Assessment of the Flexibilities of Carbohydrate Polymers by ^1H NMR Relaxation and Line Shape Analysis

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ABSTRACT: The use of the spin–spin proton magnetic relaxation time, T_2, has been assessed for the characterization of the conformational states of carbohydrate chains, and three general types of behavior have been distinguished: (i) highly rigid conformations for which T_2 is in the range of tens of microseconds; (ii) highly flexible chains for which the relaxation approximates to an exponential function with T_2 around 100 ms; and (iii) chains that are neither totally flexible nor totally rigid, but whose motions are considerably restricted by nonbonded interactions between residues. These have T_2 values in the range of milliseconds to tens of milliseconds. The NMR relaxation behavior is a useful, practical guide to the degree to which carbohydrate chain conformations are "stiffened" by noncovalent interactions, but we draw attention to several serious pitfalls that must be avoided in interpretation.

Introduction

The first stage in looking at the chain conformation of polysaccharides or of the carbohydrate moiety of glycoproteins in solution is to determine the degree of conformational order.[1,2] Some information on the average properties of these, as for other polymer molecules in solution, can be obtained from classical methods of analysis such as light scattering, small-angle X-ray scattering, sedimentation, and viscosity measurements (including the response to ionic strength[3]). These methods analyze size, shape, and/or dissymmetry of polymer molecules but suffer from the limitation that they give information only about average properties. Hence if part of the molecule is ordered whereas the remainder is disordered, the evidence will indicate that the molecules are disordered. Moreover, the interpretation of any measurement of overall dimensions using these methods must take into account many factors, among which it can be difficult to distinguish the contributions of local flexibility and local bonding geometry.[4]

One approach which we have used in earlier work[5–9] is to monitor a conformation-sensitive parameter such as optical rotation to demonstrate an order–disorder transition occurring as a result of changes in an external variable such as temperature. Unfortunately, this method too has its limitations, for it depends on the ability to identify suitable conditions for conformational change. We have, therefore, investigated the possibility of using nuclear magnetic relaxation to characterize conformational states.

A peak in the high-resolution NMR spectrum of a molecule undergoing Brownian motion can be described by a Lorentzian line shape, and the half-height width $\Delta\nu_{1/2}$ of such a peak is related to the spin–spin relaxation time (T_2) by the equation

$$T_2 = 1/\pi(\Delta\nu_{1/2})$$

Because of the inverse relation between T_2 and the correlation time for molecular motion, a biopolymer in a conformation with rapid segmental motion (i.e., in the disordered state) would be expected to exhibit a well-defined high-resolution NMR spectrum, whereas the same biopolymer in an "ordered" conformation with relatively slow segmental motion, would give rise to peaks which are so broad that they may not be detectable by conventional high-resolution NMR spectroscopy. Consequently, the percentage of the NMR signal not observed in the high-resolution NMR spectrum of a biopolymer is generally assumed to be directly related to the percentage of that biopolymer in an immobilized (i.e., ordered) conformation. This type of NMR experiment is now generally used to study the solution conformation of proteins and has recently been applied to studying the solution conformation of polysaccharides[9–13] and a glycoprotein.[14]

The solution conformation of ι-carrageenan has already been investigated extensively and is now well characterized.[15] Therefore it was chosen as a model system with which to evaluate further the potential of NMR relaxation for studies of the solution conformation of carbohydrate chains. In particular, we have explored the possibilities for characterizing the "missing signal" in the high-resolution NMR spectrum using a wide-line pulse NMR spectrometer to measure the total NMR signal.

Experimental Section

Polysaccharide Samples. ι-Carrageenan (code X-52) was supplied by Pierrefitte-Auby, Paris, France, and had been isolated from *Euchema spinosum*. It was used as the potassium salt both in the native form and after segmentation as described by Rees et al.[16] ι-Carrageenan segments as the tetramethylammonium salt were prepared as described by Morris et al.[17] Dextran was purchased from Pharmacia (code T500). Agarose and κ-carrageenan (sodium salt) were prepared in the laboratories of Marine Colloids Inc. (codes REX 5468 and REX 6696, respectively). λ-Carrageenan (potassium salt) was purchased from Pierrefitte-Auby, Paris, France (code Satia Gum B). Hyaluronic acid was used as the sodium salt and was kindly supplied by Dr. E. A. Balazs (sample C34).

