Proton NMR Relaxation Studies of Aqueous Polysaccharide Systems

B. P. Hills,* C. Cano, and P. S. Belton

AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA, England

Received September 14, 1990; Revised Manuscript Received December 6, 1990

ABSTRACT: The major features of NMR transverse water proton relaxation in polysaccharide solutions and gels can be quantitatively interpreted in terms of fast chemical exchange between water and polysaccharide hydroxyl protons. The water proton relaxation is found to be a useful monitor of the mobility and state of aggregation of the polysaccharide chains and to complement information from high-resolution NMR spectroscopy. In carrageenan gels there is evidence for microheterogeneity and this gives additional diffusive dephasing contributions. Although there is evidence that polysaccharides influence the state of the water around them (the so-called "bound" water concept), the results obtained suggest that this affects the proton transverse relaxation in a minor way compared to the potent effect of the chemical exchange mechanism.

Introduction

Many reports have shown that the addition of polysaccharides to water increases the water proton transverse relaxation rate[1] and that the extent of the enhancement depends on the state of aggregation and gelation of the polysaccharide.[2] However, the origin of this relaxation enhancement remains a matter of debate. Using simple model systems such as glucose solutions and Sephadex bead suspensions, we have recently shown that rapid proton exchange between water and carbohydrate hydroxyl groups is a major transverse relaxation mechanism in carbohydrate systems.[3] The proton exchange contribution can be calculated by the general two-site exchange formalism[3,4] in terms of well-defined quantities such as the mean lifetime of the protons on the carbohydrate (k_b, s^{-1}), the proton fraction (P_b), and the intrinsic relaxation time (T_{2b}) of the carbohydrate hydroxyl protons and their chemical shift ($\delta\omega$, ppm) from the bulk water protons.

In this paper we explore to what extent proton exchange can explain the water relaxation behavior of dilute polysaccharide solutions and gels. In particular we wish to know whether the transverse water proton relaxation is a useful monitor of the conformation and mobility of the polysaccharide in solution. Conformational changes can alter the accessibility of the polysaccharide hydroxyl groups to exchange and this should be reflected in an altered value for the proton fraction P_b and possibly in the exchange rate k_b. Variations in polysaccharide chain mobility can affect the intrinsic transverse relaxation time, T_{2b}, of the hydroxyl protons involved in exchange. Since changes in P_b, k_b, and T_{2b} have predictable consequences for the water proton transverse relaxation, we anticipate that this would be a sensitive probe of the conformation and dynamic state of the polysaccharide in solution.

Another possible relaxation mechanism involves the exchange of water protons in the bulk with those of water molecules hydrogen bonding the polysaccharide, the so-called bound water. Our recent studies[5,6] of water relaxation in solutions of the α1–4 linked glucans G_n, n = 1–7, using oxygen-17 and deuterium suggest that two water molecules per carbohydrate hydroxyl group reorient anisotropically with correlation times substantially longer than that characterizing the isotropic reorientation of water molecules in the bulk phase (τ_ω). Most of the intramolecular dipolar interaction of the bound water is averaged by rapid, near isotropic rotation characterized by a fast correlation time (τ_f), which is only ca. 5–10 times longer than τ_ω (ca. 2.4 ps). However, the residual dipolar coupling is averaged on the much slower time scale involving motions of the carbohydrate molecule and is proportional to the solution viscosity. With polysaccharides there could be additional contributions from diffusion of water over the polysaccharide surface.[7] In principle, the exchange of water protons between the bulk and bound water phases by chemical exchange and/or molecular diffusion can provide an additional relaxation pathway.[8] Unfortunately it is difficult to evaluate the contributions of these various bound water relaxation mechanisms. Our previous work on dilute protein solutions[9] suggests that, at least in dilute polysaccharide systems, their contribution is small compared to the proton exchange mechanism. Accordingly, in this paper we will ignore bound water contributions and see to what extent the data can be quantitatively interpreted by using only the proton exchange mechanism.

To provide a diverse range of conformational and dynamic behavior, we have chosen polysaccharides differing in molecular weight, chain flexibility, and glycosidic linkage. These include the homopolysaccharides: α1–6 linked glucans (dextrans), β1–3 linked glucans (laminaran and schleroglucan), and α1–4 linked glucans (maltoheptose, hydrolyzed starch, and amylose). Because of their importance as gelling agents we have included some pure ion forms of κ- and ι-carrageenan.

We limit ourselves to the water proton relaxation in this paper. In subsequent papers we will report on the deuterium (D_2O) and oxygen-17 ($H_2{}^{17}O$) relaxation in these systems.

Experimental Section

Transverse proton relaxation times were measured by using the CPMG (Carr–Purcell–Meiboom–Gill) pulse sequence $90°_x$ $[\tau\text{–}180°_y\text{–}\tau\text{–echo}]_n$. The 90–180° pulse spacing τ was varied between 25 μs and 10 ms. Data were averaged over eight acquisitions with phase cycling and a recycle delay of 15 s to avoid saturation. Sufficient echoes were recorded to give a zero baseline, and the transverse relaxation times were determined from the echo decay envelope by using an exponential unpeeling program. A Brucker MSL-100 spectrometer operating at 100 MHz was used with a high-power solenoid coil and a probe temperature thermostated at 300 K. 90° pulse widths were typically between 1 and 2 μs.

Samples were made by addition of known weights of solid to a known weight of doubly distilled, deionized water. Gels were prepared by heating in a sealed bomb at 100 °C. pH values were

