Detection of Internal Motions in Oligosaccharides by ^1H Relaxation Measurements at Different Magnetic Fields‡

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ABSTRACT: The effect of internal motions on proton relaxation data in oligosaccharides has been investigated experimentally. ^1H steady-state and transient NOEs together with ^{13}C T_1's have been measured at two magnetic field strengths. The existence of internal motions leads to additional modulations of the dipolar interaction between proton pairs, thus producing a range of spectral density functions for these interactions. As a result, it is possible to show that protons relaxing through fixed distances have a different ratio of relaxation parameters, acquired at 500 and 300 MHz, compared to those relaxing through fluctuating distances. This approach has been used to unequivocally establish for two disaccharides the existence of internal motions on the time scale of the overall tumbling.

Internal motions in biomolecules in solution have been the subject of considerable interest in recent years. In NMR spectroscopy, relaxation parameters and three-bond coupling constants are the main sources of information on the dynamics of molecules. In conjunction, molecular dynamics (MD)[1] simulations and/or other computational methods are often used to interpret the experimental data.

Internal motions in proteins and DNA have been the commonest situations studied (Allerhand et al., 1971; Clore et al., 1990; Kay et al., 1989; Keepers & James, 1982; Koning et al., 1991; Krishnan et al., 1991; Levy et al., 1981a; London & Avitabile, 1978; Post, 1992; Ribeiro et al., 1980). Using ^{13}C relaxation data and MD simulations, slow (nanosecond) and fast (picosecond) fluctuations have been described, despite the relatively close-packed structure of proteins. Internal motions increased the values of ^{13}C T_1's in flexible regions by 20–300%, compared to values from rigid parts of the protein molecule (Levy et al., 1981b). Proton NMR has also been used to estimate the correlation times of internal motions. For isotopically unlabeled compounds, ^1H NMR has considerably higher sensitivity, though the analysis is more difficult. In particular, spin diffusion complicates structural analysis based on NOE data and must be considered explicitly. Inter-proton distances in flexible parts of molecules can only be estimated from relaxation data by assuming a model for the nature of internal dynamics, and only then for particular situations (Lane et al., 1986; Lane, 1988; Olejniczak et al., 1984).

Contradictory conclusions have been drawn from studies of the dynamics of glycosidic linkages in oligosaccharides in solution. Some analyses, based on ^1H T_1, steady-state NOE, and NOESY measurements, interpreted with HSEA, MM2, or MD calculations, have concluded that a single well-defined conformation (comparable to those found in the solid state) exists in solution for sucrose, Lewis blood group oligosaccharides, and other biologically important saccharides (Bock & Lemieux, 1982; Cagas & Bush, 1990; Edge et al., 1990; McCain & Markley, 1986; Sabesan et al., 1991; Thogersen et al., 1982; Kovacs et al., 1989). Similar conclusions have been drawn regarding the conformations of mannose-containing oligosaccharides and substituted blood-group disaccharides (Homans et al., 1982; Kline et al., 1990). However, other interpretations of the theoretical and experimental data support the existence of significant populations of more than one conformer in solution. In some cases, the solvent and temperature have been shown to affect the relative abundance of the conformers (Belton et al., 1991; Carver et al., 1990; Cumming & Carver, 1987a,b; Kožár et al., 1990; Hricovíni et al., 1990; Lipkind et al., 1984; Penhoat et al., 1991; Poppe et al., 1990; Tran & Brady, 1990; Tvaroška, 1989).

Several methods have been proposed to study the internal dynamics of molecules using an analysis of ^{13}C relaxation data. For example, it has been shown that fast internal motions can be detected in macromolecules from an analysis of the magnetic field dependence of ^{13}C relaxation times and heteronuclear NOEs (Lipari & Szabo, 1982a,b). MD simulations have also been used to study dynamics in proteins (Olejniczak et al., 1984). However, for larger systems, extension of MD trajectories to times comparable with molecular tumbling requires computational resources that are not generally available. In this paper we approached this problem by using proton relaxation data obtained at two magnetic field strengths. The magnetic field dependence of ^1H steady-state and 1D transient NOEs have been compared for different protons in two disaccharides. In the presence of internal motions, dipolar interaction between protons can have additional modulation, and consequently, the spectral density functions for different protons depend on the internal dynamics of the molecule. Thus the ratio of relaxation parameters for the individual protons varies with the type of relaxation pathway and can be used as a simple parameter for mapping the internal motions in biomolecules. We have applied this approach to the study of the conformations of two disaccharides, specifically deuterated Man(α1–3)Manβ-OMe (methyl β-mannobioside, I) and Xyl(β1–4)Xylβ-OMe (methyl β-xylobioside, II) (Chart I).

