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Note

Molecular motion in free and cross-linked dextrans

ALAN J BENESI AND JOHN T GERIG
Department of Chemistry, University of California, Santa Barbara, California 93106 (U. S. A.)

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Various commercially available preparations of cross-linked dextrans (Sephadexes) have been used for a number of years as supports for the immobilization of proteins[1] and to prepare media for "affinity chromatography"[2]. The dextrans themselves have been covalently attached to proteins for the purposes of increasing protein stability or altering the immunogenicity of the protein[3]. As part of a program designed to study polysaccharide–enzyme conjugates spectroscopically, we have examined the carbon-13 n.m.r. spectra of the Pharmacia products Dextran T-40, T-250, and T-2000, and Sephadex G-75. The results presented here provide insight into the nature of molecular motions in these structures and define a type of baseline from which the dynamics of molecular motion in protein–polysaccharide structures may be measured.

EXPERIMENTAL

Materials. — Dextran T-40, T-250, and T-2000, and Sephadex G-75 were purchased from Pharmacia Fine Chemicals. Data sheets supplied with the polymers indicated that the first two dextrans have weight-average molecular weights of 44,400 and 231,000, respectively. The molecular weight of the T-2000 is approximately 2 million, according to the manufacturer. Sephadex G-75 is formed by cross-linking dextran of molecular weight 70,000 with epichlorohydrin. The water content of each polysaccharide was determined gravimetrically by drying pre-weighed samples for 36 h at 120°; water contents were 9.4, 5.8, 7.7, and 5.0% for T-40, T-250, T-2000, and G-75, respectively. The heavy-metal content of the polymers is stated by the manufacturer to be less than 5 p.p.m.

Deuterium oxide (99.8% D) was obtained from Stohler Isotope Chemicals and deuteriophosphoric acid was purchased from Alfa Inorganics. A stock buffer solution consisted of 0.1M $Na_2HPO_4 \cdot 7H_2O$ dissolved in D_2O and adjusted to a pH meter-reading (glass electrode) of 7.80 with deuteriophosphoric acid. No corrections were made for the H_2O content of the solvent.

Sample preparation. — Samples were prepared by weighing the undried polysaccharide directly into a 10-mm n.m.r. tube and then adding the appropriate weight of buffer solution. By taking into account the water of hydration of the polymers, it was determined that the H_2O content in each sample did not exceed 5%. Samples were heated to boiling to speed dissolution and ensure complete solvation[4].

Carbon-13 n.m.r. spectroscopy. — All carbon-13 spin-lattice relaxation-times were obtained with complete proton-noise decoupling on a Varian Associates CFT-20 spectrometer. The sample temperature was 36 ±1°; samples were not degassed as the contribution to relaxation from dissolved oxygen was expected to be insignificant over the range of relaxation times observed[5]. The $180-\tau-90$ inversion-recovery method was used to measure T_1 with 6000 to 18000 transients collected for each τ value. At least five τ values were used in each determination. As a check on the experimental technique, the carbon T_1 parameters for a 2M solution of sucrose were determined and, when account was taken of the differences in sample temperature, these were in good agreement with the literature values[6].

Transverse relaxation-times (T_2) were estimated from the width of the observed lines at half-maximum. A correction for the instrumental broadening of the peaks was applied.

The carbon-13 chemical shifts were determined relative to the methyl carbon signal of a trace of acetone added to the buffer solution; they were corrected to a reference of tetramethylsilane by adding 30.4 p.p.m. to the observed shifts.

Carbon-13 nuclear Overhauser effects (n.O.e.), defined as the ratios of the integrated signal-intensity with proton decoupling to the intensity without decoupling, were obtained with gated proton-decoupling[7]; the sample temperature during the n.O.e. experiments was 33 ±1°.

Viscosities. — Sample viscosities at 36.5° were obtained by using Ostwald viscometers according to a published procedure[8]. Kinetic-energy corrections were applied, but no corrections for shear rate were made as these are expected to lower the measured viscosity by 2% at most[9].