0024-9297/91/2224-2944$02.50/0

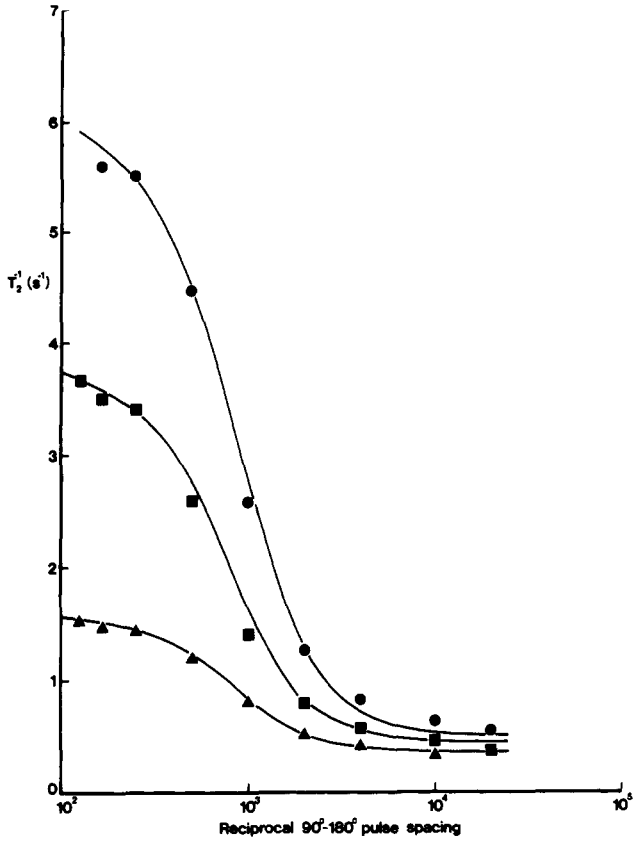


Figure 1. Experimental and theoretical relaxation dispersions for solutions of dextran (average MW 9000), pH 6–7, 300 K, at a spectrometer frequency of 100 MHz: (●) 9.85% w/w dextran, $P_b = 1.615 \times 10^{-2}$, $k_b = 1.1 \times 10^3\ s^{-1}$, $\delta\omega = 1.25$; (■) 6.40% w/w dextran, $P_b = 1.0554 \times 10^{-2}$, $k_b = 1.0 \times 10^3\ s^{-1}$, $\delta\omega = 1.15$ ppm; (▲) 1.96% w/w dextran, $P_b = 3.255 \times 10^{-3}$, $k_b = 1.0 \times 10^3\ s^{-1}$, $\delta\omega = 1.3$ ppm. Other parameters are $T_{2a} = 3$ s and $T_{2b} = 94$ ms.

adjusted by addition of trace quantities of HCl or NaOH (or DCl, NaOD in the case of deuterium oxide), prior to measurement.

Results and Discussion

Dextran Solutions. The water-soluble α1–6 linked dextrans are thought to adopt highly flexible random coil conformations in solution. Our relaxation data are consistent with this picture. Figure 1 shows the water proton transverse relaxation rates of three dextran solutions (average MW ca. 9000) of differing concentration plotted against reciprocal CPMG 90–180° pulse spacing at a spectrometer frequency of 100 MHz. The shape of the curves is characteristic of fast proton exchange and can be analyzed by the general two-site exchange formalism,[3,4] in terms of the parameters P_b, T_{2b}, T_{2a}, k_b, and $\delta\omega$. The fraction of protons in the dextran hydroxyl groups, P_b, is known from the solution concentration. The intrinsic relaxation time of the bulk water, T_{2a} (~3 s), can be measured directly. The intrinsic relaxation time of the dextran hydroxyl protons, T_{2b}, can only be obtained indirectly, either by assuming that it is the same as the relaxation time of the nonexchanging CH protons of the polysaccharide or from the proton relaxation time at short CPMG pulse spacing. At the shortest 90–180° pulse spacing (50 μs) the CPMG echo decay envelope is triple exponential with the longest relaxation time component corresponding to the water relaxation and the two faster decaying components (82 and 460 ms) corresponding to the nonexchanging dextran protons. If the dextran hydroxyl protons have similar relaxation times to the nonexchanging protons, then their average T_{2b} lies between 82 and 460 ms. The average T_{2b} can also be obtained from the observed water relaxation rate in the limit of short pulse spacing, which can be shown to be[4] $P_a T_{2a}^{-1} + P_b/(T_{2b} + k_b^{-1})$. P_b, P_a, and T_{2a}^{-1} are known and k_b is seen from the dispersion curve to be of the order of $10^3\ s^{-1}$ so that $k_b^{-1} \ll T_{2b}$. This gives an average value for T_{2b} of ca. 94 ms, which is within the range of the nonexchanging CH proton relaxation times. This value has been used in the calculation. Having determined P_b, T_{2a}, and T_{2b}, the exchange rate k_b and frequency difference $\delta\omega$ (ppm) can be obtained by numerically fitting the experimental dispersion curves. The continuous lines in Figure 1 were obtained in this way. The resulting exchange rate, $k_b = (1.1 \pm 0.1) \times 10^3\ s^{-1}$, and frequency shift, $\delta\omega = 1.25 \pm 0.05$ ppm, agree well with those previously measured for glucose solutions[3] and methanol–water mixtures.[11] It is important to note that this two-site exchange calculation takes no explicit account of possible bound water contributions. Exchange of bound and free water would not be expected to give rise to the dispersions in Figure 1 since the diffusive exchange of bound and free water occurs on a time scale many orders of magnitude faster than $10^3\ s^{-1}$. Furthermore there are unlikely to be large frequency differences between protons in bound and free water. Fitting the dispersions with the two-site formalism means that any bound water contribution would be included in the parameter T_{2b}. Since T_{2b} is of the same order as the relaxation time of the nonexchanging protons, any bound water contribution must be small.

The proton exchange is, of course, acid–base catalyzed, but in the neutral pH region around pH 6–7 previous work on methanol[11] and glucose[5] solutions has shown that the proton exchange proceeds mainly by a nonionic, cyclic, exchange mechanism. The frequency shift, $\delta\omega$, and exchange rate, k_b, deduced by fitting the dispersion curve are, of course, average values for hydroxyl protons situated on different carbon atoms in the glucose subunits. High-resolution proton spectra for glucose solutions at low temperature[23] show that $\delta\omega$ can differ by up to 0.18 ppm for hydroxyl protons on carbons 2, 3, and 4. This difference is too small to warrant fitting the observed relaxation data as a sum of separate dispersions for each site.

It is remarkable that increasing the average molecular weight of the dextran from 9000 to 2×10^6 makes very little difference to the observed relaxation behavior. As shown in Figure 2 the dispersion behavior for the high and low molecular weight dextrans is almost superimposable and the relaxation times of the nonexchanging dextran protons are also very similar, averaging to 61 ms. The lines in Figure 2 show the theoretical dependence of relaxation on concentration at three different pulse spacings, calculated with the average set of exchange parameters $k_b = 1.1 \times 10^3\ s^{-1}$, $\delta\omega = 1.1$ ppm, $T_{2a} = 3$ s, and $T_{2b} = 94$ ms. The near equivalence of the relaxation behavior for high and low molecular weight dextrans is a consequence of the flexibility of the α1–6 linkage, which allows rapid local motions that average the dipolar interactions of the dextran protons, both exchangeable and nonexchangeable. Proton exchange dispersions are even observed in suspensions of Sephadex beads in water, where the dextran chains are chemically cross-linked with epichlorhydrin, though with Sephadex bead suspensions the spatial heterogeneity can also give rise to slow diffusive exchange and multiple-exponential relaxation.[12]

Laminaran Solutions. Laminaran consists of low molecular weight (DP < 300), unbranched chains of β1–3 linked glucose units. It is water soluble but does not form a gel. In the solid state, X-ray diffraction shows that it adopts a triple-helix conformation.[13] In aqueous solution at neutral pH the changes in peak intensity of its high-resolution carbon-13 spectrum suggest that short chains having less than 20 linked glucose units are in a random

