Molecular Motion of Spin-Labeled Dextrans in Dilute Aqueous Solution

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ABSTRACT: A nitroxide spin label bound to hydroxyl groups via an ester linkage has been used to probe the dynamics of dextran polymers in dilute aqueous solution. Dextrans ranging in molecular weight from 10 500 to 2×10^6 daltons were covalently labeled at random sites. The resulting rotational correlation times demonstrate an apparent molecular weight dependence that is believed to be simply the result of averaged contributions to the apparent motion from a relatively rapid motion of the ends of the polymer chain and a slower segmental reorientation characteristic of the infinite chain.

Introduction

In recent years there has been considerable interest in the dynamic properties of macromolecules in dilute solution. These thermally driven motions span a wide range of time scales, and a variety of techniques such as dielectric relaxation, fluorescence depolarization, NMR, and inelastic light scattering have been used to investigate them.[1] One technique which is sensitive over the physically interesting range from 10^{-11} to 10^{-7} s is line shape analysis of the paramagnetic resonance spectrum of an attached spin label.[2] Spin label measurements have been performed on proteins and on a number of different synthetic polymers, but only rarely on carbohydrate polymers due to a relative lack of spin labels and spin-labeling procedures that are amenable to chemically unmodified polysaccharides. In this paper, employing a spin-labeling method developed in our laboratories for polysaccharides,[3] we describe experiments using a covalently bound nitroxide spin label as a reporter for motion of a well-characterized carbohydrate polymer, dextran, in dilute aqueous solutions.

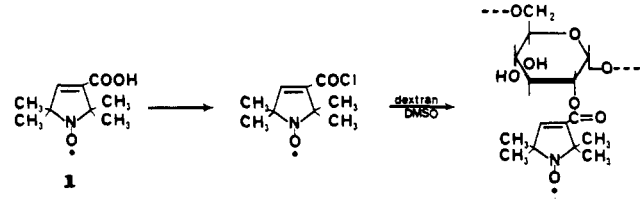
Synthesized by *Leuconostoc mesenteroides* bacteria, strain B-512, dextran was chosen as the model carbohydrate polymer for this investigation for several reasons. It consists of approximately 95% α-(1→6)-linked polymer of D-glucose, has a minimal amount of branching, and can be purchased commercially in narrow molecular weight distributions. In addition, its solution properties have been extensively studied because of its clinical use since the 1950s as a blood plasma volume extender. It is easily soluble in water and dimethyl sulfoxide, and for molecular weights above 2000 daltons (about 12 D-glucose monomers), it adopts a random coil shape in solution.[4-6] Radii of gyration, intrinsic viscosities, and other hydrodynamic properties have been determined experimentally.[4-9]

Recently, the solution dynamics of dextran have been examined by ^{1}H NMR spectroscopy,[10] where it was demonstrated that the poly-(1→6)-glycosidic linkages in dextran make it a more flexible polymer than a poly-(1→4)-linked polysaccharide, which has fewer potential bonds between monomers. Ultrasonic relaxation experiments have also recently been applied to aqueous dextran solutions.[11]

Dextrans ranging in molecular weight from 10 500 to 2×10^6 daltons have been examined in this investigation, and we find a characteristic molecular weight dependent reorientation time similar to those reported in the literature for synthetic polymers,[12-14] though our interpretation for this system is rather different. When well-characterized molecular weight fractions are available, one can often interpret the motion as a superposition of independent processes, identified as segmental motion of the infinite chain and an overall cooperative motion of the chain dependent on molecular weight.[1] For the dextran system, however, we believe the results reflect a relatively rapid motion of the ends of the polymer chain compared with the middle section and that the apparent molecular weight dependence of the motion is simply a dilution of the rapid end motions by an increasing amount of segmental reorientation characteristic of the infinite chain.

Experimental Section

Sample Preparation. Eight dextran polymer samples of average molecular weights 10 500, 17 000, 40 000, 70 000, 151 000, 250 000, 510 000, and 2×10^6 daltons were purchased from Sigma Chemical Co. The exact polydispersity of each sample was not known. The nitroxide spin label 3-(chloroformyl)-2,2,5,5-tetramethylpyrroline-1-oxyl was synthesized from 3-carboxy-2,2,5,5-tetramethylpyrroline-1-oxyl (1, Eastman Kodak Co.) according to a method previously described.[15] It was then covalently bound to the dextran polymers dissolved in dimethyl sulfoxide via a reaction with hydroxyl groups to produce an ester linkage, illustrated below.