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[1] Abbreviations: DANTE, delays alternating with nutations for tailored excitations; HSEA, hard-sphere exo-anomeric; MD, molecular dynamics; MM2, molecular mechanics; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy.

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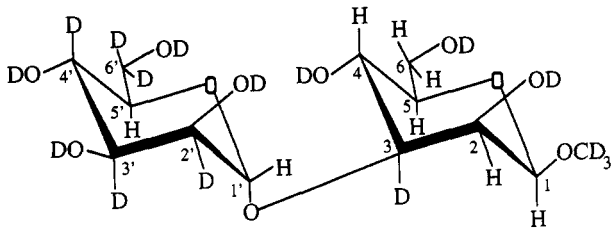
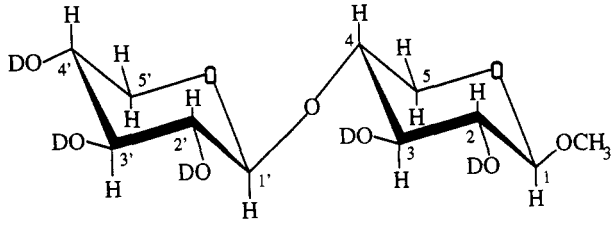
Chart I



I



II

MATERIALS AND METHODS

The time course of change in the longitudinal magnetization of the ith proton which is dipolar-coupled to $n-1$ protons, can be described by eq 1 (neglecting cross-correlation) (Solomon, 1955):

$$\frac{d[I_z(t)-I_z(0)]_i}{dt} = -\rho_i[I_z(t)-I_z(0)]_i - \sum_{j\neq i}\sigma_{ij}[I_z(t)-I_z(0)]_j \quad (1)$$

$I_z(t)_i$ and $I_z(0)_i$ are the z components of the magnetization of nucleus i at times t and 0, respectively. ρ_i is the direct relaxation rate of the ith proton, and σ_{ij} is the cross-relaxation rate between nuclei i and j. For protons:

$$\rho_i = ({}^6/_5)\pi\hbar^2\gamma_H{}^4\sum_j[({}^1/_3)J_{ij}{}^0(\omega) + J_{ij}{}^1(\omega) + 2J_{ij}{}^2(\omega)] \quad (2)$$

$$\sigma_{ij} = ({}^6/_5)\pi\hbar^2\gamma_H{}^4[2J_{ij}{}^2(\omega) - ({}^1/_3)J_{ij}{}^0(\omega)] \quad (3)$$

where ω is the Larmor frequency and γ_H is the proton gyromagnetic ratio. The spectral density function $J_{ij}(\omega)$, for magnetic dipole–dipole relaxation of an isotropically tumbling, rigid molecule has a simple form:

$$J_{ij}{}^n(\omega) = \frac{r_{ij}{}^{-6}}{4\pi}\frac{\tau_o}{1+(n\omega\tau_o)^2} \quad (4)$$

where τ_o is the tumbling time of the molecule. However, J_{ij} differs if internal motions are present in the molecule. The exact form of $J_{ij}(\omega)$ depends on the nature of the overall and internal motions, and a number of expressions corresponding to different approximations have been described (Lipari & Szabo, 1982a,b; London & Avitabile, 1978; Krishnan et al., 1991; Olejniczak et al., 1984; Ribeiro et al., 1980; Tropp, 1980; Wittebort & Szabo, 1978; Woessner, 1962). For example, if one supposes that overall and internal motions can be regarded as uncorrelated, internal motions are faster than the overall tumbling of a molecule, and fluctuations are considered in the orientations of interproton vectors as well as interproton distances, then $J_{ij}{}^n(\omega)$ can be approximated (Lipari & Szabo, 1982a):