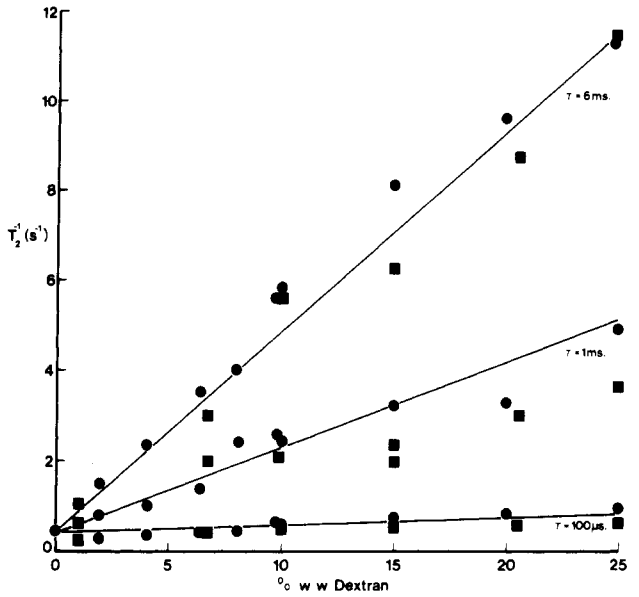


Figure 2. Dependence of the transverse water proton relaxation rate at three 90–180° CPMG pulse spacings (τ) on dextran concentration: (●) dextran average MW 9000; (■) dextran average MW 2×10^6. Parameters used to calculate the theoretical lines are $P_b = 3x/(3x + 1800)$, $\delta\omega = 1.1$ ppm, $\omega_0 = 100$ MHz, $k_b = 1.1 \times 10^3$ s^{-1}, $T_{2a} = 3$ s, $T_{2b} = 94$ ms for an x% w/w dextran concentration.

coil state, whereas longer chains having more than 49 linked glucose units adopt a more rigid, stable conformation, presumably a triple helix.[14] This makes laminaran an interesting example for water relaxation studies, since the laminaran hydroxyl protons involved in triple-helix formation are not expected to be readily accessible to exchange with water protons, either because they are involved in intramolecular hydrogen bonding or because there is a sheath of tightly bound water surrounding, and stabilizing, the triple helix,[15] making formation of cyclic proton exchange transition states energetically unfavorable. This is consistent with the observation of Nardin and Vincenden[16] that, in a polydisperse sample of laminaran dissolved in D_2O containing 25 g/L LiCl at room temperature, only ca. 70% of the hydroxyl protons were exchanged with deuterium over a 2-h period. Presumably the remaining 30% of hydroxyl protons belonged to the longer chain component involved in helix formation.

Figure 3 shows the transverse relaxation dispersion observed for a 10.8% w/w laminaran solution at pH 6.5 and at 300 K. Curve a shows the theoretical dispersion assuming only 70% of the laminaran hydroxyl protons are accessible to exchange and are associated with laminaran molecules in the random coil state. The same exchange parameters as random coil dextran ($\delta\omega = 1.23$ ppm, $k_b = 1.1 \times 10^3$ s^{-1}) have been used with a value for T_{2b} of 30 ms. This value was deduced by fitting the relaxation data at short CPMG pulse spacings, assuming a relaxation time for bulk water (T_{2a}) of 3 s. It can be seen that this set of assumptions gives excellent agreement with experiment. On the other hand curve b in Figure 3 shows the dispersion curve calculated with the same parameters but assuming that all of the laminaran hydroxyl protons are accessible to exchange. Clearly this assumption fails to describe the observed relaxation data. The dispersion analysis therefore provides independent confirmation of the results of Nardin and Vincenden[16] that ca. 30% of the laminaran hydroxyl protons are inaccessible to exchange. The echo decay envelope at a 90–180° pulse spacing of 50 μs shows two fast components with relaxation times of 8 ms and 490 μs arising from the nonexchanging protons. With the shortest relaxation time component (490 μs) there

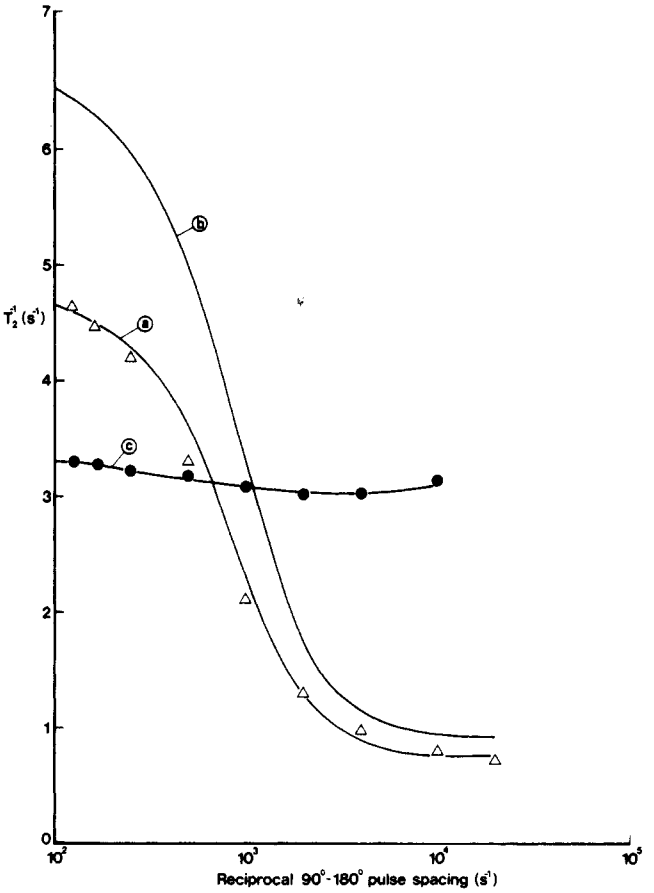


Figure 3. Experimental and theoretical water proton transverse relaxation rates for laminaran and schleroglucan. Curve a: Theoretical dispersion for a 10.78% w/w laminaran solution, pH 6.5, 300 K, calculated assuming only a 70% accessibility ($F = 0.7$); $P_b = 1.2354 \times 10^{-2}$, $k_b = 1.1 \times 10^3$ s^{-1}, $\delta\omega = 1.23$ ppm, $T_{2a} = 3$ s, $T_{2b} = 30$ ms, $\omega_0 = 100$ MHz. Triangles are experimental points. Curve b: As curve a except that a 100% accessibility for proton exchange has been assumed ($F = 1.0$), $P_b = 1.764\,95 \times 10^{-2}$. Curve c: The experimental relaxation rates for a 2.6% w/w schleroglucan gel at 300 K, $\omega_0 = 100$ MHz. The curve is a guide to the eye.

