Effect of Cross-Correlation between Dipolar and Chemical Shift Anisotropy Relaxation Mechanisms upon the ¹³C Relaxation Rates in Pentasaccharide Fragment from Heparin

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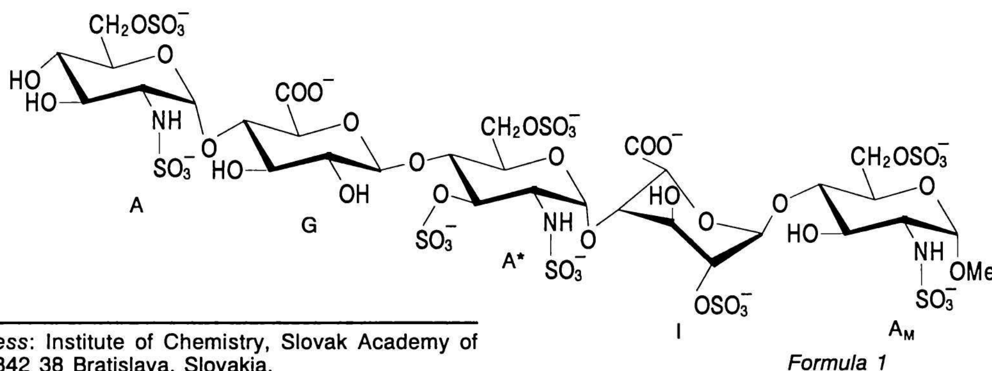
Received 7 January 1994

Nuclear magnetic resonance (NMR) spectroscopy is one of the most convenient methods for studying motional properties of molecules in solution [1]. NMR relaxation data contain information regarding the rates of overall and internal motions in chemical systems, and over the past two decades a number of studies dealt with dynamics of molecules in solution, especially those with biological activity. The interpretation of the homonuclear NMR relaxation data was usually based on Solomon's equations [2], though these were intended to describe the exchange of the two longitudinal Zeeman terms due to cross-relaxation in a system only with two spins. In the two-spin system consisting of unlike 1/2 spins (I, S), relaxed both by intramolecular dipolar (DD) and chemical shift anisotropy (CSA) interactions, however, the two-spin order state can be created due to cross-correlation effect between DD and CSA relaxation mechanisms [3]. In this case, the time dependence of the longitudinal magnetization modes is described by the following equation [4]

$$d[S_z(t)]/dt = -[\sigma_{IS}\,\Delta I_z + (\rho_S + \rho_{CSA})\,\Delta S_z + \mu_{IS,S}\,2\Delta I_z\,S_z] \qquad (1)$$

where Δ represents a deviation from thermal equilibrium. ρ_S and σ_{IS} are the spin-lattice relaxation rate and cross-relaxation rate [2], respectively, ρ_{CSA} is the chemical shift anisotropy term, and $\mu_{IS,S}$ is the cross-correlation term [3, 4]. Similar expressions exist for transverse relaxation rates [5]. It has been generally accepted that cross-correlation influences the recovery of magnetization only negligibly and thus this phenomenon has been largely neglected. However, rigorous theoretical analysis [4] and some recent experimental studies demonstrated that this universal practise was not always justified [6, 7]. For example, it was found that in some proteins the interference effect can influence ^{15}N spin-lattice and spin-spin relaxation rates up to 10 % and 25 %, respectively [7]. Since heteronuclear relaxation data are often used to derive overall molecular and internal motion correlation times, this relatively large effect may affect the obtained data, if not considered properly. Consequently, the estimated motional properties of biomolecules are not precise. The above-mentioned observations of interference effects in proteins have prompted us to estimate the extent of DD—CSA cross-correlation in ^{13}C relaxation data for pentasaccharide AGA*IA$_M$ (Formula 1) since, to our knowledge, these effects have not been quantified in heteronuclear relaxation data of carbohydrates. Dynamics in the pentasaccharide is also of great interest due to its biological properties — the molecule



Formula 1

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Table 1. ^{13}C NMR T_1 and T_2 Data for the Pentasaccharide AGA*IA$_M$ in Aqueous Solution Recorded at 11.7 T and 298 K

Saccharide unit	Atom	δ	$^{13}C\ T_{1cc}/s$	$^{13}C\ T_1/s$	$^{13}C\ T_{2cc}/s$	$^{13}C\ T_2/s$
A	C-1	99.65	0.33	0.29	0.24	0.16
	C-2	60.06	*	*	*	*
	C-3	73.21	0.32	0.29	0.24	0.17
	C-4	71.01	0.31	0.28	0.22	0.17
	C-5	71.79	0.31	0.28	0.23	0.17
	C-6	68.60				
			0.318 (0.010)	0.285 (0.006)	0.233 (0.010)	0.168 (0.005)
G	C-1	103.30	0.28	0.25	0.25	0.18
	C-2	74.84	*	*	*	*
	C-3	78.17	0.29	0.26	0.24	0.19
	C-4	78.91	0.30	0.26	0.22	0.18
	C-5	79.14	0.30	0.26	0.22	0.17
			0.293 (0.010)	0.258 (0.005)	0.233 (0.015)	0.180 (0.008)
A*	C-1	98.13	0.27	0.22	0.19	0.16
	C-2	58.74	*	*	*	*
	C-3	78.26	0.28	0.23	0.21	0.17
	C-4	74.90	0.25	0.23	0.20	0.18
	C-5	71.61	0.26	0.23	0.22	0.17
	C-6	68.31				
			0.265 (0.013)	0.228 (0.005)	0.205 (0.013)	0.170 (0.008)
I	C-1	101.68	0.28	0.25	0.18	0.14
	C-2	79.40	0.29	0.25	0.21	0.17
	C-3	72.50	0.29	0.24	0.21	0.17
	C-4	78.12	0.30	0.26	0.21	0.18
	C-5	72.43	0.28	0.24	0.20	0.17
			0.288 (0.008)	0.248 (0.008)	0.202 (0.013)	0.166 (0.015)
A$_M$	C-1	100.29	0.34	0.28	0.23	0.16
	C-2	59.79	*	*	*	*
	C-3	71.85	0.35	0.30	0.24	0.18
	C-4	78.37	0.36	0.30	0.24	0.18
	C-5	70.60	0.34	0.30	0.23	0.17
	C-6	69.05				
			0.348 (0.010)	0.295 (0.010)	0.235 (0.006)	0.173 (0.010)