$$J_{ij}{}^n(\omega) = \frac{1}{4\pi}\left\{S^2\langle r_{ij}{}^{-3}\rangle^2\frac{\tau_o}{1+(n\omega\tau_o)^2} + [\langle r_{ij}{}^{-6}\rangle - \langle r_{ij}{}^{-3}\rangle^2 S^2]\frac{\tau_c}{1+(n\omega\tau_c)^2}\right\} \quad (5)$$

where S^2 is the generalized order parameter describing spatial restriction of internal motions and $\tau_c{}^{-1} = \tau_o{}^{-1} + \tau_i{}^{-1}$. τ_i is the effective correlation time which is a measure of the rate of internal motion. $\langle r_{ij}{}^{-6}\rangle$ and $\langle r_{ij}{}^{-3}\rangle^2$ are the time-averaged distances between protons i and j. If internal motions are very fast compared to overall tumbling (i.e., on a picosecond time scale compared to a nanosecond time scale for tumbling), $J_{ij}{}^n(\omega)$ simplifies to (Tropp, 1980; Olejniczak et al., 1984)

$$J_{ij}{}^n(\omega) = \frac{1}{4\pi}S^2\langle r_{ij}{}^{-3}\rangle^2\frac{\tau_o}{1+(n\omega\tau_o)^2} \quad (6)$$

On the other hand, if τ_i is very slow compared to τ_o, $J_{ij}{}^n(\omega)$ has the form

$$J_{ij}{}^n(\omega) = \frac{1}{4\pi}\langle r_{ij}{}^{-6}\rangle\frac{\tau_o}{1+(n\omega\tau_o)^2} \quad (7)$$

In both cases (eqs 6 and 7), the frequency dependence of the relaxation parameters would be expected to be identical for all protons.

Equation 5 can be rearranged into the following form:

$$J_{ij}{}^n(\omega) = \frac{\langle r_{ij}{}^{-6}\rangle}{4\pi}\left\{S^{*2}\frac{\tau_o}{1+(n\omega\tau_o)^2} + [1-S^{*2}]\frac{\tau_c}{1+(n\omega\tau_c)^2}\right\} \quad (8)$$

where

$$S^{*2} = S^2[\langle r_{ij}{}^{-3}\rangle^2/\langle r_{ij}{}^{-6}\rangle]$$

and S^{*2} includes effects of both radial ($\langle r_{ij}{}^{-3}\rangle^2/\langle r_{ij}{}^{-6}\rangle$) and angular ($S^2$) components of motional averaging. Equation 8 is identical in form to that used by Koning et al. (1991). However, it should be noted that in the presence of radial averaging their parameter S^2 would be identical to S^{*2}, as defined above.

Because τ_c and S^{*2} can vary for different protons in the molecule, it can be anticipated that the ratio of relaxation parameters measured at 300 and 500 MHz will differ from proton to proton. In particular, protons relaxing primarily through fixed distances will have $S^{*2} = S^2$, since there will be no radial averaging. On the other hand, protons relaxing through fluctuating distances will experience both radial and angular averaging so that $S^{*2} \neq S^2$. We denote the S^2 values for the C–H vectors as $S^2_{C\text{-}H}$ and for the fixed distance H–H vectors as $S^2_{H\text{-}H}$. The S^{*2} values for the protons relaxing through fluctuating distances are denoted by $S^{*2}_{H\text{-}H}$.

