Protein Rotational Relaxation as Studied by Solvent ^1H and ^2H Magnetic Relaxation†

Klaas Hallenga‡ and Seymour H. Koenig*

ABSTRACT: Earlier studies of the magnetic field dependence of the nuclear spin magnetic relaxation rate of solvent protons in solutions of diamagnetic proteins have indicated that this dependence (called relaxation dispersion) is related to the rotational Brownian motion of solute proteins. In essence, the dispersion is such that $1/T_1$ (the proton spin-lattice relaxation rate) decreases monotonically as the magnetic field is increased from a very low value (~10 Oe); the dispersion has a point of inflection at a value of magnetic field which depends on protein size, shape, concentration, temperature, and solvent composition. The value of the proton Larmor precession frequency ν_c at the inflection field appears to relate to τ_R, the rotational relaxation time of the protein molecules. We have measured proton relaxation dispersions for solutions of various proteins that span a three-decade range of molecular weights, and for one sample of transfer ribonucleic acid. We have also measured deuteron relaxation dispersions for solutions of three proteins: lysozyme, carbonmonoxyhemoglobin, and *Helix pomatia* hemocyanin with molecular weight 900 000. A quantitative relationship between both proton and deuteron dispersion data and protein rotational relaxation is confirmed, and the point is made that magnetic dispersion measurements are of very general applicability for measuring the rotational relaxation rate of macromolecules in solution. It has been previously shown that the influence of proton motion on the relaxation behavior of the solvent is not due to exchange of solvent molecules between the bulk solvent and a hydration region of the protein. In the present paper, we suggest that the interaction results from a long range hydrodynamic effect fundamental to the situation of large Brownian particles in an essentially continuum fluid. The general features of the proposed mechanism are indicated, but no theoretical computations are presented.

Protein molecules in water solution increase the longitudinal (spin-lattice) magnetic relaxation rates $1/T_1$ of solvent water nuclei, the magnitude of the increase depending on the strength of an externally applied static magnetic field H_0. The effect is greatest at low fields; for example, for 100 mg/ml solutions of globular protein of molecular weight about 10^5, and for $H_0 \lesssim 200$ Oe, $1/T_1$ increases by an order of magnitude from its value in protein-free solvent. As H_0 is increased to ~2 kOe, $1/T_1$ decreases to within a factor two of its value for pure solvent and remains field independent until H_0 reaches about 20 kOe. At higher fields, $1/T_1$ again decreases slowly toward the pure solvent value (Koenig and Schillinger, 1969; Gupta and Mildvan, 1975). The range 200 Oe to 2 kOe for H_0 corresponds to a proton Larmor frequency range of 0.8–8 MHz, and a frequency range a factor of about six lower for deuterons and ^{17}O, well below the frequencies of traditional commercial NMR[1] instrumentation. Thus measurements of the type discussed here require specialized instrumentation (Koenig and Schillinger, 1969; Noack, 1971), and only a small amount of such data has been published to date.

The magnetic field dependence of $1/T_1$ for solvent protons has been studied for a range of sample conditions (pH, temperature, concentration) for solutions of the diamagnetic proteins apotransferrin, 84 000 daltons (Koenig and Schillinger, 1969), and carbonmonoxyhemoglobin, 64 000 daltons (Lindstrom and Koenig, 1974). Less extensive data have been reported for lysozyme, 14 000 daltons (Koenig et al., 1975), carbonic anhydrase, 30 000 daltons (Fabry et al., 1970), concanavalin A, 54 000 daltons (Koenig et al., 1973), serum albumin and ovalbumin, 63 000 daltons (Kimmich and Noack, 1970a,b,c; Blicharska et al., 1970), carbonmonoxyhemoglobin (Kimmich, 1971), reduced ceruloplasmin, 150 000 daltons (Koenig and Brown, 1973), and fully associated and reduced hemocyanin, 9×10^6 daltons (Koenig et al., 1975).

The magnetic field dependences of $1/T_1$ for solvent deuterons in ^2H_2O have been reported for solutions of lysozyme and hemocyanin, and at two values of field for ^{17}O relaxation in solutions of lysozyme enriched in $H_2{}^{17}O$ (Koenig et al., 1975).