RESULTS

Fig. 1 displays representative carbon-13 spectra of a dextran (T-40) as well as the cross-linked dextran, Sephadex G-75. Six major lines are in evidence, representing the six carbon atoms of the D-glucose monomeric residues in each polysaccharide. The signals near 98 and 66 p.p.m. are readily assigned[10] to carbon atoms C-1 and C-6; the remaining resonances are tentatively assigned as indicated in Table I. These latter assignments are based on previous carbon-13 n.m.r. studies of oligomers of D-glucose[11], and are probably the most prone to ambiguity as regards the assignment of C-4 and C-5. Corresponding chemical-shifts for each polysaccharide examined were found to be similar.

A minor resonance at about 60 p.p.m. was occasionally observed. The dextrans used here are mainly connected by α-D-(1→6) linkages, but also contain a small

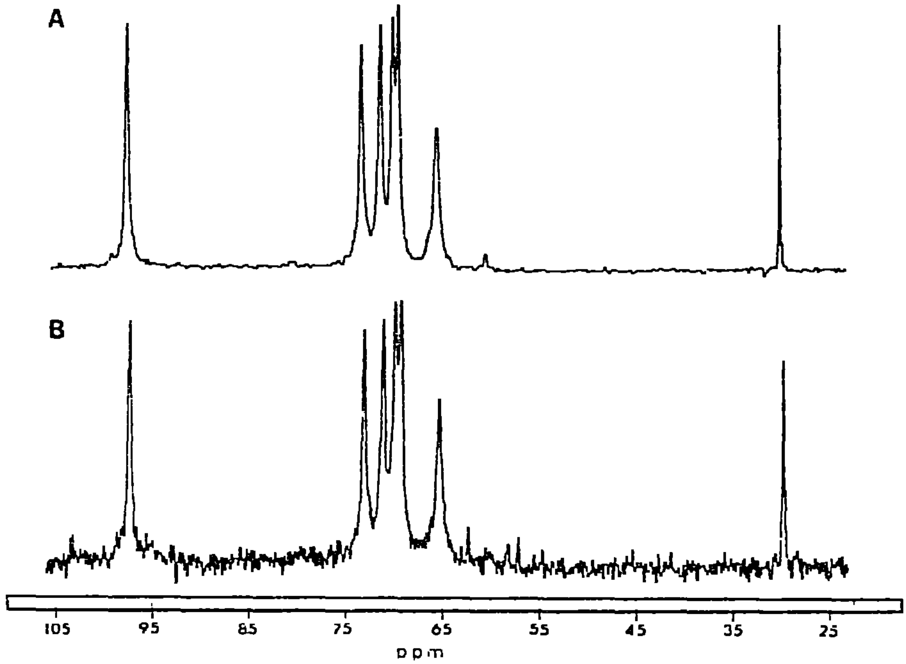

Fig. 1 The carbon-13 n.m.r. spectrum of 18.5% Dextran T-40 (A), and Sephadex G-75 (B). The chemical-shift scale is given relative to tetramethylsilane: the sharp signal at 30.4 p.p.m. is due to the methyl carbon of a trace amount of acetone in the samples. For each spectrum, about 40,000 transients were accumulated.

TABLE I

CARBON-13 N.M.R. DATA FOR DEXTRANS AND SEPHADEX G-75

Carbon atom	*δ, p.p.m.*[a]	T_1, *sec*[b]			*Dextran T-2000*	*Sephadex G-75*
		Dextran T-40	*Dextran T-250*[c]			
C-1	97.8	0.064	0.056,	0.057	0.067	0.070
C-3	73.6	0.061	0.047,	0.054	0.057	0.061
C-2	71.6	0.062	0.045,	0.059	0.060	0.060
C-4	70.3	0.060	0.047,	0.052	0.054	0.060
C-5	69.8	0.063	0.054,	0.052	0.060	0.061
C-6	65.8	0.035	0.030,	0.032	0.028	0.033
Sample viscosities,	cP	16	79,	800	252	gel

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[a] Chemical shift in p.p.m. downfield from Me_4Si; the shifts for all samples were identical within ±0.2 p.p.m. [b] Estimated error, ±0.01 sec. [c] The second column gives results for a 31% (w/w) solution of dextran T-250; all other dextran solutions were 18.5% (w/w). The gel sample was 6.4% (w/w).

percentage (~6%) of α-D-(1→3) bonds[12]. The minor signal is assigned to the unlinked C-6 atoms; other minor signals are doubtless present, but are either unresolved from the main bands or lost in the noise.