^{1}H steady-state and transient NOE experiments were carried out on Bruker AM 300 and 500 spectrometers at 300 K. Steady-state NOE values were determined by difference spectroscopy. Both total time of irradiation and total time of relaxation delay (including acquisition time) were 25 s, which is more than 5 times the T_1 of the slowest relaxing proton, H-1′ (4.74 s). In order to obtain good thermal equilibrium, the sample was stored in the probe at least 3 h before measurement. The total number of transients varied between 512 and 1024. Three independent measurements at both fields were carried out. In ^{13}C T_1 measurements, broad-band-

decoupled ^{13}C spectra were collected with 12 τ values. A total of 2000 transients were accumulated at each τ value to obtain satisfactory signal/noise ratios. We found no evidence of nonexponential recovery. For the calculation of the tumbling times, a value of 112 pm was used for the C–H distance (McCain & Markley, 1986). Four independent 1D transient NOE experiments were acquired for each mixing time (Kessler et al., 1986) using a selective 90° pulse generated by a DANTE-Z sequence (Boudot et al., 1989, Poppe & van Halbeek, 1991); mixing times varied from 400 to 800 ms. At shorter mixing times, NOE intensities at 500 MHz were small (≈1%), and consequently, the error of the measurement increased. In both types of relaxation experiments, digitization of the spectra was 0.1 Hz/point. To avoid spinning artifacts, all relaxation measurements were obtained on nonspinning samples.

Specifically deuterated methyl β-mannobioside (I) was prepared in our laboratory (Shah, Grey, and Carver, in preparation) by a modification of the synthesis of specifically deuterated 2-propyl-3-*O*-α-mannopyranosyl-β-D-mannopyranoside (Dime et al., 1987). Methyl β-xylobioside (II) was obtained as previously described (Hricovíni et al., 1990). Compounds were dissolved (as 10 and 50 mM solutions for I and II, respectively) in 99.99% D_2O. Argon was bubbled through the solutions in order to remove oxygen, and the tubes were then sealed.

RESULTS

Methyl β-Mannobioside. The disaccharide, methyl β-mannobioside, is a synthetic analogue of part of the trimannosyl core common to N-linked glycoproteins. The conformation of the 1–3 linkage in these types of mannosides has been the subject of considerable discussion. Views on the internal dynamics within the linkage still differ (Edge et al., 1990; Homans et al., 1982; Homans, 1990; Brisson & Carver, 1983; Carver et al., 1990). In order to detect the rigidity and/or flexibility of the 1–3 linkage more clearly in relaxation measurements, the disaccharide was specifically deuterated. On the nonreducing unit only protons H-1′ and H-5′ remained; on the reducing end the methyl protons and H-3 were deuterated. Thus, the number of relaxation pathways for protons in the molecule is considerably reduced. As a result, the contribution of the long-range dipole–dipole relaxation pathways is enhanced. For example, protons H-1′ and H-5′ are predominantly relaxed through protons of the β-Man as evidenced by the observed pattern of NOEs [Cumming et al. (1986); see below]. Because inter-proton distances spanning the glycosidic linkage are more sensitive to fluctuations in the glycosidic torsion angles, the relaxation parameters become more sensitive to the existence of such motions. Steady-state NOE values collected at 300 and 500 MHz are shown in Table I. Saturation of H-2 caused changes of intensities of signals of H-1 and H-4 (intraunit), and of H-1′ and H-5′ (across the glycosidic linkage). Due to limited relaxation pathways, three-spin effects were not observed except on H-5 and H-5′. Standard deviations indicate good reproducibility of the measurements for large NOEs; for small NOEs (≈1%) reproducibility was not as good. The ratio of enhancements detected at 300 and 500 MHz [NOE(300/500)], varied from 1.2 to more than 2.0. This range of values reflects the different sources of modulation of the dipole–dipole interaction between different protons. The value of the ratio for H-1′ and H-5′, which relax across the linkage, was close to 1.2. A higher ratio, ≈1.5, was obtained for the H-1 and H-4 protons, which relax primarily through interaction with protons at fixed distances within the β-linked mannose. Saturation of H-1 caused changes of H-2 and H-5 (intraunit) and of H-5′ (three-spin effect across the glycosidic linkage via H-2). The value of NOE(300/500) is 1.7 and 1.5 for both protons relaxing within the same unit, H-2 and H-5, respectively, thus corresponding to that found for the H-1 and H-4 protons when H-2 was irradiated. Because the only term which can affect the ratio NOE(300/500) is the spectral density function for the relaxation of individual protons, different values of the ratio indicate the presence of internal motions about the glycosidic linkage.