Table I
Effect of Increasing Concentration on Parameters Related to the Accessibility and Mobility of Laminaran Hydroxyl Protons

laminaran concn, % w/w	F^a	T_{2b}(OH),[b] ms	T_{2b}(CH) slow, ms	T_{2b}(CH) fast, μs
2.59	0.53	50	28	180
4.75	0.675	40	11.8	350
7.92	0.60	30	8.6	263
10.78	0.70	30	7.9	490

[a] Exchangeable fraction of laminaran hydroxyl protons. [b] From the water relaxation.

were very few data points, so this relaxation time is only an order of magnitude estimate. In D_2O a 2.6% laminaran solution gave two fast decaying components of 14.6 ms and 89 μs. Although we cannot assign these components with certainty, it is probable that the longer relaxation time component (8–15 ms) is associated with low molecular weight laminaran in the random coil state, while the shorter component (89–490 μs) is associated with longer chain laminaran in the more rigid triple helix state. Table I lists the results of analyzing a series of laminaran solutions of increasing concentration. The relaxation times T_{2b}(OH) and T_{2b}(CH) (slow component) tend to shorten with increasing laminaran concentration as the viscosity increases, while the fracton (F) of laminaran hydroxyl protons accessible to exchange appears to increase slightly

with increasing laminaran concentration. It is, however, doubtful whether this later trend is significant because the errors in determining F increase with decreasing concentration as the amplitude of the relaxation dispersion [i.e., T_2^{-1}(long pulse spacing) − T_2^{-1}(short pulse spacing)] decreases.

The proton exchange mechanism can therefore provide a quantitative description of transverse water proton relaxation in laminaran solutions and suggests that there are subpopulations of random coil and helical conformations associated with chains of different length.

Schleroglucan. Like laminaran, the backbone of schleroglucan consists of a linear chain of α1–3 linked glucose units. It differs in having a single pendant β1–6 linked glucose unit on every third glucose unit along the backbone. In the solid state schleroglucan adopts a triple-helix structure similar to laminaran with the pendant glucose units projecting radially outward from the helix.[5] In solution schleroglucan forms thick gels, which CPMAS spectra suggest have a high percentage of ordered triple-helix structure.[17] Surprisingly, Nardin and Vincenden[16] found that only about 18% of the total number of schleroglucan hydroxyl proton in a 2% w/v gel exchanged with deuterium over a 2-h period in D_2O at 90 °C. Even supposing all the hydroxyl groups in the backbone chains were inaccessible for deuterium exchange, the four hydroxyl groups on each pendant β1–6 linked glucose would still be expected to give a 33% exchange percentage (F = 0.33). Evidently helix association in the gel state or a tight hydration sheath causes this to be considerably lower. Such a low accessibility should have observable consequences in the water proton relaxation.

The relaxation time of the nonexchanging CH protons for a 2.06% w/w schleroglucan gel in D_2O is about 32 μs, as seen in the single, fast-decaying component of the proton FID. This very short value is consistent with the expected rigidity of the triple-helix conformation in the gel. It is also consistent with the lack of any observed dependence of water proton transverse relaxation rate on CPMG pulse spacing (Figure 3). The absence of any T_2 dispersion when T_{2b} is very short can be understood analytically. When $P_b \ll 1$, which is the case with our dilute schleroglucan gels, the water proton transverse relaxation rate at long pulse spacings such that $\tau \gg (2k_b)^{-1}$ (τ is the 90–180° pulse spacing) is given by the Swift–Connick expression[10]

$$T_2^{-1}(\text{long } \tau) = P_a T_{2a}^{-1} + P_b k_b \left\{ \frac{T_{2b}^{-2} + T_{2b}^{-1} k_b + (\delta\omega)^2}{(T_{2b}^{-1} + k_b)^2 + (\delta\omega)^2} \right\} \quad (1)$$

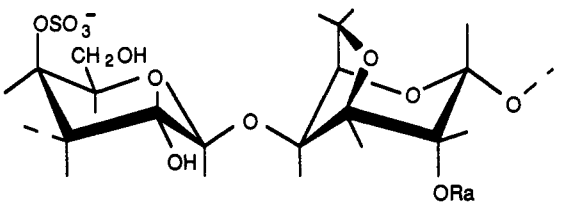
If T_{2b} is short such that the terms in $(\delta\omega)^2$ are negligible, this reduces to

$$T_2^{-1}(\text{long } \tau) = P_a T_{2a}^{-1} + P_b/(T_{2b} + k_b^{-1}) \quad (2)$$

This is the same expression expected in the short pulse spacing limit.[4] Hence under the conditions that $\delta\omega \ll T_{2b}^{-1}$, no dependence of relaxation rate (T_2^{-1}) on pulse spacing is observed. If the exchangeable schleroglucan hydroxyl protons have a similar short relaxation time, T_{2b}, of about 32 μs, as observed for the nonexchanging protons, then it is safe to assume further that, in the neutral gel, $k_b^{-1} \gg T_{2b}$, so that the relaxation is exchange rate limited and eq 2 reduces to $T_2^{-1} = P_a T_{2a}^{-1} + P_b k_b$, at all pulse spacings. If a fraction F of the schleroglucan hydroxyl protons is accessible to exchange, this becomes $T_2^{-1} = T_{2a}^{-1} + F(P_b)_{max} k_b$, where $(P_b)_{max}$ is the theoretical value assuming 12 exchangeable protons per repeating unit. A plot of T_2^{-1} against $(P_b)_{max}$ should therefore be linear with slope Fk_b and intercept T_{2a}^{-1}. Figure 4 shows that this is indeed the case and the experimental slope is 667 s^{-1}. Taking F equal to 0.18 from the data of Nardin and Vincenden yields a value for the proton exchange rate, k_b, of 3.7×10^3 s^{-1}. This result is of the correct order of magnitude for proton exchange, especially if the pH of the gel deviates somewhat from neutrality. As a further test we measured the water relaxation time of the same gel at a very acidic pH value (≤2). This was done by allowing 0.1 M HCl to diffuse into the gel for several hours at 5 °C. We reasoned that since proton exchange is acid–base catalyzed the exchange rate, k_b, at very low pH values should become very fast compared to the relaxation rate, T_{2b}^{-1}. If so, the water proton relaxation should change from the exchange-limited case to the "T_{2b}-limited" case for which $T_2^{-1} = T_{2a}^{-1} + F(P_b)_{max} T_{2b}^{-1}$. This would enable the relaxation time of the exchangeable schleroglucan hydroxyl protons (T_{2b}) to be estimated and compared with that measured for the nonexchanging CH protons. Assuming F remains unchanged at 0.18, the observed relaxation rate (3.1 s^{-1}) for the acidified gel gives a value for T_{2b} of 216 μs. This is to be compared with a relaxation time of ca. 167 μs for the nonexchanging protons in a 2% schleroglucan gel prepared in D_2O and acidified with DCl. The agreement is reasonable but both relaxation times (216 and 167 μs) are significantly longer than those in the unacidified gel (ca. 32 μs), which suggests that the acid treatment has weakened the gel structure somewhat.