Preparation of Solutions. The solvent contribution to the proton NMR signal was reduced by successive dissolution of the polysaccharide in deuterium oxide and subsequent freeze-drying. Solutions of hyaluronic acid were prepared by dissolution in deuterium oxide at room temperature. Other polysaccharide solutions were prepared by dissolution in deuterium oxide in a sealed tube followed by heating in an autoclave at 15 lb in.$^{-2}$ for 20 min. Solutions other than hyaluronic acid and λ-carrageenan were filtered through 3-μm Millipore filters. (Hyaluronic acid and λ-carrageenan solutions were too viscous to pass through the filters.)

Spectroscopic Methods. Optical rotation measurements were made at 436 nm with a Perkin-Elmer 241MC polarimeter with a 1-cm path length cell.

High-resolution proton NMR spectra were measured on a Varian XL-100 Fourier transform spectrometer, a Bruker WP200 Fourier transform spectrometer, and a Varian HR300 Fourier transform spectrometer (TNO, Delft, Holland). Integrated peak areas were referred to an external standard of pyrazine in D_2O, contained in a coaxial capillary tube inside the NMR sample tube. Theoretical line shapes containing both one and two Lorentzian functions were fitted to the well-resolved anomeric proton peak in the high-resolution NMR spectra of ι-carrageenan segments, using a least-squares minimization routine. The NMR spectrum of ι-carrageenan segments was computer simulated with each peak

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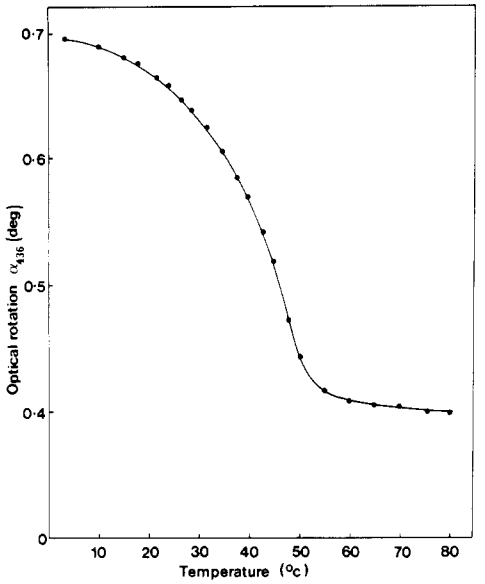

Figure 1. Order–disorder behavior of 5% (w/v) ι-carrageenan segments (potassium salt) as monitored by optical rotation (436 nm, 1-cm path length).

having a Lorentzian line shape, in a manner similar to that described by Welti.[18] A composite computer-simulated spectrum was also produced in which each peak was now the sum of two Lorentzian functions, and the parameters used to describe this type of line shape were those evaluated from the anomeric proton peak in the recorded NMR spectrum.

The direct measurement of the decay of total magnetization of polysaccharide protons in solution was determined with a Bruker SXP spectrometer operating at a frequency of 60 MHz using the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence.[19] The decay of magnetization was recorded directly on a Didac 800 signal averager. To enable the total decay of magnetization to be recorded, the sampling rate of the signal averager was reduced in two steps as a function of time. The slowest relaxing component was due entirely to the residual protons in the solvent, and for several of the samples this was eliminated by increasing the repetition rate of the CPMG sequence to suppress its amplitude and limiting the acquisition time of the signal averager to that required for the solute protons to relax to equilibrium. The resultant decays were then analyzed on a Harris /4 computer by two different approaches: (i) curve fitting of the decay data by a summing of discrete exponential components using an iterative least-squares method and (ii) deconvolution of the decay by Fourier methods to obtain an estimate of the actual distribution of relaxation times.[20]

The free induction decay (FID) immediately following a 90° pulse was recorded with a Biomation 805 transient recorder as an interface between the NMR spectrometer and the signal averager.