The curves showing the dependence of $1/T_1$ on H_0 (referred to throughout as dispersion curves) inflect at values of H_0 that correspond to Larmor precession frequencies ν_c (for ^1H, ^2H, and ^{17}O as the case may be) related to the orientational relaxation times τ_R of the various solute protein molecules by (Koenig and Schillinger, 1969):

$$\nu_c \simeq \sqrt{3}/(2\pi\tau_R);\ \tau_R = 4\pi\eta r^3/kT \qquad (1)$$

Here η is the solvent viscosity, r the radius of the protein, k Boltzman's constant, and T the absolute temperature. This result is for approximately spherical proteins; there appears to be more than one inflection frequency for solutions of highly anisotropic proteins like apotransferrin (Koenig and Schillinger, 1969) and serum albumin (Kimmich and Noack, 1970a). It has now become quite clear, from dispersion data to date, that solvent water molecules somehow sense the rotational Brownian motion of the protein molecules, in that measurements of $1/T_1$ of *solvent* nuclei yield information about the motion of *solute* macromolecules.

In the present paper we report results of a quantitative study of solvent proton relaxation dispersion in aqueous solutions of proteins ranging in molecular weight from 10^4 to 10^7, and for

† From IBM Thomas J. Watson Research Center, Yorktown Heights, New York 10598. *Received March 8, 1976.*

‡ Present address: Vrije Universiteit Brussel, Faculteit der Wetenschappen, Organische Chemie, Brussels, Belgium.

[1] Abbreviations used: NMR, nuclear magnetic resonance; tRNA, transfer ribonucleic acid; rms, root mean square; TP, apotrypsin; TF, apotransferrin; CP, ceruloplasmin.

one sample of tRNA. We have also studied the temperature dependence of the dispersion for several protein solutions and have measured the dispersion of both ^1H and ^2H relaxation rates for solutions of three proteins in solvents containing a range of proton to deuteron ratios. The study was intended to document thoroughly the dispersion phenomena, with the intention of deducing the mechanism of protein–solvent interaction responsible for the observations. Earlier data (Koenig and Schillinger, 1969; Lindstrom and Koenig, 1974) had been interpreted in terms of a two site model (Daskiewicz et al., 1963): water being either in the bulk or attached to the protein molecules, with rapid exchange between the two. Except for the one unsettling feature that the number of water attachment sites on the protein turned out to be but a few percent of the number of water molecules required to form a monolayer on the protein, the model fits the results quite well. It has since been demonstrated (Koenig et al., 1975), however, that such a model is at best overly simplistic and, in all likelihood, inappropriate. Though the actual mechanism underlying the phenomenology remains undetermined, we believe it relates to certain fundamental hydrodynamic effects of the Brownian motion of large particles on the long time motion of the solvent molecules.

Experimental Procedures

Materials. The proteins used in this study were chosen to span a wide range of molecular weights. The particular proteins used were those for which the protein x-ray structure and primary sequence were both known, when possible, and for which solutions were stable and readily characterizable. For each sample, buffer composition, pH, and ionic strength were judiciously selected to assure that the state of aggregation of the protein molecules in solution was known to be monodisperse. Protein concentration was determined from measurements of optical density at 278 nm, unless otherwise indicated.

Hen egg-white lysozyme, obtained salt-free and lyophilized from Worthington, was used with no further purification. The molecular weight (14 500) and molecular structure are well known from x-ray studies (Blake et al., 1967), and the hydrodynamic behavior has been carefully studied by laser light scattering and related to the structure by Dubin et al. (1971) Samples were 50 mg/ml protein (using $E_{1cm}^{1\%} = 25$) in deionized water, pH 4.5; no significant aggregation is anticipated under these conditions (Bruzzesi et al., 1965).

Human carbonic anhydrase B was prepared by Pesando (1975) from pooled human blood, and furnished as a powder obtained by lyophilization of distilled water solutions of the highly purified, 100% active enzyme. Samples were 47 mg/ml in deionized water (using $E_{1cm}^{1\%} = 16.3$ (Armstrong et al., 1966)). The molecular weight (30 000) is known from the amino acid sequence (Andersson et al., 1972). Hydrodynamically, human carbonic anhydrase B behaves very much like the human C isozyme (Derrien and Laurent, 1969), which from x-ray data (Liljas et al., 1972) should behave as a sphere.

Apoconcanavalin A was prepared by Brewer (Koenig et al., 1973) from Miles-Yeda native protein by demetalization at low pH. Samples were 21 mg/ml (using $E_{1cm}^{1\%} = 11.4$ (Agrawal and Goldstein, 1967)), pH 5.6, in 0.1 M potassium acetate buffer, 0.9 M KCl. The protein should be dimeric with molecular weight 54 000 under these conditions (McKenzie et al., 1972). Hydrodynamically, the dimer should have an orientational relaxation time, as measured in the present experiments, about 10% greater than that of a sphere of equivalent volume (Koenig et al., 1973).