The proton exchange mechanism therefore appears to confirm that the schleroglucan gel network is extremely rigid and that the hydroxyl protons have a low accessibility for exchange with water. It also explains the observed dependence of relaxation rate on concentration and pulse spacing. It is noteworthy that no explicit reference has been made to contributions from bound water. In the above two-site proton exchange analysis any bound water contribution would be incorporated in a somewhat enhanced value for the exchange parameter, k_b. While this remains a possibility, the fact that the value derived for k_b (3.7×10^3 s^{-1}) is of the correct order of magnitude for proton exchange suggests that bound water contributions are minor.

Sodium κ-Carrageenan. The repeating unit of sodium κ-carrageenan is shown below:



Iota Ra = SO_3^-
Kappa Ra = H

Structure of the repeat unit of carrageenan

Figure 5 shows the experimental dependence of the single-exponential water proton transverse relaxation rate on pulse spacing for a 4.47% w/w gel in 0.2 M NaCl at pH 6.8 measured at 300 K. At this relatively high ionic strength the sample forms a soft gel having a slightly milky appearance. This semiopaque quality of the gel suggests some form of microheterogeneity and examination with a light microscope reveals a granular structure on a distance scale of 1–10 μm. This microheterogeneity is most probably a result of a microphase separation process,[18] whereby the gel is formed by association of many chains into a three-dimensional network of microfibers having a thickness of 1–10 μm. The existence of two microphases

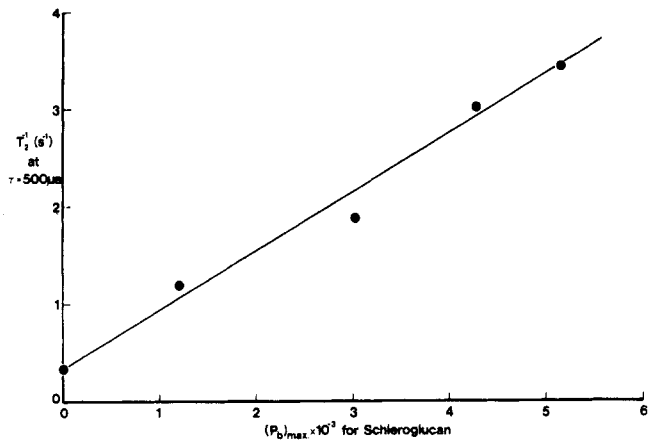


Figure 4. Dependence on concentration, $(P_b)_{max}$, of the water proton transverse relaxation rate at a CPMG 90–180° pulse spacing of 500 μs for schleroglucan gels at 300 K.

has important consequences for the interpretation of the relaxation data. At the shortest 90–180° pulse space (50 μs) there are, besides the water relaxation, two faster decaying components in the CPMG echo decay envelope, having relaxation times of ca. 12.7 ms (0.54%) and 150 μs (1.52%). These two faster components could correspond either to nonexchanging protons in the two microphases or, more probably, to carrageenan protons in the gel-rich phase where the shorter 150-μs component corresponds to helices associated into more rigid junction zones[19] with the microfibers. The assignment is not critical since both possibilities lead to similar expressions for the water proton relaxation rate in the limit of short pulse spacings. To see this we first assume that the two relaxation times $T_{2b}^{(1)}$ (12.7 ms) and $T_{2b}^{(2)}$ (150 μs) are associated with carrageenan hydroxyl protons in two separate microphases labeled (1) and (2). Then we can write

$$T_{2b}^{-1}(\text{short } \tau) = \{P_a^{(1)}T_{2a}^{-1} + P_b^{(1)}/(T_{2b}^{(1)} + k_b^{-1})\} + \{P_a^{(2)}T_{2a}^{-1} + P_b^{(2)}/(T_{2b}^{(2)} + k_b^{-1})\} \quad (3)$$

where the two terms in braces correspond to the two separate microphases. (Possible contributions from diffusive exchange between the two phases are ignored.) But since $P_a^{(1)} + P_a^{(2)} = P_a = (1 - P_b^{(1)} - P_b^{(2)})$, this expression can also be written as

$$T_2^{-1}(\text{short } \tau) = P_aT_{2a}^{-1} + P_b^{(1)}/(T_{2b}^{(1)} + k_b^{-1}) + P_b^{(2)}/(T_{2b}^{(2)} + k_b^{-1}) \quad (4)$$

which would also result from associating both $T_{2b}^{(1)}$ and $T_{2b}^{(2)}$ with molecular structures of different mobility within a single microfiber phase surrounded by bulk water. For simplicity we therefore refer in the following to two "domains" without specifying whether these correspond to the spatially distinct microphases seen in the microscope or merely to different molecular aggregates within a single phase. The sum $P_b = P_b^{(1)} + P_b^{(2)}$ can be calculated from the known gel concentration, assuming three exchangeable carrageenan hydroxyl protons per repeating disaccharide unit, with the result $P_b = 2.949 \times 10^{-3}$ for a 4.47% w/w sodium κ-carrageenan gel. T_{2a} has the bulk water value (3 s), which leaves $P_b^{(2)}$ and k_b as the only independent, unknown variables. If we assume k_b has a value of ca. 10^3 s^{-1} in line with dextran and glucose, $P_b^{(2)}$ can be calculated from eq 4 with the result $P_b^{(2)} = 0.696 \times 10^{-3}$ and $P_b^{(1)} = 2.253 \times 10^{-3}$. Knowing these values from the relaxation rate at short pulse spacing, it is straightforward to calculate the relaxation rates at all other pulse spacings as $T_2^{-1} = T_2^{-1(1)} + T_2^{-1(2)} - T_{2a}^{-1}$ where $T_2^{-1(i)}$ is the relaxation rate for domain i calculated by using the two-site exchange