Table I: ^1H Steady-State NOEs (%) for Selectively Deuterated Methyl β-Mannobioside Measured at 500 and 300 MHz when H-1 and H-2 Were Irradiated[a]

irrad	NOE	500 MHz	300 MHz	NOE (300/500)
		Relaxation through Fluctuating Distances		
H-2	H-1′	4.9 (0.16)	5.9 (0.22)	1.2
	H-5′	12.6 (0.20)	15.5 (0.0)	1.2
H-1	H-5′	−1.9 (0.12)	−2.2 (0.36)	1.2
		Relaxation through "Fixed" Distances		
H-2	H-1	10.9 (0.38)	16.4 (0.0)	1.5
	H-4	0.9 (0.32)	2.4 (0.14)	2.7
	H-5	−1.3 (0.10)	−1.7 (0.14)	1.2
H-1	H-2	11.4 (0.12)	19.6 (0.49)	1.7
	H-5	12.0 (0.04)	18.4 (0.16)	1.5

[a] Steady-state NOE values were obtained at 300 K at both magnetic fields. Measurements were made three times for each experiment, and the averaged values with standard deviations are shown.

Table II: Transient NOEs (μ_i, %) for Methyl β-Xylobioside Measured at Three Different Mixing Times at 500 and 300 MHz at 300 K[a]

H-1′ irrad					
mix time (ms)	$\omega/2\pi$ (MHz)	μ_i H-4	H-5e	H-3′	H-5a′
400	300	5.2 (0.12)	2.5 (0.10)	3.9 (0.07)	4.4 (0.17)
	500	3.4 (0.26)	1.8 (0.07)	2.3 (0.07)	
600	300	8.5 (0.14)	4.1 (0.22)	6.4 (0.14)	6.4 (0.20)
	500	5.5 (0.10)	2.9 (0.10)	3.7 (0.15)	3.3 (0.28)
800	300	11.3 (0.35)	5.4 (0.14)	9.0 (0.58)	9.1 (0.15)
	500	7.6 (0.10)	3.8 (0.10)	5.2 (0.30)	5.4 (0.17)

[a] The anomeric H-1′ proton was irradiated. Averaged values from four independent measurements for each mixing time are shown. The standard deviations are in parentheses.

Methyl β-Xylobioside. Recently, Hricovíni et al. studied the three-dimensional structure of methyl β-xylobioside in different solvents (Hricovíni et al., 1990; Hricovíni et al., 1991). The magnitudes of the three-bond carbon–proton coupling constants across the glycosidic linkage varied with solvent and temperature and indicated the presence of more than one conformer in solution. These experimental data agree with quantum-chemical PCILO calculations where four minima with populations of more than 10% were found. In contrast to coupling constants, interresidue steady-state NOE measurements in water and methanol did not change with temperature. This insensitivity was predicted by the theoretical calculations.

To support these findings of flexibility about the glycosidic bond, relaxation measurements at 300 and 500 MHz were obtained for methyl β-xylobioside. For this disaccharide transient NOEs (μ_i) rather than steady-state NOEs were analyzed (Table II). Proton H-1′ on the glycosidic linkage was irradiated and transient NOEs were observed on H-3′, on H-5a′ (intraunit), and on H-4 and H-5e (interunit) at several mixing times. Though relatively long mixing times were used, NOE intensities increased linearly (within experimental error) and were relatively strong.

Table III: Ratio of Transient NOEs [μ_i(300/500)] for Methyl β-Xylobioside

	μ_i(300/500)			
mix time (ms)	H-4	H-5e	H-3′	H-5a′
400	1.5	1.4	1.7	
600	1.5	1.4	1.7	1.9
800	1.5	1.4	1.7	1.7

The ratios of the values of μ_i at the two magnetic field strengths are shown in Table III. For protons H-4 and H-5e the values are ≈1.4 and 1.5, respectively. For protons H-3′ and H-5a′ the ratios are higher, ≈1.7–1.9, respectively. Thus, the ratio of the values of μ_i measured at both fields differed depending on whether relaxation pathways are intra- or interresidue in a manner similar to the ratio NOE(300/500) for methyl β-mannobioside. Lower values of the μ_i(300/500) are found (Table III) for protons relaxing across the glycosidic linkage due to the presence of internal motions.