Results and Discussion

Order–Disorder Transition for Carrageenan. ι-Carrageenan is an alternating copolymer of 1,3-linked β-D-galactose 4-sulfate and 1,4-linked 3,6-anhydro-α-D-galactose 2-sulfate with occasional "interruptions" in the sequence when D-galactose 6-sulfate or D-galactose 2,6-disulfate occurs in place of the 4-linked anhydride residues. At high temperatures the polysaccharide has a random coil conformation whereas at low temperatures the chains associate to form a three-dimensional network containing double-helical regions terminated by those 4-linked residues that are incompatible with helix formation.[15] The ι-carrageenan chains may be chemically degraded[5,16] to give a preparation of carrageenan segments containing only the regular copolymer sequences. These segments have lost the ability to form a gel network but show conformational changes indicative of a coil–double helix transition. The

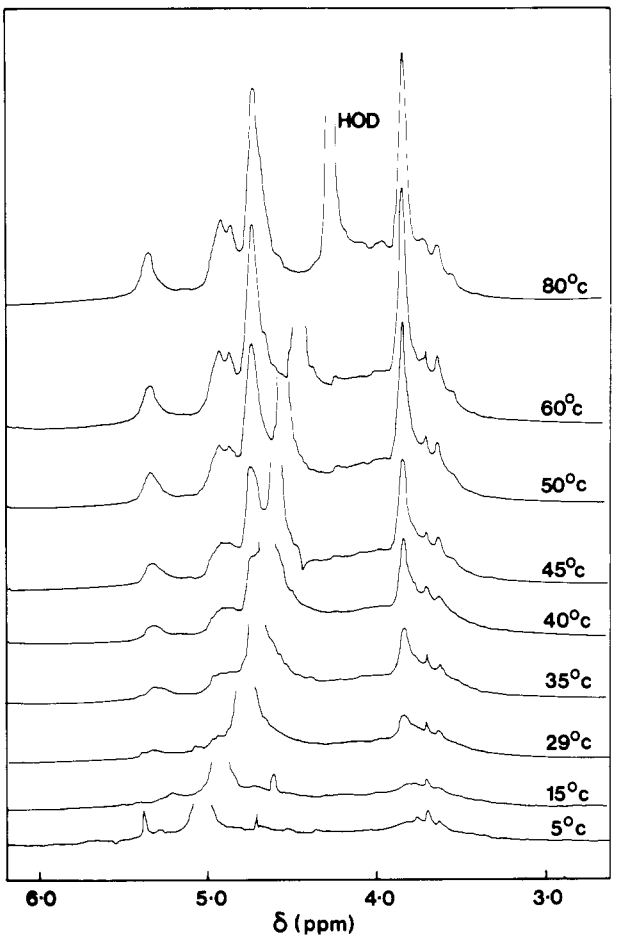

Figure 2. Temperature dependence of the ^1H NMR spectrum at 200 MHz for 5% (w/v) ι-carrageenan segments (potassium salt) in deuterium oxide. A concentric capillary tube containing pyrazine in deuterium oxide was used as an external intensity reference.

change of optical rotation with temperature for a 5% solution of ι-carrageenan segments (potassium salt) is shown in Figure 1. This temperature-induced shift in optical rotation is typical of the coil-to-double helix transition for ι-carrageenan.[5]

The temperature dependence of the high-resolution ^1H NMR spectrum of the same 5% solution of ι-carrageenan segments is shown in Figure 2. It can be seen that there is a decrease in the intensity of the NMR spectrum as the temperature falls and the double-helix content increases. This decrease in intensity is not due to aggregation of the double helices, because a similar result was also obtained for ι-carrageenan in the tetramethylammonium form, for which it has been reported that there is no evidence of the double helices aggregating.[17] It is important to note that the peaks are not broadened or shifted as a function of decreasing temperature. Therefore, there is no evidence of any time averaging of the NMR signals from the coil and double helix. This is consistent with a previous study of ι-carrageenan by ^{13}C NMR[10] and by calorimetry[7] which also pointed to a "two-state all-or-none" model for the transition.

These conclusions are confirmed by the obvious bimodal nature of the free induction decay (FID) for the ι-carrageenan segments observed using a wide-line pulse NMR spectrometer (Figure 3). The decay of magnetization for ι-carrageenan segments in the double-helical conformation is very rapid and has a characteristic relaxation time of approximately 30 μs (Figure 3a). This relaxation time is equivalent to a peak having a line width of approximately