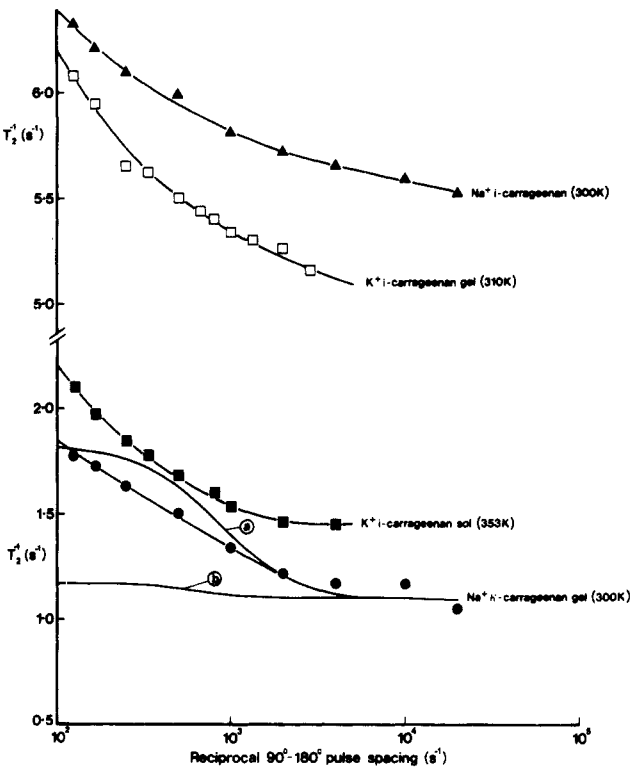


Figure 5. Experimental and theoretical water proton transverse relaxation rates for several pure ion forms of carrageenan. (●) 4.47% w/w sodium κ-carrageenan gel in 0.2 M NaCl at 300 K, pH 7; (□, ■) ca. 10% w/w potassium ι-carrageenan, (□) gel at 310 K, (■) sol at 353 K; (▲) 9.89% w/w sodium ι-carrageenan at 300 K, pH 7 (no added NaCl). Curve a: The theoretical proton exchange dispersion calculated as the sum of dispersions characterized by $P_b^{(1)} = 2.253 \times 10^{-3}$, $T_{2b}^{(1)} = 13$ ms, $P_b^{(2)} = 6.966 \times 10^{-4}$, and $T_{2b}^{(2)} = 150$ μs. Other parameters are $\delta\omega = 1.3$ ppm, $T_{2a} = 3$ s, $k_b = 10^3$ s^{-1}, and $\omega_0 = 100$ MHz. Curve b: The theoretical proton exchange dispersion calculated as the sum of dispersions characterized by $P_b^{(1)} = 3.549 \times 10^{-4}$, $P_b^{(2)} = 2.594 \times 10^{-3}$, and $k_b = 300$ s^{-1}. Other parameters as in curve a. Curves other than a and b are only guides for the eye.

equations with an assumed hydroxyl proton chemical shift of about 1.25 ppm on the basis of dextran and glucose data. The relaxation rate of bulk water T_{2a}^{-1} needs to be subtracted since it would otherwise be counted twice. As with schleroglucan the component having a short T_{2b} of only 150 μs does not give rise to any pulse spacing dependence, so the predicted variation (curve a in Figure 5) originates from the component characterized by a T_{2b} of 12.7 ms. Comparison of curve a with the experimental data in Figure 5 shows that while the proton exchange mechanism adequately accounts for the relaxation rate at short pulse spacings it fails to reproduce the monotonic increase in relaxation rates as the 90–180° pulse spacing becomes longer than about 500 μs.

The theoretical curve a is, however, based on an assumed proton exchange rate, k_b, of 10^3 s^{-1}. The independent observation that raising the temperature gives an exchange minimum in the water relaxation rate[2] suggests that at 300 K the proton exchange rate is slow, such that $k_b^{-1}\delta\omega > 1$. Curve b in Figure 5 has been calculated by assuming a k_b of only 300 s^{-1} to illustrate that lowering k_b markedly reduces the amplitude of the dispersion. Nevertheless this still does not explain the observed monotonic increase in relaxation rate. Such monotonic increases can, however, arise when water molecules diffuse in systems having microheterogeneity. This is because different microregions are associated with slightly different water proton resonance frequencies so that diffusion of water between them causes enhanced dephasing, which increases with increasing pulse spacing. The spatial variations in resonance

frequencies are caused by magnetic susceptibility differences and their associated field gradients as well as by fast proton exchange in carrageenan gel with spatially varying concentration. We have previously reported similar monotonically increasing water proton relaxation rates in the model system consisting of a packed suspension of Sephadex beads (G25–50) in water, where the beads have a mean radius of 25 μm.[12] The criterion for observing single-exponential relaxation in these spatially heterogenous systems is, approximately, that $(a^2/D)|T_2^{-1(1)} - T_2^{-1(2)}| \ll 1$, where a is the mean dimension of the heterogeneity, D is the water self-diffusion coefficient, and $T_2^{(i)}$ is the intrinsic relaxation time of microregion i.[21] Since a is of the order of 10 μm or less, D is ca. 10^{-6} cm^2 s^{-1} and $T_2^{(1)}$ and $T_2^{(2)}$ are calculated to be 2.0 and 1.06 s, respectively; for k_b = 300 s^{-1}, the ratio $(a^2/D)|T_2^{-1(1)} - T_2^{-1(2)}$ is only 0.045, which is consistent with the observed monoexponential water proton relaxation.

The exchange minima observed in carrageenan gels as the temperature is raised[2] are predicted by the proton exchange model. Equation 4 shows that exchange minima are expected at temperatures for which $T_{2b}^{-1(1)} = k_b$ and $T_{2b}^{-1(2)} = k_b$. This occurs when k_b is 78.7 s^{-1} ($T_{2b}^{(1)}$ = 12.7 ms) and 6.7 × 10^3 s^{-1} ($T_{2b}^{(2)}$ = 150 μs). The later exchange rate is in the correct range for carbohydrate hydroxyl proton exchange at elevated temperatures. Once the gel has melted $T_{2b}^{(2)}$ changes from 150 μs to tens of milliseconds, so an exchange minima is no longer observable.[2]

The observation[2] that plots of T_2^{-1} at fixed pulse spacing against carrageenan concentration (P_b) are linear but have an intercept at zero concentration that is significantly higher than T_{2a}^{-1} is a simple consequence of the existence of two domains in the sample. Since $P_b = P_b^{(1)} + P_b^{(2)}$, eq 4 can be written as

$$T_2^{-1}(\text{short } \tau) = P_a T_{2a}^{-1} + P_b^{(1)}/(T_{2b}^{(1)} + k_b^{-1}) + (P_b - P_b^{(1)})/(T_{2b}^{(2)} + k_b^{-1}) \quad (5)$$

In general $P_b^{(1)}$ is expected to depend in a complicated way on the gel concentration (P_b) but, provided $P_b \ll 1$, $P_b^{(1)}$ can be written as a polynomial in P_b and terms higher than first-order neglected. Then $P_b^{(1)} = \alpha + \beta P_b + \ldots$ Substituting this into eq 5 gives

$$T_2^{-1}(\text{short } \tau) = (T_{2a}^{-1} + \alpha I) + P_b(T_{2b}^{(2)} + k_b^{-1})^{-1} + \beta I P_b \quad (6)$$

where

$$I = (T_{2b}^{(1)} + k_b^{-1})^{-1} - (T_{2b}^{(2)} + k_b^{-1})^{-1} \quad (7)$$