DISCUSSION

The experimental measurements reported in this paper are not sufficient to define all the parameters in eq 8 which describe the motional properties of the disaccharides. However, some can be estimated. For example, Hricovíni et al. (1991) have reported methine ^{13}C T_1's of 0.78 s for II at 298 K and 300 MHz. Measurements at 500 MHz (not shown) gave identical values, indicating that, at these temperatures and concentrations, the longitudinal relaxation times of the carbons are not frequency-dependent. Similar results were found for I (data not shown) with methine ^{13}C T_1's of 0.55–0.57 s at both field strengths. With these additional data it is possible to estimate that the value of the overall tumbling time must be close to 200 ps for I and 170 ps for II. Longer values of τ_o would lead to frequency dependence for the carbon T_1's, and shorter values would lead to ratios of the NOEs and σ's that are less than those observed (for all values of S^2 and τ_i). However, even for these τ_o values, there is a range of values of S^2_{H-H}, S^2_{C-H}, S^{*2}_{H-H}, and τ_i that is consistent with the experimental values, assuming that eq 8 is appropriate.

Lipari and Szabo (1982b) have defined the conditions for which eq 8 gives reasonably accurate estimates of the parameters. They considered three cases: (i) $\tau_i/\tau_o < 0.01$; (ii) $\tau_i/\tau_o < 0.1$ with $S^2 > 0.01$ and $\omega\tau_i < 0.5$ ($\tau_i < 80$ ps for the disaccharides); and (iii) $S^2 > 0.3$. For case i to hold for the disaccharides, τ_i would have to be a few picoseconds, which seems unduly rapid. A more probable condition is that in case ii, particularly since preliminary in vacuo molecular dynamics simulations [Carver et al. (1990); unpublished results] indicate that transitions between low-energy conformers occur approximately every 10 ps, on average. This assumption is inconsistent with the results of recent disaccharide simulations with explicit solvent reported by Edge et al. (1990). However, the latter simulations were obtained using a parametrization derived from AM1 calculations. Tvaroška and Carver (1991) have shown that AM1 gives poor estimates for the torsion energies about the glycosidic bond. Hence the conclusions of Edge et al. (1990) regarding the rate of transitions between conformers are likely to be unreliable, and in the absence of further information, τ_i values near 10 ps seem justified.

For the disaccharides, the assumption of τ_i values of 20 and 17 ps for I and II, respectively, satisfies $\tau_i/\tau_o \leq 0.1$ and also the second condition under (ii), since $\omega\tau_i$ would be approximately 0.1. If, as a first approximation, the further assumption is made that the protons relaxing through fluctuating distances, the protons relaxing through fixed distances, and the carbons can each be characterized by single values of S^{*2}_{H-H}, S^2_{H-H}, and S^2_{C-H}, respectively. Then, it is possible to find values which when inserted into eq 8 yield calculated values in good agreement with the observed values (Table IV).

Table IV

	parameter	calcd	obsd
Compound I (τ_0 = 200 ps; τ_i = 20 ps)			
NOE ratio (fixed distances)	S^2_{H-H} = 1.0	1.56	1.6 ± 0.1
NOE ratio (flexible distances)	S^{*2}_{H-H} = 0.18	1.2	1.2 ± 0.1
T_1 ^{13}C (500 MHz)	S^2_{C-H} = 0.52	0.63	0.56 ● 0.05
T_1 ^{13}C (300 MHz)	S^2_{C-H} = 0.52	0.54	0.56 ± 0.05
Compound II (τ_0 = 170 ps; τ_i = 17 ps)			
σ ratio (fixed distances)	S^2_{H-H} = 0.62	1.7	1.7 ± 0.1
σ ratio (flexible distances)	S^{*2}_{H-H} = 0.23	1.44	1.45 ± 0.1
T_1 ^{13}C (500 MHz)	S^2_{C-H} = 0.41	0.84	0.78 ● 0.05
T_1 ^{13}C (300 MHz)	S^2_{C-H} = 0.41	0.75	0.78 ± 0.05