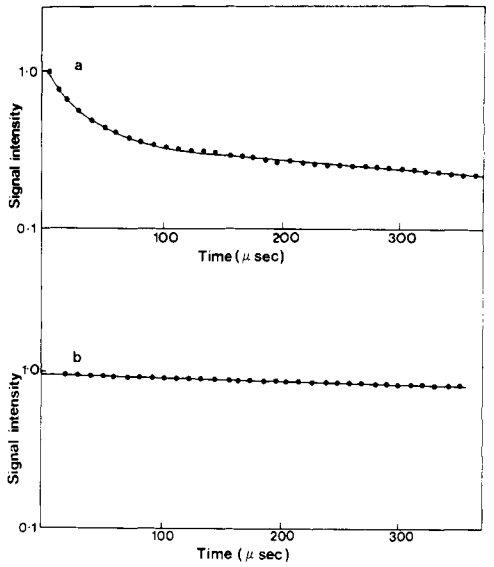

Figure 3. Semilogarithmic plot of the initial part of the free induction decay following a 90° pulse for 2% (w/v) ι-carrageenan segments (potassium salt) in deuterium oxide in (a) a partially ordered (double helix) conformation (280 K), showing the rapid decay of magnetization for ι-carrageenan segments in the double-helical conformation (the very slow decay of magnetization which can also be seen is due to the small proportion of segments in the random coil formation and to residual protons in the solvent) and (b) a totally disordered (random coil) conformation (353 K), showing very slow decay of magnetization (again the residual protons in the solvent contribute to the slow-decaying signal).

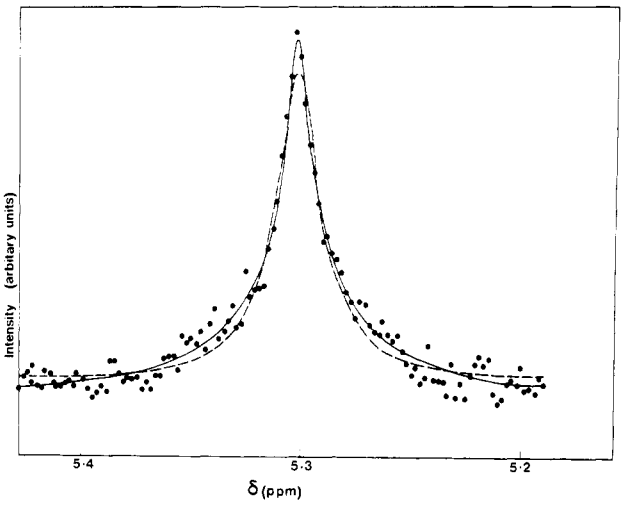

Figure 4. Theoretical line shapes containing both one and two Lorentzian functions, respectively, computer fitted to the well-resolved anomeric proton peak in the 300-MHz ^{1}H spectrum of 2% ι-carrageenan segments at 80 °C: (●) observed results; (---) Lorentzian line shape with a half-height width ($\Delta\nu_{1/2}$) of 4.2 Hz; (—) line shape described by two Lorentzian functions, with 46% of the signal having a $\Delta\nu_{1/2}$ of 31.8 Hz and 54% of the signal having a $\Delta\nu_{1/2}$ of 3.7 Hz.

10 kHz. Therefore, it is not surprising that no high-resolution NMR signal is observed for ι-carrageenan segments in the double-helical formation. In constrast, the decay of magnetization for the ι-carrageenan segments in the random coil conformation is relatively so slow that the dominant relaxation process of the FID is now the inhomogeneity of the electromagnet (T_2 approximately 5 ms) (Figure 3b).

The process of resolving the fast and slow relaxation processes to use the result as an estimate of the proportions of order and disorder present is complicated by the contribution from the residual solvent protons. The magnitude of this may, however, be determined from the decay of magnetization following the CPMG pulse sequence,[19] which eliminates the effect of magnetic field inhomogeneity on the spin–spin decay of magnetization. Hence the use of wide-line measurements to determine the degree of order/disorder directly requires the total decay of magnetization to be resolved into at least three separate processes, namely, the rapid relaxation associated with the ordered component, the slower relaxation associated with the flexible chain, and the still slower relaxation of solvent nuclei. Naturally, and as we discuss in more detail below, this analysis may be further complicated in that it is often impossible to fit the relaxation of the flexible chain by a single-exponential process. The measurements can also be very tedious and time-consuming due to the long relaxation time of the solvent protons (T_2 approximately 5–10 s).

As we show later, these problems are not insurmountable but first we consider the relationship between chain flexibility and parameters of the high-resolution NMR spectrum.

Line Shape Analysis and Relaxation Behavior of Flexible Conformations of Carbohydrate Chains. Close inspection of the high-resolution NMR spectrum of ι-carrageenan segments (Figure 2) reveals that the line shape of each peak cannot be described accurately by a simple Lorentzian function.