* Not determined due to overlap. Chemical shifts δ are referenced to internal acetone (δ = 30.0). Longitudinal relaxation times (column 4, $^{13}C\ T_{1cc}$), recorded without suppression of cross-correlation between dipolar and CSA relaxation mechanisms, longitudinal relaxation times (column 5, $^{13}C\ T_1$), recorded with suppression of cross-correlation, transversal relaxation times (column 6, ^{13}C T_{2cc}), recorded without suppression of cross-correlation between dipolar and CSA relaxation mechanisms, transversal relaxation times (column 7, $^{13}C\ T_2$), recorded with suppression of cross-correlation. Averaged values and standard deviations are listed in the last row for each residue.

corresponds to the binding site of heparin and it specifically binds with a plasma protein antithrombin III leading to anticoagulant and antithrombotic activity.

^{13}C spin-lattice and spin-spin relaxation times have been determined with two-dimensional double INEPT [8]. Six different relaxation delays were used in T_1 experiments, namely, 20 ms, 70 ms, 120 ms, 200 ms, 300 ms, and 450 ms. In the T_2 experiments the CPMG (Carr—Purcell—Meiboom—Gill) pulse trains were 6 ms, 24 ms, 48 ms, 72 ms, 96 ms, 150 ms, and 270 ms long. The cross-peak volumes were measured in each T_1 and T_2 experiment using the UNIXNMR software package running on a Bruker X32 computer. T_1 and T_2 relaxation times were also measured with modified sequences [7] to suppress the effect of cross-correlation between dipolar and chemical shift anisotropy relaxation mechanisms where a train of 180° proton pulses was applied during the relaxation interval or during the CPMG spin-echo, respectively, leading to an interchange of multiplet components in the spin system. T_1 and T_2 values were then calculated from a two-parameter best fit to the experimental cross-peak volumes using the nonlinear Levenburg—Marquart algorithm. Standard deviations of the fit were typically 1—2 %, in cases where cross-correlation effects were neglected and the decays were fitted to a single exponential, standard deviations varied between 3—8 %. Other experimental details will be described in a full paper on the dynamics of the pentasaccharide [9].

The numerical values of ^{13}C spin-lattice and spin-spin relaxation times for AGA*IA$_M$ are listed in Table 1. $^{13}C\ T_{1cc}$ values, the longitudinal relaxation times collected without suppression of interference effects,

varied between 0.25 s and 0.36 s for different carbons in the monosaccharide units. ^{13}C T_1 values, spin-lattice relaxation times recorded with suppression of DD—CSA cross-correlation, are shown in column 3. These values ranged from 0.22 s up to 0.30 s, thus all longitudinal times were considerably (10—15 %) shorter than the T_{1cc} values. This rather large and unexpected effect of interference, observed in the pentasaccharide, is even stronger than that detected in several proteins. This phenomenon can, however, be explained by the smaller molecular size of AGA*IA$_M$, compared to the size of proteins investigated. In such case, where the longitudinal relaxation rate approaches the T_1 minimum, the C—H spin flip rate is comparable with the rate of decay of multiplet components and thus DD—CSA interference is considerable. Even higher differences were observed in spin-spin relaxation rates. ^{13}C T_{2cc} relaxation times (without suppression of cross-correlation) varied between 0.18 s and 0.25 s, ^{13}C T_2 (with suppression of interference) values were 0.14 s—0.19 s, thus the effect was about 20—25 % in this case and it was found to be comparable with that observed in proteins [7, 8]. There were also variations in the magnitudes of longitudinal relaxation times between the individual monosaccharide units, which indicate anisotropic overall molecular motion. However, more detailed description of dynamics of the molecule in aqueous solution will be presented elsewhere [9].

As it can be expected, the neglect of relatively large influence of DD—CSA cross-correlation is also transferred into derived correlation times and into other parameters which characterize dynamics of biomolecules in solution, if calculated from ^{13}C data. The present data thus indicate that the interpretation of ^{13}C relaxation data in oligosaccharides, collected without suppression of these effects, may lead to a partially biased motional picture of studied molecules in solution. Whether the phenomenon of DD—CSA interference is important also in carbohydrate polymers is currently under investigation.

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Translated by M. Hricovíni