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The reaction mixture was exhaustively dialyzed against 30% ethanol and then against distilled water to remove gross unbound spin label, and the spin-labeled dextran samples were finally lyophilized. The details of the labeling procedure are given elsewhere.[3]

Gram quantities of dextran of each average molecular weight were labeled and it was determined that the low molecular weight dextrans (i.e., 10 500–151 000 daltons) had a spin concentration of approximately one spin label per 200 carbohydrate monomer units and that the high molecular weight samples (i.e., 250 000 to 2×10^6 daltons) possessed spin concentrations of about one per 500 carbohydrate monomers. In parallel experiments where the amount of spin label was varied in the derivatization procedure, it was established that at the above label concentrations there were no contributions to line widths from spin exchange or dipole–dipole interactions between spins.[3]

Before each ESR experiment, the lyophilized spin-labeled dextran powders were dissolved in distilled water (15 mg/3 mL) and dialyzed for 4 h against distilled water at 55 °C followed by

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an additional 4 h at room temperature. This method effectively removed the small amount of remaining unbound nitroxide label, and the resulting solutions were found to be stable in the ESR cavity from 15 to 65 °C. The 0.5% dextran concentration was chosen in order to make negligible any polymer–polymer interactions. Nitrogen gas was bubbled through all dextran solutions prior to taking data, although oxygen broadening effects were never observed.

Data were taken on a standard Varian X-band spectrometer, Model V4500, with 100-kHz field modulation. Sample temperature was varied by placing the sample holder in a quartz Dewar through which chilled/heated nitrogen gas was passed. Temperature was monitored by a thermocouple wire inserted into the Dewar to the depth of the sample. Field sweep calibrations were made by using an aqueous solution of the spin label 2,2,6,6-tetramethylpiperidine-1-oxyl (Tempo). Line widths and hyperfine splittings were determined from these calibrations.

ESR Line Width Analysis. In a preliminary attempt to interpret the data the motionally narrowed ESR spectra were analyzed with the Kivelson theory[16] for rapid isotropic motion to obtain rotational correlation times (τ_c). Assuming Lorentzian lines shapes and negligible nonsecular broadening, one obtains the following independent expressions for τ_c:

$$\tau_c(1) = \tau_c = \frac{3^{1/2}\pi\Delta\nu(0)}{2C}\left[\left(\frac{h(0)}{h(-1)}\right)^{1/2} + \left(\frac{h(0)}{h(+1)}\right)^{1/2} - 2\right] \quad (1)$$

$$\tau_c(2) = \tau_c = \frac{3^{1/2}\pi\Delta\nu(0)}{2B}\left[\left(\frac{h(0)}{h(-1)}\right)^{1/2} - \left(\frac{h(0)}{h(+1)}\right)^{1/2}\right] \quad (2)$$

where $h(M_I)$ are the peak-to-peak intensities of the absorption derivative $^{14}_{7}$N hyperfine lines and $\Delta\nu(0)$ is the peak-to-peak width of the central line (in Hz). C and B are defined as follows:

$$C = b^2/8, \qquad B = 4b\Delta\gamma B_0/15$$

where

$$b = (2/3)[A_{zz} - (A_{xx} + A_{yy})/2] = (A_{zz} - A_{iso}) \qquad (\text{rad/s})$$

and

$$\Delta\gamma = -\frac{|\beta|}{\hbar}[g_{zz} - (g_{xx} + g_{yy})/2]$$

B_0 is the external magnetic field strength and g_{ii} and A_{ii} are the principal axis values of the g and hyperfine tensors, respectively. Because eq 1 and 2 make use of different spectral information a comparison of the two τ_c's provides an internal consistency check for our main assumptions: isotropic motion and small inhomogeneous line broadening.

For spin-labeled dextrans in aqueous solution, the isotropic hyperfine splitting A_{iso} was found to be 16.1 G and A_{zz}, determined from the frozen solution, was 36.8 G, yielding a value for b of 365.3 $\times 10^6$ rad/s. $\Delta\gamma$ was approximated from single-crystal values of g_{xx}, g_{yy}, and g_{zz} for a typical pyrroline spin label, resulting in a value of 3.885 $\times 10^4$ rad/(s·G). Rotational correlation times calculated from (1) and (2) generally agreed to within 5%, implying that the value of $\Delta\gamma$ used was a reasonable approximation.