This shows that if there is microheterogeneity (i.e., two domains) a plot of T_2^{-1} against P_b gives a straight line of slope $(T_{2b}^{(2)} + k_b^{-1})^{-1} + \beta I$ and intercept $(T_{2a}^{-1} + \alpha I)$. A positive value of the incremental intercept, αI, means that there is proportionally more of the more rigid domain at the minimum gelling concentration than at higher concentrations. It is noteworthy that if both $T_{2b}^{(1)}$ and $T_{2b}^{(2)}$ become much shorter than k_b^{-1} so the relaxation is exchange rate limited, then $I \to 0$ and the intercept becomes simply T_{2a}^{-1}. This may well be the case with deuterium (D_2O) relaxation in carrageenan gels for which $T_{2b}^{(1)}$ and $T_{2b}^{(2)}$ are determined by fast quadrupolar relaxation mechanisms. Schleroglucan gels, which were discussed in the previous section, have an intercept equal to T_{2a}^{-1}, at least within experimental error. This is expected since the gels are transparent and there is only a single very short T_{2b} associated with the nonexchanging protons. Accordingly there is no evidence for microheterogeneity (i.e., more than one domain) in schleroglucan gels.

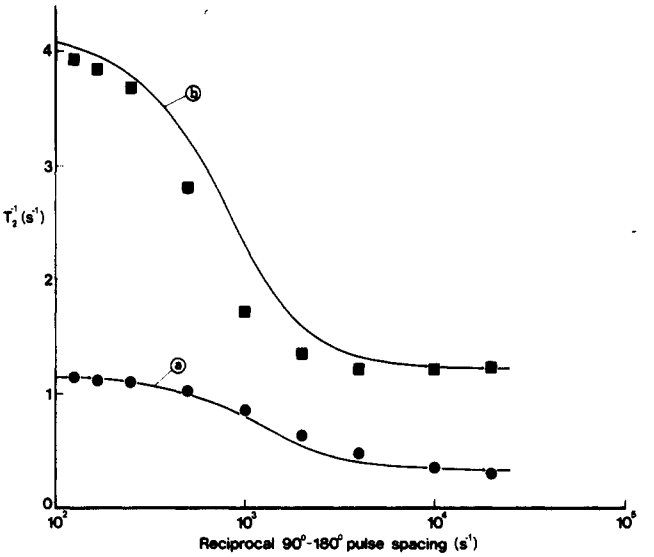


Figure 6. Experimental and theoretical water proton relaxation dispersion curves for maltoheptose and hydrolyzed starch. (●) Experimental relaxation rates for a 2.08% w/w maltoheptose solution, pH 7, 300 K. (■) Experimental relaxation rates for a 10.75% w/w gel of hydrolyzed potato starch at 300 K. Curve a. Curve a: Theoretical proton exchange dispersion calculated with P_b = 3.7236 × 10^{-3}, k_b = 1.8 × 10^3 s^{-1}, $\delta\omega$ = 1.1 ppm, T_{2a} = 3 s, T_{2b} = 0.7 s, and ω_0 = 100 MHz. Curve b: Theoretical proton exchange dispersion calculated as the sum of two dispersions characterized by $P_b^{(1)} = 10^{-2}$, $T_{2b}^{(1)}$ = 1 s, $P_b^{(2)}$ = 7.787 × 10^{-4}, and $T_{2b}^{(2)} < 100$ μs. Other parameters are k_b = 1.1 × 10^3 s^{-1}, T_{2a} = 3 s, $\delta\omega$ = 1.1 ppm, and ω_0 = 100 MHz.

The above analysis therefore provides a consistent framework for interpreting the subtle relaxation phenomena found in carrageenan gels. Unfortunately, the magnitude of the exchange rate, k_b, at 300 K has not been determined except that it must be considerably lower than for dextran and glucose. This may be a consequence of the ionic nature of carrageenan gels where charged sulfate groups spaced along the backbone, together with their associated counterions, may sterically hinder proton exchange between water and the carrageenan hydroxyl groups or so structure the water that the cyclic transition states needed for proton exchange at neutral pH[5,11] are energetically unfavorable.

Although we have so far considered only sodium κ-carrageenan a very similar picture is found with other pure ion forms of carrageenans. In Figure 5 we have included the experimental data for a more concentrated (ca. 10%) potassium ι-carrageenan gel at 310 K and its corresponding sol state after heating to 353 K as well as for sodium ι-carrageenan at 300 K. It can be seen that all these systems exhibit the monotonic dependence on pulse spacing, which is characteristic of water diffusion in microheterogeneous systems. It is interesting to note that even in the sol state of sodium ι-carrageenan at pH 7 at low ionic strength (no added NaCl) the viscous solution remains slightly opaque, suggesting the persistence of microphases over a distance scale of micrometers.

Maltoheptose, Hydrolyzed Starch, and Amylose. Because of the rigidity of their gel networks, starch, amylose, and amylopectin gels would not be expected to show any proton exchange dispersion in the water proton transverse relaxation rate as the pulse spacing is varied. A gel of potato starch that has been partially hydrolyzed for use in electrophoresis does, however, show a typical proton exchange dispersion (Figure 6). This dispersion undoubtedly originates from the hydrolytic treatment, which creates short, flexible chains attached to the gel network or a distribution of low molecular weight oligosaccharides dissolved in the gel. The CPMG echo decay

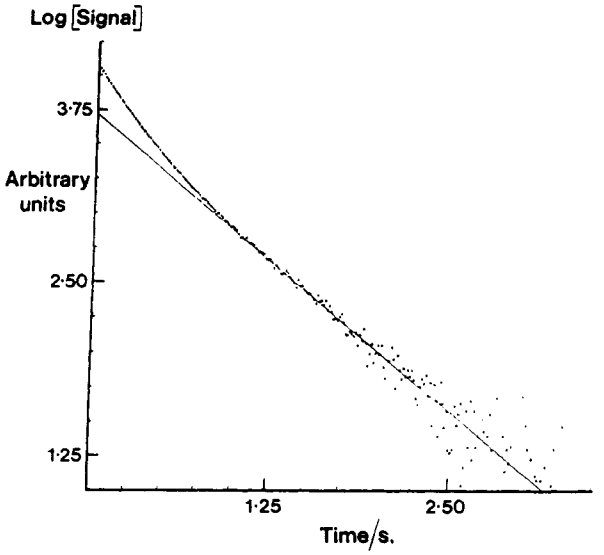


Figure 7. Biexponential decay observed in an amylose gel at 300 K using a 90–180° CPMG pulse spacing of 1 ms. The component relaxation times are 0.506 and 0.194 s.