The ability to find a self-consistent set of values of the parameters which, when inserted into eq 8, generate values of the relaxation parameters in agreement with those observed does not in itself prove that the Lipari and Szabo expressions (1982a,b) are valid approximations for the disaccharides. Neither does it mean that the derived parameter values correspond to physical reality. It is possible that τ_i is in fact closer in value to that of τ_o. If this were true, then eq 8 would not be applicable and the only way to compare the theory and experiment would be to calculate the relaxation parameters directly from molecular dynamics simulations with explicit solvent. These simulations would have to be long compared to the overall tumbling in order to adequately sample all the possible motions. Thus, even for these rapidly tumbling molecules, this constraint means the simulations would need to be for several nanoseconds.

However, if eq 8 is applicable to the disaccharides, then the values in Table IV would indicate that the internal motions affecting the transglycosidic relaxation pathways are very similar in the two compounds, suggesting this time scale is characteristic of the torsional potentials for glycosidic rotations.

It is significant for the analysis of oligosaccharide relaxation parameters that the averaging of the angular component of the dipole–dipole interaction is incomplete, (as reflected in the departure of S^2 and S^{*2} from unity). Because of this incomplete averaging, it is not possible to extract estimates of τ_o from ^{13}C T_1's using eq 4. Two recent studies have reported discrepancies between ^{13}C- and ^1H-derived correlation times, neglecting internal motions: Cagas and Bush (1990) for the Lewis blood group oligosaccharides and Sabesan et al. (1991) for a hexasaccharide fragment of a ganglioside. In both these studies, the discrepancy was ignored and the data were fitted to a single τ_o. The analysis given above for the disaccharides indicates a possible explanation for the reported discrepancies. If incomplete averaging is occurring as a result of internal motions on a time scale comparable with the overall tumbling, then the S^2 can be different for the protons and the carbons. As a result, the assumption that $S^2 = 1$ (neglect of internal motions and hence incomplete averaging) will lead to incorrect and discrepant estimates for τ_o from the two kinds of measurement using eq 4. In previous studies (McCain & Markley, 1986), the lack of field dependence in the ^{13}C T_1 values was interpreted as suggesting an isotropically tumbling rigid model for sucrose. However, it has been demonstrated above that a lack of field dependence in ^{13}C T_1's is totally compatible with the existence of internal motions, provided

that incomplete angular averaging is taken into account appropriately. Thus, the above results support the presence of an equilibrium between several conformers in solution and indicate that neither constant values of one of the observable NMR parameters with variations in solvent and temperature nor a lack of frequency dependence in the ^{13}C T_1's necessarily indicates rigidity of the molecule.

CONCLUSIONS

A decade ago, Jardetzky pointed out some of the problems connected with the interpretation of NMR data of biomolecules in solution (Jardetzky, 1980). In the presence of internal motions, one can only detect time-averaged NMR parameters because the interconversion between conformers is fast compared with the time scale of the experiment. A single NMR-derived structure, therefore, may not correspond to the geometry of any conformer actually present in significant amounts in the solution. Recently, these difficulties in the interpretation of experimental data have been shown for the small cyclic decapeptide, antamanide, where it was necessary to assume the presence of four conformers in solution (Kessler et al., 1988). In NMR experiments one detects only a single value for each parameter, e.g., cross-relaxation rate or coupling constant, and on the basis of these data exclusively it is difficult to evaluate whether it corresponds to a single and/or time-averaged structure.

In this paper we have shown that internal motions in disaccharides I and II can be detected by a comparison of ^1H relaxation parameters obtained at different magnetic fields. The ratio of relaxation data for individual protons, measured at two frequencies, varied for protons relaxing through fixed distances compared to those relaxing through fluctuating distances. As a result, the ratio of cross-relaxation rates can reveal the existence of internal motions. The existence of internal motions is consistent with the previous demonstration of the existence of multiple low-energy minima for compounds I and II (Imberty et al., 1990; Hricovíni et al., 1990, 1991). However, the results reported here do not permit unequivocal determination of the time scale of internal motions, simply that they are comparable with the overall tumbling time. Full quantitative analysis will require MD simulations with solvent molecules to monitor the motional properties. Such simulations are in progress.

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