Spin-lattice relaxation-times (T_1) for the six major resonances in each dextran sample were determined and are recorded in Table I. Within the probable experimental error (10–20%), the T_1 values for carbon atoms 1–5 are virtually identical (average value 58 msec) and are independent of the degree of polymerization of the polymer, the sample viscosity, or the presence of cross-links. The relaxation time for C-6 also varied little from sample to sample, and had an average value of 32 msec.

Transverse relaxation-times (T_2) averaged 53 msec for carbons 1–5 and 29 msec for carbon 6. As with the spin-lattice relaxation-times, the T_2 relaxation parameters were essentially independent of polymer molecular weight or extent of cross-linking.

Lastly, nuclear Overhauser enhancements were estimated for the signals in the Dextran T-2000 sample. Carbon atoms 1–5 exhibited an enhancement factor of 1.7 ±0.3, whereas carbon-6 was enhanced by 2.0 ±0.3 upon proton irradiation.

Our observations regarding the virtual independence of carbon-13 relaxation-times on polymer size are reminiscent of a previous report by Allerhand and Hailstone[13]. These authors found that solutions of various polystyrenes in tetrachloroethylene had similar carbon-13 relaxation-times for corresponding atoms, when the molecular weight of the polymer was greater than about 10,000. Surprisingly sharp ^{13}C n.m.r. lines are often observed for polymers[13–15], and this is generally attributed to the ability of small segments of the polymer to execute rapid, local motions that are relatively independent of the overall conformation of the polymer in solution[10].

The observation that carbon-6 in the D-glucose residue is relaxed twice as efficiently as the other carbon atoms in the structure, which have only one proton directly attached, suggests that the relaxation of all carbon atoms in these polymers is overwhelmingly dominated by dipolar interactions[5,14,17]. The theoretical framework provided by Woessner may be used to relate the experimental spin-lattice and transverse relaxation-times, as well as the Overhauser effect, to the details of the molecular motions responsible for these effects[17–19]. For many polymers, the motion of a given monomeric unit appears to be nearly isotropic and, for the backbone carbon atoms, the relaxation times and nuclear Overhauser effect may be described in terms of a single correlation time (τ_c) for isotropic reorientation[13,14,20].

We have applied the equations of Woessner for isotropic reorientation in an attempt to find a single value of τ_c that was at once consistent with the T_1, T_2, and n.O.e. values observed in this work. It was not possible to fit this model to our data and, for the next level of approximation, the monomeric unit was assumed[*] to resemble an ellipsoid of revolution undergoing anisotropic reorientation[19,21]. Under

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[*] For a recent consideration of anisotropic molecular motion as it relates to carbon-13 spectroscopy, see ref. 21.

this assumption, the motion is described in terms of a rotational diffusion-constant for motion around the symmetry axis ($R_{\parallel}$) and a diffusion constant ($R_{\perp}$) for molecular tumbling around the axes perpendicular to the symmetry axis.

A wide range (10^6–10^{12} rad.sec^{-1}) of values of the diffusion constants ($R_{\parallel}$ and $R_{\perp}$) was searched with the goal, once again, of finding a set that would be consistent with the relaxation-times observed and the Overhauser enhancements. In making these calculations, the carbon–hydrogen bond-length was assumed to be 1.09 Å and the angle between the C–H internuclear vector and the symmetry axis of the ellipsoid was assumed to be 90°. When $R_{\parallel}$ and $R_{\perp}$ have the values 4×10^9 rad.sec^{-1} and 2.5×10^8 rad.sec^{-1}, respectively, fairly good agreement with the experimental results may be obtained; with these parameters the calculated values for T_1, T_2, and the n.O.e. were 59 msec, 47 msec, and 2.1, respectively. Variations in these diffusion coefficients within 10% of the values mentioned did not greatly alter the nature of the agreement between the calculated and observed values. Our results thus suggest that, in the dextran polymers and in the gel, molecular motion of the glucose residues about the axis roughly defined by the polymer-chain direction is about sixteen times easier than reorientation normal to the chain direction.

An alternative rationalization of our results in terms of a distribution of correlation times for isotropic motion may also be possible[22] This was not attempted, as it would be anticipated that the distribution function would be altered considerably by cross-linking of the polymers or be dependent on the molecular weight of the polymer.

The number of cross-links present in Sephadex G-75 appears to be proprietary information; it must be rather small as no carbon-13 n.m.r signals assignable to the cross-linking reagent were observed.

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