Since eq 1 and 2 are based on line width differences between individual hyperfine components, they remain valid even when the ESR lines are subject to inhomogeneous broadening (indeed, this is their beauty) as long as all three hyperfine lines are broadened equally. Small broadening asymmetries will be introduced, though, when the inhomogeneous field distribution associated with hyperfine couplings to nearby methyl protons is convoluted with the natural line shape, as first pointed out by Poggi and Johnson.[17] This potential source of systematic error is most important in the lowest molecular weight sample (where lines are the sharpest). For this sample we can estimate, with our inhomogeneous $\Delta H \lesssim 0.6$ G, that the correlation times of eq 1 may be 5–10% too short. No correction has been made for this effect.

Finally, several groups have observed that the rigidity of the ester linkage minimizes the motional freedom of the spin label with respect to the polymer backbone,[18-20] so the rotational correlation time determined from the ESR spectrum should reflect the motion of the polymer. This ab initio expectation is confirmed, of course, by the strong molecular weight dependence described below.

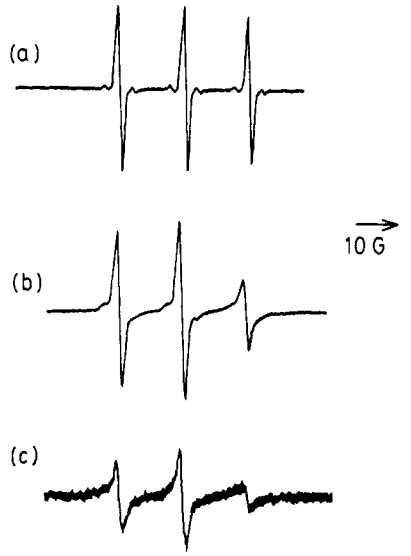

Figure 1. Absorption derivative spectra recorded at room temperature (B_0 = 3230 G, power level 1 mW, modulation amplitude 0.4 G, time constant 0.3 s, sweep rate 100 G/10 min): (a) spin label 1 in H_2O; (b) 10 500 MW dextran (5 mg/mL H_2O); (c) 2 × 10^6 MW dextran (5 mg/mL H_2O).

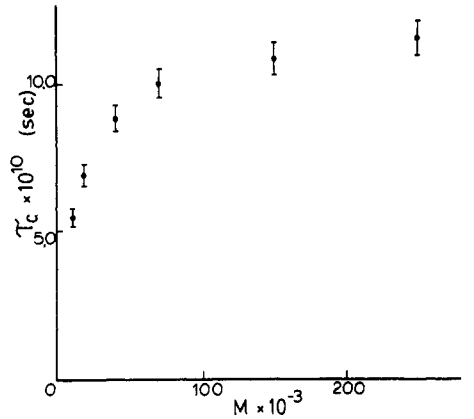

Figure 2. Rotational correlation time vs. molecular weight for spin-labeled dextrans in 0.5% aqueous solution at 20 °C, calculated from eq 1.

Results and Discussion

Typical room-temperature ESR spectra are illustrated in Figure 1. Under all experimental conditions, including measurements at other temperatures and in other solvents not described here, the rotational correlation times increased smoothly with increasing molecular weight. The possibility that the apparent faster motion of the low molecular weight samples is an artifact due to the presence of unbound spin label was carefully investigated. For example, small amounts of spin label 1 were added to spin-labeled dextran solutions. The resulting changes in peak intensities and line widths show unambiguously that the ESR spectrum of the 10 500 molecular weight spin-labeled dextran can not be reproduced by adding free label, in any amount, to a high molecular weight sample.

A plot of room-temperature correlation time vs. molecular weight is shown in Figure 2. In order to analyze the data in terms of rates, assuming one or more independent processes to be involved in the motion, correlation rates (τ_c^{-1}) were plotted vs. $1/M$. As Figure 3 illustrates, a plot of r_c vs. $1/M$ appears to have the form

$$r_c = r(M) + r_\infty \quad (3)$$

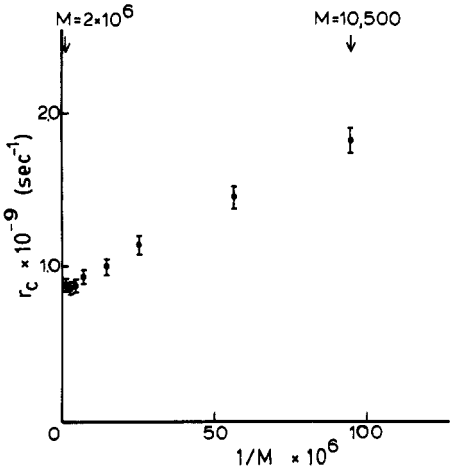

Figure 3. Rate $1/\tau_c$ vs. $1/M$ for spin-labeled dextrans in 0.5% aqueous solution at 20 °C.