envelope of a 10.75% gel observed with a 50 μs 90–180° pulse spacing shows two faster decaying components with mean relaxation times of 20.5 ms and 40 μs. The shorter, 40 μs-relaxation time probably corresponds to CH protons in the more rigid gel network, while the longer 20.5-ms component arises from the more flexible hydrolyzed chains and/or the dissolved oligosaccharides. Figure 6 shows the experimental proton exchange dispersion for the purified oligosaccharide maltoheptose at pH 7, which can be satisfactorily fitted with an exchange rate of 1.8×10^3 s^{-1} and a T_{2b} of 0.7 s. The polydispersity of the hydrolyzed starch gel prevents any accurate theoretical analysis of its dispersion curve, but the existence of a dispersion in a partially hydrolyzed gel can be demonstrated theoretically by modeling it as a solution of oligosaccharides with an average T_{2b} of 1 s dissolved in a rigid starch gel having a very short T_{2b} of less than 100 μs (Figure 6, curve b).

The gelling and retrogradation properties of amylose solutions have been extensively investigated.[18,20] Cooling aqueous amylose solutions can produce either homogeneous gels or complex two-phase systems where there is gelation accompanied by precipitation or gel lump formation, depending on the degree of polymerization, concentration, and rate of cooling. The transverse relaxation behavior of the water protons is a sensitive probe of these complex phase phenomena. If the dimension a characterizing the scale of the gel heterogeneity is sufficiently large that the ratio $(a^2/D)|T_2^{-1(1)} - T_2^{-1(2)}|$ becomes greater than unity, multiple-exponential relaxation is predicted.[12,21] Figure 7 shows the experimental biexponential relaxation in the CPMG echo decay envelope of an amylose gel, where the gel has become opaque due to phase separation. The two phases have intrinsic relaxation times of 0.506 and 0.194 s that are independent of pulse spacing. These components no doubt are determined by fast proton exchange between water and amylose hydroxyl protons in the separate phases. However, contributions from diffusive exchange and the bound water relaxation mechanism cannot be discounted.

Conclusion

By assuming proton exchange to be the major transverse relaxation mechanism, we have succeeded in showing that the diverse range of water relaxation behavior observed in dilute polysaccharide systems can be interpreted quantitatively and related to the rigidity and state of aggregation of the polysaccharide chains. A recurring difficulty in the analysis has been the unique determination of the parameters, P_b, k_b, and T_{2b}. For this reason it is best to combine the water relaxation measurements with other NMR techniques that give complementary information on the state of the biopolymer. For example, deuterium exchange methods[16] can provide an independent determination of the accessibility of hydroxyl protons to exchange and hence of the parameter P_b. Line widths in high-resolution carbon-13 spectroscopy can give some indication of the dynamic state of the biopolymer chains,[22] while high-resolution CPMAS spectra of polysaccharide gels can, in suitable circumstances, be related to the conformational state of the biopolymer.[17,20]

The results obtained here suggest that in dilute polysaccharide systems bound water has no significant part to play in proton relaxation phenomena. It is not clear if this is the case in more concentrated systems and it is a matter of considerable interest to determine what effects polysaccharides do have on the behavior of water. Deuterium and ^{17}O relaxation time measurements can cast considerable light on this question and we hope to report on results of these in forthcoming papers.

Acknowledgment. We gratefully acknowledge funding from the Ministry of Agriculture, Fisheries and Food and wish to thank M. Vincenden for the gift of schleroglucan.

References and Notes

(1) Suggett, A. In *Water, a Comprehensive Treatise*; Franks, F., Ed.; Plenum Press: New York, 1976; Volume 4, Chapter 6.
(2) Lewis, G. P.; Derbyshire, W.; Ablett, S.; Lillford, P. J.; Norton, I. T. *Carbohydr. Res.* **1987**, *160*, 397–410.
(3) Hills, B. P.; Wright, K. M.; Belton, P. S. *Mol. Phys.* **1989**, *67*, 1309–1326.
(4) Carver, J. P.; Richards, R. E. *J. Magn. Reson.* **1972**, *6*, 89–105.
(5) Hills, B. P. *Mol. Phys.*, in press.
(6) Belton, P. S.; Ring, S. G.; Botham, R. L.; Hills, B. P. *Mol. Phys.*, in press.
(7) Halle, B. *Mol. Phys.* **1987**, *61*, 963–980.
(8) Ahmad, S. B.; Packer, K. J.; Ramsden, J. M. *Mol. Phys.* **1977**, *33*, 857–874.
(9) Hills, B. P.; Takacs, S. F.; Belton, P. S. *Mol. Phys.* **1989**, *67*, 903–937.
(10) Swift, T. J.; Connick, R. E. *J. Chem. Phys.* **1962**, *37*, 307–320.
(11) Hills, B. P. *J. Chem. Soc., Faraday Trans.* **1990**, *86*, 481–487.
(12) Hills, B. P.; Wright, K. M.; Belton, P. S. **1989**, *67*, 193–208.
(13) Deslandes, Y.; Marchessault, R. H.; Sarko, A. *Macromolecules* **1980**, *13*, 1466–1471.
(14) Saito, H. In *Solution Properties of Polysaccharides*; Brant, D. A., Ed.; ACS Symposium Series 150, American Chemical Society: Washington, DC, 1981; pp 125–147.
(15) Chuah, C. T.; Sarko, A.; Deslandes, Y.; Marchessault, R. H. *Macromolecules* **1983**, *16*, 1375–1382.
(16) Nardin, R.; Vincenden, M. *Macromolecules* **1989**, *22*, 3551.
(17) Stipanovic, A. J.; Giammatteo, P. J. In *Water Soluble Polymers*; Glass, J. E., Ed.; Advances in Chemistry 223; American Chemical Society: Washington, DC, 1989; pp 1–9.
(18) Miles, M. J.; Morris, V. J.; Ring, S. G. *Carbohydr. Res.* **1985**, *135*, 257–269.
(19) Morris, E. R.; Rees, D. A.; Robinson, G. *J. Mol. Biol.* **1980**, *138*, 349–362.
(20) Gidley, M. J.; Bulpin, P. V. *Macromolecules* **1989**, *22*, 341–346.
(21) Belton, P. S.; Hills, B. P.; Raimbaud, E. R. *Mol. Phys.* **1988**, *63*, 825–842.
(22) Gorin, P. A. J. *Adv. Carbohydr. Chem. Biochem.* **1981**, *38*, 13–104.
(23) Harvey, J. M.; Symons, M. C. R.; Naftalin, R. J. *Nature* **1976**, *261*, 435–436.

Registry No. Carrageenan, 9000-07-1.