Hemoglobin A was prepared from citrate and dextrose treated pooled human blood by the procedure of Drabkin (1946). The stock solution was stored at 4 °C under CO atmosphere. Part of the stock solution was diluted tenfold with 99.9% ^2H_2O and reconcentrated to the original protein concentration by ultrafiltration at 4 °C under CO overpressure. A final deuterated stock solution, obtained by repeating this process three times during a 48-h period, had no more than 0.2% solvent protons, as indicated by the intensity of the proton NMR signal. The concentrations of both the deuterated and undeuterated stock solutions were adjusted to 115 mg/ml (using $E_{1cm}^{1\%} = 8.4$ at 54 nm (Chiancone et al., 1970)). Samples with arbitrary proton to deuteron ratios could be prepared by mixing appropriate volume ratios of aliquots of the stock solutions. The samples were virtually free of methemoglobin as judged from a comparison of the optical absorption spectra in the visible before and after addition of a few mg/ml of dithionite. The molecular weight (64 500), structure and sequence are well known (cf. Perutz, 1968), and the hydrodynamic properties are well established (cf. Schlecht et al., 1969).

Alkaline phosphatase was furnished by Zukin (1974), who purified the enzyme after isolation from *E. coli* using a procedure somewhat modified from that of Torriani (1966). The molecular weight is about 80 000. The sample used was 71 mg/ml (using $E_{1cm}^{1\%} = 7.7$ (Rothman et al., 1963)).

Yeast alcohol dehydrogenase, obtained lyophilized from pH 7.5 buffer from Calbiochem (A grade), was redissolved in deionized water and centrifuged to remove a small amount of aggregated material. The molecular weight is about 155 000 (Kagi and Vallee, 1960). Samples were 32 and 68 mg/ml (using $E_{1cm}^{1\%} = 2.6$ (Hayes and Velick, 1954)) and gave stable relaxation results for about 8 h at room temperature.

Nonspecific human γ-immunoglobulin, obtained from Miles, was dissolved in 0.1 M phosphate buffer, pH 7.2. The sample was 45 mg/ml (using $E_{1cm}^{1\%} = 14.3$ (Little and Donahue, 1968)). The molecular weight is 160 000 (Edsall, 1953), and the molecule is known to be Y shaped (Valentine and Green, 1967) and to behave hydrodynamically as though it were highly aspherical (Yguerabide et al., 1970).

α-Hemocyanin, isolated from the snail *Helix pomatia* according to the procedure of Konings et al. (1969a), was furnished, freeze-dried, by van Driel. The powder was redissolved in deionized water and dialyzed against 0.1 M phosphate buffer solution, pH 7.5, to remove sucrose from the freeze-dried preparation. The association–dissociation equilibrium as a function of pH and ionic strength and the form of the many possible aggregates of hemocyanin have been extensively studied for samples prepared from the same starting material as used here (Konings et al., 1969b; Siezen and van Bruggen, 1974; Siezen and van Driel, 1974). At pH 7.0 and ionic strength $\mu = 0.1$, a monodisperse solution of fully associated hemocyanin molecules, 9×10^6 daltons, can be prepared. Increasing μ to 1.0 yields a sample with ~90% of the hemocyanin associated to 4.5×10^6 daltons ($\frac{1}{2}$ the fully associated weight), whereas at pH 7.9, and $\mu = 1.0$, a compact structure (C form) of 9×10^5 daltons ($\frac{1}{10}$ the full weight) forms with essentially 100% abundance. At pH 9.0 and $\mu = 0.1$, the C form alters to a looser structure (L form) of the same molecular weight. Finally two forms of molecules of $\frac{1}{20}$ the weight of the fully associated form (4.5×10^5 daltons) can be prepared: one, relatively compact, forms at pH 10 and $\mu = 0.01$; a second with much lower sedimentation constant forms at pH 11. Samples

of all but the last form were prepared at concentrations of 11.5 mg/ml (using $E_{1cm}^{1\%} = 14.2$ at pH 9.2, borax buffer (Heirwegh et al., 1961)). In addition, some samples in solvent containing 80% ^2H, 20% ^1H, were prepared at pH 7.2 and 9.7, $\mu = 0.1$, to obtain fully and $1/10$ associated molecules, respectively. These samples were diluted tenfold with their corresponding buffers, and reconcentrated by ultrafiltration to 30.7 and 26.5 mg/ml, respectively. This allowed the study of the dispersion of ^2H and ^1H relaxation in the same samples. A subsequent mass spectroscopic analysis of the isotopic content gave 74% ^2H, 26% ^1H.