indicating a rate that is the sum of a molecular weight dependent part that scales with mass (perhaps a cooperative mode) and a part, r_∞, that is independent of molecular weight (a local segmental mode). Expressing eq 3 as

$$r(M) = r_c - r_\infty = kM^x \tag{4}$$

a plot of ln $r(M)$ vs. ln M will have a slope equal to the mass exponent x. Setting r_∞ equal to the high molecular weight limit, a least-squares fit of ln $r(M)$ vs. ln M yielded a mass exponent of -1.10 ± 0.03. When rate corrections for methyl proton hyperfine broadening were introduced, the decreased rates at low molecular weight led to an estimated reduction in the mass exponent of 5–10%. Thus $r(M)$ appears to scale as $1/M$, and we can write

$$r_c = A/M + B \tag{5}$$

Molecular weight effects in polymer motion have been reported in the literature. In some studies, a molecular weight dependence has been ascribed to a contribution to the motion from overall rotation of the molecule as a whole.[12] We have calculated rotational correlation times for end-over-end tumbling of dextrans in aqueous solution of 20 °C using the expression derived by Riseman and Kirkwood[21,22] for rotational diffusion of random coils

$$r_{eoe} = 6D_{rot} = 6RT/4M\eta_0[\eta] \tag{6}$$

where η_0 is the solvent viscosity in poise and $[\eta]$ is the intrinsic viscosity. The factor of 6 (instead of 2) arises from the fact that the ESR experiment involves relaxation of a second-rank tensor quantity rather than a vector. Intrinsic viscosities were obtained from the literature.[7,8] Even for the lightest polymer the tumbling rate calculated from eq 6 is smaller than $r(M)$ by about a factor of 30, indicating that the contribution of overall tumbling is negligible.

There are many other cooperative modes of motion in a long polymer besides end-over-end tumbling, of course, the best known being those associated with the works of Rouse and Zimm.[23,24] These lowest order Rouse–Zimm modes have occasionally been invoked as the source of a molecular weight dependence for spin label relaxation,[13,14] but it is simply a mistake to apply them to relaxation of a randomly oriented spin dipole; their only relevance is to relaxation processes involving a change in mean segment length. (Although irrelevant, it may help fix the time scales involved to mention that the lowest order Rouse–Zimm mode gives a characteristic relaxation rate a factor of 10 less than the excess rate found in the lightest dextran.) The Rouse–Zimm modes do serve to illustrate an important point though, in that they give relaxation rates that scale at least as rapidly as $(1/M)^{3/2}$ with mass. One can present arguments on quite general grounds that any cooperative mode will vary at least that rapidly with mass, in clear contradiction to the slower mass dependence of eq 5.

Our interpretation of the ESR spectra is that they are composites representing two independent processes. Bearing in mind that the dextran polymers are randomly labeled at hydroxyl groups that are uniformly spaced along the chain, if the correlation time of a particular label should depend upon the point of attachment of that label, then the observed signal will be a superposition of spectra with a range of times, and not representative of any true correlation time. Specifically, our hypothesis is that there are modes of motion uniquely available to the ends of the polymer chain (extending roughly a Kuhn length down the chain) as well as short-range segmental reorientation modes that span the entire length of the polymer. At any given point on the chain the rates for these two types of motion will be additive. The observed composite ESR signal will shift with molecular weight, and only for the heaviest polymers will the end effects be negligible and a true homogeneous correlation time be observed.

This hypothesis can be checked independently of any detailed knowledge of the end modes, and in fact independently of any particular understanding of the ESR line shapes. Assume that a polymer of molecular weight M consists of two pieces: (a) a length of 5250 MW on each end, whose signal is identical with that of the 10 500 MW samples, and (b) a length of $(M - 10500)$ in the center whose signal is identical with the $M = \infty$ signal. If the 5250 MW end piece is long enough to take full account of the end modes, these assumptions are plausible and a simple superposition of the 10 500 MW signal and the $M = \infty$ (or 2×10^6) signal, weighted so the ratio of the number of nitroxide radicals reflects the mass ratio, should reproduce the observed signal.

This procedure works remarkably well. Since the maximum line width ratio between corresponding hyperfine lines of the two primitive signals is <1.5, there is no obvious indication that the superposition signal is composite (in contrast to the frequently encountered situation where one of the primitives is a very sharp free label signal.) But when these synthesized spectra are converted to artificial correlation rates through eq 1, they generate a line that is essentially indistinguishable from $r_c = A/M + B$, in excellent agreement with the experimental data, as Figure 4 illustrates.