A well-resolved peak at δ 5.30 is sufficiently separated from the rest of the spectrum for line shape analysis. (This is assigned to the anomeric proton of the 3,6-anhydro-α-D-galactopyranoside residue.[18]) Such analysis demonstrated that a two-component line shape containing both a "broad" and a "narrow" Lorentzian line shape was required for an adequate description (Figure 4).

Computer simulation of the total high-resolution NMR spectrum demonstrates that all other peaks also have non-Lorentzian line shape. The spectrum computed with all the peaks having a similar two-component line shape (Figure 5b) gave a better approximation of the observed spectrum (Figure 5c) than the corresponding spectrum computed with all peaks having a simple Lorentzian line shape (Figure 5a). The quality of the data was insufficient to allow a more detailed analysis to establish whether the line shapes could be fitted even better by three or more components.

Closer analysis of the spin–spin decay of magnetization following a CPMG pulse sequence for ι-carrageenan segments in the random coil conformation (2% solution at 80 °C) showed that at least three separate exponential functions are required for an adequate description of the overall decay. The slowest relaxation (T_2 approximately 8 s) was due entirely to residual solvent protons and will be omitted from the following discussion. The remaining two relaxation processes (see Table I) must, however, be associated with the random coil chain. Since their relative amplitudes correspond to those seen in the high-resolution spectrum for the "broad" and "narrow" lines, it seems likely that they have the same common origin. The alternative possibility, that the two relaxations derive from distinct sets of differently chemically shifted peaks in the high-resolution spectrum having different line widths, is unlikely because all the protons in the ι-carrageenan structure have been assigned to narrow peaks in the high-resolution NMR spectrum,[18] each of which has approximately the same line shape and width (see above). Seven of the peaks in the 200-MHz high-resolution NMR spectrum of 2% ι-carrageenan segments were sufficiently resolved for analyses. Measurement of the individual spin–spin decays of

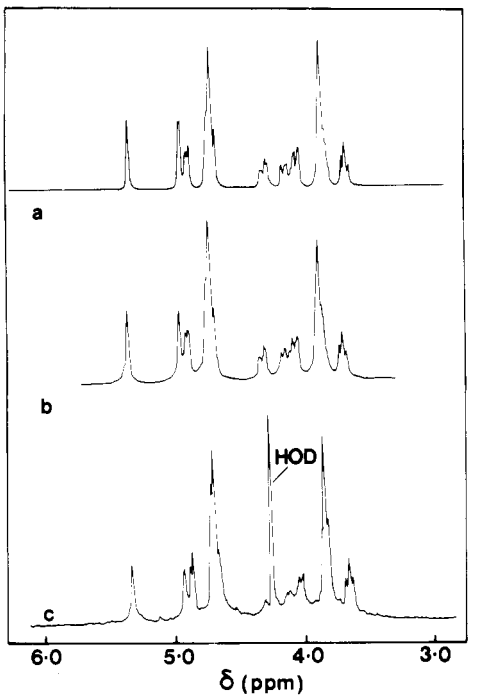

Figure 5. Computer-simulated ^1H NMR spectra for 2% (w/v) ι-carrageenan segments at 80 °C: (a) simulated with all resonance peaks having a Lorentzian line shape and a half-height width ($\Delta\nu_{1/2}$) of 4.2 Hz; (b) simulated with all resonance peaks having a line shape described by two Lorentzian functions, with 46% of this signal having a $\Delta\nu_{1/2}$ of 31.8 Hz and 54% of the signal having a $\Delta\nu_{1/2}$ of 3.7 Hz; (c) experimental result.

Table I
Spin-Spin (T_2) Relaxation Behavior of the Solute Protons for Various Saccharide Solutions in D_2O at 80 °C[a]

	component 1		component 2	
sample	amplitude, %	T_2, ms	amplitude, %	T_2, ms
2% glucose	100	1500		
2% dextran	100	191		
2% agarose	69	118	31	24
2% κ-carrageenan	62	113	38	25
2% κ-carrageenan segments	52	109	48	20
0.2% ι-carrageenan segments	46	109	54	16
2% ι-carrageenan	52	68	48	11
2% λ-carrageenan	50	60	50	6
1.5% sodium hyaluronate	47	27	53	4

magnetization for these peaks revealed that none of them relaxed exponentially but that they were all of a similar nonexponential form to that encountered with the total NMR signal observed with the wide-line pulse NMR spectrometer. Furthermore, all the peaks studied decayed at a similar rate, with T_2 values (as determined from the half-life of the decay) in the range 60–115 ms, with a mean of 95 ms. This small range of differences between the T_2 values for the individual peaks is probably a reflection of the chemically distinct protons involved being separated from their nearest neighbors by slightly different distances because T_2 is proportional to r^6.

