direct comparison with the cross-section (through the U-4 signal) in the 2D NOESY spectrum (Fig. 3A) shows that the (U-4)–(A-6′) cross-peak originates from direct transfer of magnetization and also that two other signals, A-5 and U-3, in the 2D SNOESY spectrum have comparable relative intensities. Thus there is no measurable contribution from spin-diffusion in the cross-peak volumes. Comparable intensities in 2D NOESY experiments were also observed for other signals in different sets of SNOESY experiments indicating that the mixing times used in the NOESY experiments were short enough to prevent the indirect transfer of magnetization. The experimental inter-proton distances have been obtained using the reference distance of 1.85 Å between the (A-6)–(A-6′) protons, and they are listed in Table 2. The (A-6)–(A-6′) cross-peak has been chosen as the reference because it is well-separated in the spectrum and it belongs to the strongest cross-peak. The other potential reference cross-peak, (A-1)–(A-2), overlapped with the cross-peak originating from impurities and was thus discarded. The intra-unit cross-peaks in the epoxide residue were not considered as suitable for reference because although the analysis showed that the $^5H_0$ conformer prevailed, a contribution from the $^0H_5$ conformer could not be ruled out. It must also be noted that the spectral density function for the rigid, isotropically tumbling molecule has been used for the calculation. Some of the inter-proton distances could be reproduced by the molecular modeling, however, several discrepancies between the experimental and the computed data were also observed within the monosaccharide units. For example, the experimental distance of 3.0 Å between A-3 and A-5 is longer

Table 3
^{13}C chemical shifts (δ, ppm, referenced to external TSP), spin-lattice and spin–spin relaxation times (values in ms) of **1** collected at two magnetic field strengths 9.4 T (T_1(100.6)) and 7 T (T_1(75.8)) in aqueous solution at 308 K

	δ	T_1(100.6)	T_1(75.8)	T_2(75.8)
A-1	99.0	259	198	24
A-2	60.6	265	211	20
A-3	72.6	[a]	[a]	[a]
A-4	79.8	269	216	20
A-5	71.8	[a]	[a]	[a]
A-6	69.1	151	122	12
U-1	97.5	292	227	17
U-2	54.5	269	211	17
U-3	53.5	254	189	21
U-4	72.7	[a]	[a]	[a]
U-5	71.8	[a]	[a]	[a]
U-6	177.9	[b]	[b]	[b]

[a] Not resolved.
[b] Not measured.

than calculated (2.5 Å). Similarly, the distances between the U-3 and U-4 protons in the epoxide residue differ from each other—the experimental distance is 2.4 Å, the calculated 2.6 Å. However, higher deviations were obtained in distances across the linkages. The most unexpected experimental distance was obtained between the U-4 and A-6′ protons (2.3 Å). This very short distance could not be reproduced by any conformation of the glycosidic linkage, of the (C-5)–(C-6) linkage (whose calculated distance was 3.3 Å), or by time-averaged distances based on the weighted average from more conformations, even taking into account the high energy conformers. The inappropriate application of the spectral density function (for isotropic motion of a rigid molecule) in the calculation of the distances from the experimental data could be a possible explanation for the above disagreement.

The ^{13}C chemical shifts, and spin-lattice and spin–spin relaxation times for **1**, collected at 100.6 MHz and 75.8 MHz, are listed in Table 3. Carbon chemical shifts agree with those published [2], except that for U-5, where the value of 71.8 ppm was found instead of 78.3 ppm as shown in the ^{13}C spectrum of Fig. 4. T_1 values collected at 75.8 MHz ranged from 190 ms up to ~230 ms for the methine carbons and for the methylene carbon T_1 was 122 ms. Spin-lattice relaxation times obtained at 100.6 MHz were between 254 ms and 292 ms for the C–H carbons; for the CH_2 carbon we obtained the value of 151 ms. The spin–spin relaxation times, measured by CPMG spin-echo at 75.8 MHz, were considerably shorter than T_1 indicating slow overall molecular motion and they varied from 17 to 24 ms for C–H carbons; for the CH_2 carbon the value of T_2 was 12 ms.

A precise interpretation of the relaxation data requires an appropriate theoretical model which characterizes overall molecular tumbling and internal motions. A number of models for both isotropic and anisotropic overall motions have been proposed, with or