The spectral superpositions not only gave correlation rates that fit the experimental data, but in addition both model peak ratios $R = h(0)/h(-1)$ and $R' = h(0)/h(+1)$ fit the corresponding experimental values independently. This is most readily shown by using linear combinations of the rates given by (1) and (2)

$$\tau_c(7) = \frac{\tau_c(1) + B/C\tau_c(2)}{1 + B/C} = \frac{3^{1/2}\pi\Delta\nu(0)}{C + B}\left[\left(\frac{h(0)}{h(-1)}\right)^{1/2} - 1\right] \tag{7}$$

$$\tau_c(8) = \frac{\tau_c(1) - B/C\tau_c(2)}{1 - B/C} = \frac{3^{1/2}\pi\Delta\nu(0)}{C - B}\left[\left(\frac{h(0)}{h(+1)}\right)^{1/2} - 1\right] \tag{8}$$

Figure 5 shows the experimental correlation rates determined from eq 7 and 8, which make use of R and R' separately, plotted vs. $1/M$, with the model curves superim-

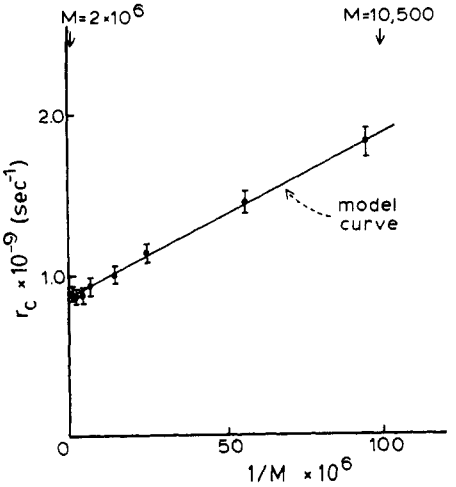

Figure 4. Experimental rate vs. $1/M$ for spin-labeled dextrans in 0.5% aqueous solution at 20 °C, with model curve superimposed.

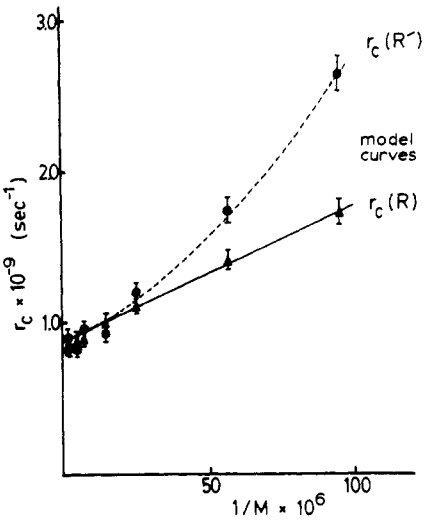

Figure 5. Experimental rates vs. $1/M$ for spin-labeled dextrans in 0.5% aqueous solution at 20 °C, calculated from eq 7 and 8, with model curves superimposed.

posed. Again, there is excellent agreement.

Figure 5 is a useful plot for a second reason. The agreement shown between $r_c(R)$ and $r_c(R')$ at high molecular weight tends to support our assumptions of isotropic motion, uniformity of sites, and small inhomogeneous hyperfine broadening. As one goes to smaller molecular weights the rates are expected to diverge, since composite signals will not give internally consistent rates. Unfortunately, the unresolved hyperfine broadening also produces inconsistencies as the motion becomes more rapid, and we have not been able to unambiguously separate these effects.

A recent NMR study reported in the literature lends support to our interpretation of the data. ^{13}C T_1 measurements on selectively deuterated polystyrene polymers indicate that the effecive correlation times of backbone carbons in long chains decrease in going from the center toward the ends.[25]

In summary, the ESR spectra of spin-labeled dextrans in dilute solution appear to be composites representing two types of motion: a local segmental motion that spans the entire length of the polymer and rapid end motions whose contribution becomes more pronounced as molecular weight decreases. These end modes can be characterized by two parameters, a strength and a range. Since the 10500 MW signal successfully incorporates the end modes, an upper bound on the range is about 20 monomers with a corresponding average end mode rate of about $2r_\infty$. An upper limit on the strength of the end modes can also be estimated. Spin-labeled methylglucose (roughly equivalent to a dextran monomer) was synthesized[3] by a procedure similar to that used for the dextrans and yielded a room-temperature correlation time of 1.0×10^{-10} s, implying an upper bound on the end-mode rate of about $10r_\infty$. A more detailed description of the relaxation rate as a function of the distance from the polymer end would appear to be an interesting but open experimental and theoretical problem.

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