We conclude that the multiexponential decay of magnetization observed with the wide-line spectrometer is directly related to the non-Lorentzian line shape of the individual peaks in the high-resolution NMR spectrum. In principle, therefore, the line shapes of individual peaks in the high-resolution NMR spectrum could be deduced more precisely from the form of the overall spin–spin decay of magnetization.

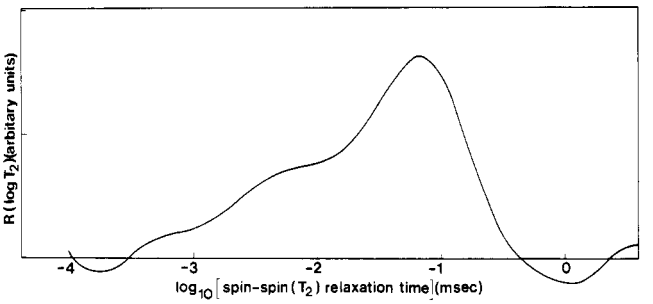

Figure 6. Distribution profile of the exponential components present in the decay of magnetization following a CPMG pulse sequence for 6% ι-carrageenan segments in deuterium oxide at 353 K as determined by the deconvolution method of Clark and Lillford.[20]

Mathematical Analysis of the Relaxation of Flexible Chains. Further analysis of the decay function was somewhat limited by the quality of the experimental data. However, again using ι-carrageenan segments in the random coil form, it was found that as the quality of the data improved, the number of exponential functions required to describe that data also increased. For example, when the decay of magnetization for a 6% solution of ι-carrageenan segments at 80 °C was accumulated over a longer time period (10 times that used previously), it required four separate exponential functions for an adequate description: T_2 = 127 ms (30%), T_2 = 31 ms (40%), T_2 = 5 ms (21%), and T_2 = 1.2 ms (9%). The need to invoke an increased number of discrete exponential processes to describe the improved data is exactly what would be expected if a continuous distribution of relaxation times were involved. For ι-carrageenan this possibility was further explored by applying data analysis based on deconvolution by Fourier series.[20] In this approach it was assumed only that the decay was exponentially based, and a Fourier series approximation to the underlying exponential distribution function $R(\log T_2)$ was extracted from it without further information being required. For ι-carrageenan the resulting solution for the distribution $R(\log T_2)$ (limited to a finite number of Fourier terms determined by the extent and accuracy of the data) is shown in Figure 6. This appears to be consistent with the continuous-distribution interpretation, but in view of past experiences with the deconvolution approach and the problems which can arise when using it to distinguish between continuous distributions and groups of closely spaced discrete exponentials, another method of data simulation and analysis was also applied. This involved simulating the decay of magnetization (including random noise) on the basis of either the discrete or the continuous exponential description and then analyzing these decays by the transform approach.[20] Solutions for $R(\log T_2)$ for the two models (summed to various numbers of Fourier terms) were then compared with corresponding experimental results. The continuous-distribution model provided the better explanation of the experimental data for ι-carrageenan. From these results (which will be described in detail in a future publication) it seems likely that descriptions in terms of two discrete components for the decay of the wide-line signal and for the high-resolution line shapes for ι-carrageenan segments (Table I and Figure 4) are merely first approximations to the physical reality of a continuous distribution.