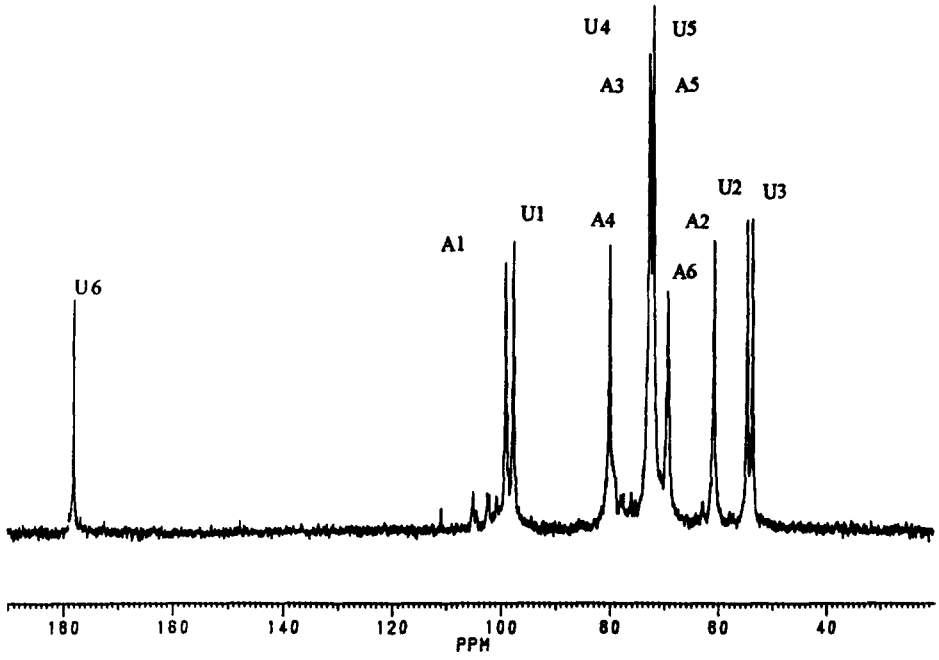

Fig. 4. ^{13}C 75.8-MHz spectrum of the heparin epoxide. The spectrum was obtained with 5500 scans, the signals were referenced to external DSS.

without internal motions [12–14]. However, some of these models are not applicable in our case. Several theoretical approaches are rather complicated, i.e. there are too many parameters to be determined in order to fit the experimental data—typically for molecules with no axial symmetry. Some other models have been developed for different types of molecules with specific chain arrangement [14], and therefore they cannot correctly interpret relaxation data of polysaccharides. In addition, if internal motions are expected in the molecule, the polysaccharide chain is less likely to adopt an ordered shape and a random coil type conformation might be present in solution. Both hydrogen bonds and the solvent effects have influence on the conformation as well, thus the resulting shape of the polysaccharide in solution depends on which of these effects is dominant [17]. Consequently, the choice for the appropriate spectral density function is not trivial since the exact shape of the molecule is usually not known and some feasible theoretical model should be applied. We have tested several models for motional behavior of the polysaccharide and have computed NMR parameters using the appropriate spectral density functions and compared the results with the experimental data. The model for a rigid, isotropically tumbling molecule could not interpret the measured ^{13}C T_1 and T_2 values. The values of the computed parameters ^{13}C T_1 ($T_1(100.6) \sim 240$ ms, $T_1(75.8) \sim 150$ ms) and T_2 ($T_2(75.8) \sim 70$ ms) for overall correlation time $\tau_o \sim 3$ ns were found to be the closest compared to the experimental values ($T_1(100.6) \sim 270$ ms,

$T_1(75.8) \sim 210$ ms) and T_2 ($T_2(75.8) \sim 20$ ms), however, the difference between these two data sets was large enough to demonstrate that this simple model is not appropriate for the description of motional properties of heparin epoxide. Application of this model to the calculation of inter-proton distances based on $^1H-^1H$ NOEs also explained the observed discrepancies between the experimental and computed data. Similarly, calculated relaxation times using spectral densities within the model free approach [12] for a flexible, isotropically tumbling molecule were not comparable with the observed values. The smallest differences were for $\tau_o \sim 4$ ns, $S^2 \sim 0.8$ and $\tau_c \sim 200$ ps: $T_1(100.6) \sim 310$ ms, $T_1(75.8) \sim 200$ ms and $T_2(75.8) \sim 65$ ms. Finally, since heparin epoxide is a linear $(1 \rightarrow 4)$ linked polysaccharide, we assumed that the shape of the molecule could be considered as a prolate ellipsoid (assuming that the main axis of rotation is along the polysaccharide chain spanning the glycosidic oxygens and the C-1 and C-4 carbons in the pyranose units) and we used the spectral density function for a symmetric top model. Using spectral density functions for this model of the rigid molecule with $\tau_{\parallel} = 2$ ns, $\tau_{\perp} = 40$ ns and $\theta = 90°$, the following values were obtained: $T_1(100.6) \sim 300$ ms, $T_1(75.8) \sim 200$ ms and $T_2(75.8) \sim 21$ ms. Though these values adequately interpret the carbon relaxation times, differences were observed for proton cross-relaxation rates. The value of $\sigma \sim -3$ s^{-1} was obtained which was not comparable with either of the observed cross-relaxation rates of protons (A-3)–(A-5) ($\sigma_{35} = -1.4$ s^{-1}) or (A-2)–(A-4) ($\sigma_{24} = -0.8$ s^{-1}). As mentioned, the angle θ was set to 90°, assuming that such an orientation of the C–H relaxation vectors and the (A-3)–(A-5) vector, with respect to the symmetry axis, might be considered as a reasonable approximation. Experimental evidence for the value of the θ angle close to 90° for C–H vectors has been reported in a recent study of amylose conformation [18]. A more accurate determination of orientations of individual relaxation vectors could possibly be obtained using sophisticated relaxation models but these require considerably more experimental data. Even in that case, the precision of the orientations of relaxation vectors obtained from such an approach still remains questionable because the exact shape of the molecule in solution and the precise orientation of its symmetry axis are unknown. We consider that employing these approximations is a necessary compromise between the feasibility of the experiment and the physical picture of heparin epoxide. The best fit with the experimental data was obtained when we introduced the internal motions within the symmetric top model. The model-free spectral density function has been used for fitting since it is relatively simple for evaluation and it has been derived without the assumption of any particular structural arrangement. Thus, we generated the following parameters: $T_1(100.6)$, $T_1(75.8)$, $T_2(75.8)$, $\sigma_{35}(500)$ and $\sigma_{24}(500)$ for a wide range of values of $\tau_{\parallel}$, $\tau_{\perp}$, τ_e and S^2 using the eqs 1, 2, 3, and 5. The following values gave the best fit to the experimental data: $\tau_{\parallel} = 0.8$ ns, $\tau_{\perp} = 42$ ns, $\tau_e = 150$ ps, $S^2_{C-H} = 0.65$ and $S^2_{A2-A4} = 0.62$ for which $T_1(100.6) = 276$ ms, $T_1(75.8) = 222$ ms, $T_2(75.8) = 33$ ms, $\sigma_{35} = -1.7$ s^{-1} (with $\theta = 90°$), and $\sigma_{24} = -0.85$ s^{-1} (with $\theta = 45°$). The values of the correlation times $\tau_{\parallel}$ and $\tau_{\perp}$ indicate quite strong motional anisotropy of heparin epoxide in aqueous solution where the ratio $\tau_{\perp}/\tau_{\parallel}$ is ~ 50. Both values of $\tau_{\parallel}$ and $\tau_{\perp}$ are outside the extreme narrowing limit. The calculated magnitude of the internal motion correlation time, τ_e, is 150 ps, i.e. considerably shorter than either of the overall correlation times. It must be also mentioned that the effect of cross-correlation between