This model is also conceptually more plausible than the "two-component" model since the latter would require the polysaccharide to exhibit two discrete molecular mobilities

whereas all the evidence available on the molecular state under these conditions points to a "random coil" conformation.[15]

Molecular Origin of Non-Lorentzian Line Shapes and Nonexponential Relaxation. Some insight into the reason for the distribution of relaxation times was obtained from a comparison of the decays of magnetization for a variety of polysaccharides in solution. The distribution of relaxation times for each was characterized by two exponential functions, bearing in mind that this probably does not correspond to physical reality but is simply for convenience to obtain numerical indices for any differences in the distribution profiles (Table I). No very rapidly relaxing component (T_2 approximately 30 μs) was observed in the free induction decay for any of these samples under the conditions studied. This indicates that the distribution profiles in Table I do represent the total NMR signals. For three of the polysaccharides under different conditions where they are known to exist in ordered conformations, namely, agarose, ι-carrageenan, and ι-carrageenan segments at 29 °C, free induction decay measurements did show the expected rapid relaxations. These had T_2 values of approximately 10 μs (agarose) and approximately 30 μs (ι-carrageenan and ι-carrageenan segments).

The distribution profile of the decay of magnetization for ι-carrageenan segments in the random coil form at 80 °C is independent of concentration between 0.2% and 2.0% (Table I) and therefore the origin of the distribution function must be intramolecular rather than intermolecular in nature. Moreover, the shape of the distribution function is shown to be independent of molecular weight for ι-carrageenan (Table I) and therefore it cannot be explained in terms of polydispersity of the samples.

Table I shows the differences between the decays of magnetization for a range of different saccharides. A single exponential function is found to be sufficient to describe the decay of magnetization for glucose and dextran, whereas two functions are required for the other polysaccharides studied. We point out that these structures differ in their molecular mobility. Because of the low molecular weight of glucose, it should be able to tumble freely in solution. The 1,6-glycosidic linkage in dextran permits a high degree of internal freedom of rotation about the glycosidic system. All the other polysaccharides here have 1,4-linkages and do not have such freedom of internal segmental motion. We propose that the multiexponential decays of magnetization arise from restricted mobilities. Table I was constructed with the substances listed in order of increasingly restricted molecular or segmental motion.[4] It is immediately apparent that the degree of multiexponentiality parallels the degree of restriction.

NMR signals of a similar line shape to these have been reported for both liquid crystalline systems[21] and cross-linked polymer gels.[22] The origin of the line shape effects were attributed to some residual static dipolar interaction caused by molecular anisotropy. When the segmental motion in polysaccharides in solution is sufficiently anisotropic as a result of the restricted rotation about the glycosidic bonds, it is possible that there is a similar residual static dipolar interaction to give rise to multiexponential decay of magnetization. An alternative explanation could be that steric restrictions cause a gradation of molecular motion down the polysaccharide chain, and hence lead to different T_2 values being associated with different segments at any instant, and that the conformational interconversions are too slow for averaging on the NMR time scale. Whichever is the correct explanation, this study has highlighted the existence of multiexponentiality and its clear correlation with restriction of segmental motion of polysaccharides.

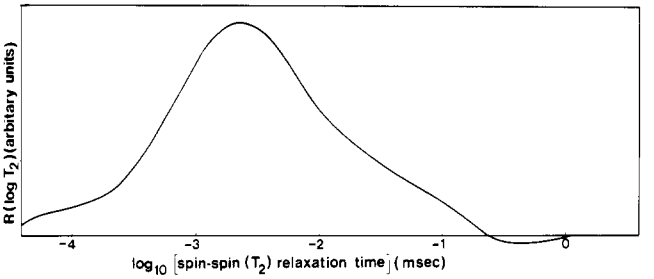

Figure 7. Distribution profile of the exponential components present in the decay of magnetization following a CPMG pulse sequence for 5% (w/v) sodium hyaluronate in deuterium oxide at 353 K as determined by the deconvolution method of Clark and Lillford.[20]

Scope for the Application of Relaxation and Line Shape Measurements. The traditional high-resolution NMR method for determination of the degree of order and disorder for macromolecules in solution uses the area of a well-resolved peak in the NMR spectrum.[11,14] It is assumed that the proportion of the signal which is visible is a direct expression of that part of the structure which is in a disordered conformation and that the remainder is associated with an ordered component which is so immobilized that it cannot be observed in the high-resolution NMR spectrum. We have now shown, however, that the line shape of each peak in the high-resolution NMR spectrum may need to be described by a distribution of Lorentzian line shape functions; i.e., the lineshape is super-Lorentzian. Such peaks can appear to be relatively sharp but on close inspection they may have very broad "wings" which make it very difficult to determine the total area. If some of this "wing" area of the super-Lorentzian peak is excluded from the measured integral, the proportion of ordered conformation will be grossly overestimated. It is now clear that this has occurred, for example, for sodium hyaluronate in solution. It was previously reported[11] that only 40% of the NMR signal is observed in the high-resolution NMR spectrum of sodium hyaluronate. One interpretation was that two distinct molecular mobilities exist, with 60% of the sample being in an immobilized (i.e., ordered) conformation and the remaining 40% being mobile (i.e., in coil conformation). With the improved wide-line instrumentation now available, we have been able to show that the spin–spin decay of magnetization for 2% sodium hyaluronate in solution at 29 °C is multiexponential and deconvolutes into two exponential functions with T_2 = 15 ms (42%) and T_2 = 2 ms (58%). Analysis of the free induction decay revealed no other components which decay more rapidly and, therefore, we conclude that the total NMR signal can be described by these two exponential functions. As in the case of ι-carrageenan, this "two-component" description (Figure 7) is actually an approximation of a continuous distribution of relaxation times, with the center of distribution at 3 ms for sodium hyaluronate, compared with 60 ms for ι-carrageenan, pointing to even more restricted internal motion.

Conclusions

This study has shown that polysaccharides in rigid ordered conformations may be characterized by proton spin–spin relaxation times as short as tens of microseconds. Polysaccharides in conformations that are nominally flexible may retain sufficient restrictions on interresidue motion to give rise to high-resolution peaks which have a

super-Lorentzian line shape. The profile of this line shape corresponds to the analysis of the overall spin–spin decay of magnetization using a wide-line pulse spectrometer. The magnitude of this effect appears to be a useful index of the extent to which nonbonded interactions stiffen the chain.

Gross inaccuracies may result if quantitative measurements of the relative proportions of order and disorder are attempted simply on the basis of the area of the "visible signal" in the high-resolution spectrum. The complex NMR signals for polysaccharides in solution are better interpreted by using a combination of wide-line pulse and high-resolution NMR techniques when it is possible to derive both the extent of conformational ordering and the relative magnitude of any constraints on chain flexibility in the disordered state. These methods, of course, presuppose a molecular weight that is sufficiently high for spin relaxation to be dominated by segmental motion rather than overall tumbling of the molecule.

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CIDEP and Spin–Lattice Relaxation Time Studies of Some Polymeric Phenoxy Radicals in Solution

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ABSTRACT: Photooxidation of polymeric phenols by duroquinone in solution produced both primary semiquinone and phenoxy radicals which exhibited the chemically induced dynamic electron polarization phenomenon. Measurements were obtained for the spin–lattice relaxation time T_1 and the line widths of the 2,4,6-tri-*tert*-butylphenoxy radical and polymeric derivatives of this radical: the polymer chain replaced the *tert*-butyl group at the 4-position. Also the vitamin E radical was investigated. The observed T_1's for the polymeric radicals were not significantly greater than for the monomeric phenoxy radical but the lines were broadened somewhat. The vitamin E radical, however, had a much larger T_1. A simple model in which the polymeric radicals were treated as rotating rods was used to explain these observations.

Introduction

The photochemical reactions between quinones and phenols in solution have been extensively used as model systems in our laboratory for chemically induced dynamic electron polarization (CIDEP) studies.[1] In addition to the mechanistic and kinetic information that can be obtained from CIDEP studies, another important physical parameter, the spin–lattice relaxation time, T_1, can be estimated from the polarization decay of a transient radical.[2] Freed and co-workers[3] have investigated the spin–lattice relaxation times of the benzoquinone series, while we have employed the time-resolved CIDEP technique to extend the semiquinone T_1 study to include the transient naphthosemiquinone and anthrasemiquinone series.[4,5] We found that T_1 is just as sensitive to the number of rings as it is to the size of the semiquinone radicals. In most of these systems the molecular size contribution toward the orientational correlation time τ_2 is a power of the effective molecular radius. An excellent recent analysis of T_1 of transient radicals in solution has been presented by Fessenden and co-workers.[2]

In this paper we examine a number of polymeric phenoxy radicals. These are derivatives of the 2,6-di-*tert*-butylphenoxy radical with a very long hydrocarbon chain attached to the 4-position of the ring. Also we looked at the radical formed from vitamin E. Although these are hardly spherical molecules, they nonetheless should have a large effective molecular radius. This in turn would imply large values for τ_2 and hence T_1; moreover, T_1 would be expected to be sensitive to chain length. Our experimental results are not in agreement with such expectations; they indicate that the length of the polymer chain has a

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