dipolar and chemical shift anisotropy relaxation mechanisms [19] has been neglected in the analysis. Furthermore, since the sample was naturally polydisperse, the assumption of single correlation times $\tau_{\parallel}$ and $\tau_{\perp}$ for all molecules with a different chain length is not justified. Thus, the experimentally obtained motional parameters correspond to an average of the ensemble of molecules with various molecular sizes with a mean value of $M_w \sim 12{,}000$.

Recently, theoretical conformational analysis of the pentasaccharide corresponding to the binding site of the heparin for antithrombin III has been carried out [20]. Several energy minima, obtained by molecular mechanics calculations, were found on the Ramachandran-type ϕ,φ map. The experimental $^1H-^1H$ NOE data were mostly consistent with the calculated values, however, several discrepancies were also observed. Differences between the computed and the experimental NOEs were found both within the monosaccharide unit as well as across the glycosidic linkage. Other comprehensive experimental analyses using relaxation measurements of this pentasaccharide, carried out at various magnetic field strengths [21], showed that the full interpretation of the data requires consideration of anisotropic overall molecular motion in addition to the presence of internal motions about the glycosidic linkages. This situation seems analogous to the present case where in heparin epoxide we observed differences between the experimental and the calculated NOEs, when the spectral density function for isotropic motion was used. Other observations supporting the overall anisotropic motion of polysaccharides have also been reported [22,23]. For heparin in aqueous solution the axial ratio found was 50 [23], i.e. nearly the same value as in heparin epoxide found in this study. The similar molecular size, and therefore the chain length, which probably dominate the overall motional properties of these molecules in solution, is likely very similar and might be the cause of this behavior. The magnitudes of correlation times, however, are remarkably different. For heparin epoxide, the magnitudes of the $\tau_{\parallel}$ and $\tau_{\perp}$ were found to be longer as a consequence of considerably lower experimental temperature and higher concentration. The same ratio of $\tau_{\perp}/\tau_{\parallel}$ data might suggest that the overall shape of both molecules is similar in aqueous solution. However, the rate of internal motions on the glycosidic linkages and the values of the order parameters could not be compared because these data have not been presented for heparin.

In summary, in this first part of our study, the experimental NMR analysis was focused on the characterization of the dynamics of heparin epoxide in aqueous solution. We have used ^1H NOE data in combination with ^{13}C relaxation rates measured at different magnetic field strengths. The NMR-derived interproton distances, using spectral densities for isotropic motion, were mostly consistent with the distances obtained in molecular mechanics calculations. In several cases, however, a considerable discrepancy between the experimental and the computed distances was observed. A good fit of the experimental data was possible only if we used spectral density function for a symmetric top model. The above evidence therefore supported the assumption that the overall motion of the polysaccharide must be considered as anisotropic. In addition, the full interpretation of the experimental data required evaluation of internal motions of the glycosidic linkages suggesting that heparin epoxide is a flexible polymer in aqueous solution. Finally, the numerical values of correlation times indicate that the rate of internal motion is on a picosecond timescale.

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