In addition to the protein samples, several samples of nonspecific bakers' yeast tRNA (Boehringer Mannheim Corp.) were prepared with concentrations about 60 mg/ml. The molecular weight of tRNA is about 26 000 and the structures of some specific ones are known. The molecules tended to aggregate within several hours after sample preparation, so that satisfactory relaxation could only be obtained on freshly prepared samples.

Relaxation Measurements. Relaxation rates of solvent protons and deuterons were measured using apparatus considerably improved, but differing little in principle, from that described previously (Koenig and Schillinger, 1969). Relaxation rates can be measured for H_0 continuously variable from 1 Oe to 7 kOe, corresponding to Larmor frequencies from 4 kHz to 30 MHz for protons, and to an upper limit of 16 kOe for deuterons, corresponding to a deuteron Larmor frequency of 8 MHz. Basically, the system involves cycling H_0 through a sequence of preassigned values. H_0 is switched rapidly from either a large or very low value to the field at which data are desired. The resulting nonequilibrium magnetization of the nuclear spin system approaches equilibrium exponentially with a time constant T_1. After some variable time τ, H_0 is switched to a precise resonant field to which a radio frequency system is tuned, and the magnetization, proportional to that at time τ, is measured by applying a 90–180° pulse sequence in the usual NMR fashion. The integrated intensity of the resulting spin echo is recorded. The data points are automatically logged and sent to a time-shared computer for data reduction under control of the experimenter at a typewriter terminal. This process is repeated for 31 values of τ and a least-squares fit to an exponential is performed for the 31 pairs of data values for τ and integrated echo amplitude. The results for $1/T_1$ generally have an rms deviation of about 1%, but small systematic uncertainties limit the results to ±1.5%. H_0 is produced by a superconducting solenoid that can be switched over a range of 7 kOe in about 20 ms by a specially designed power supply (Redfield et al., 1968). Though the superconducting coil is a substantial improvement over the previous air core, room temperature, copper solenoid which had an upper limit of 1.5 kOe, nonreproducible field inhomogeneities limit its performance at high fields, thereby setting an upper limit of about 30 MHz for proton relaxation measurements. The data presented for protons at fields corresponding to 50 MHz were taken at fixed field in a conventional iron core magnet.

Data Reduction. We represent the magnetic relaxation dispersion, by analogy with procedures used for dielectric relaxation dispersion, by the Cole–Cole expression (Cole and Cole, 1951):

$$1/T_1 = (1/T_{1w}) + D + A\,\mathrm{Re}\,[1/(1 + (i\nu/\nu_c)^{\beta/2})]$$
$$= (1/T_{1w}) + D + \frac{A(1 + (\nu/\nu_c)^{\beta/2}\cos(\pi\beta/4))}{1 + 2(\nu/\nu_c)^{\beta/2}\cos(\pi\beta/4) + (\nu/\nu_c)^{\beta}} \quad (2)$$

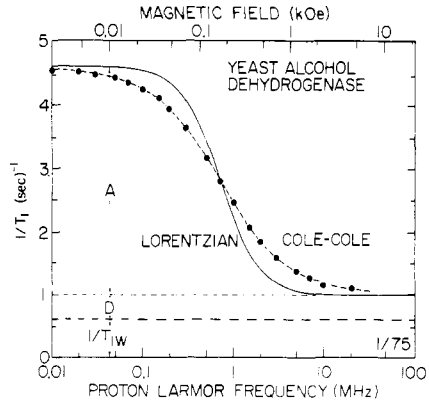

FIGURE 1: Dispersion of the solvent water protein magnetic relaxation rate $1/T_1$ for a 65 mg/ml solution of yeast alcohol dehydrogenase, molecular weight 160 000, at 5.9 °C. The solid circles are the experimental data points. The dashed line through the points results from a least-squares fit of the Cole–Cole expression, eq 2, to the data. Two of the parameters of the fit are indicated: A, the amplitude of the main dispersive contribution; and D, a residual high frequency contribution; $1/T_{1w}$ is the proton relaxation rate in protein-free solvent. The solid line that intersects the dashed line is a Lorentzian curve, eq 3, obtained using the same values for A, D, $1/T_{1w}$, and ν_c as for the solid line.

Here $1/T_{1w}$ is the relaxation rate of the water nuclei in the protein-free buffer, ν the Larmor precession frequency of the nuclei at H_0, and D, A, ν_c, and β are parameters to be determined from a fit of eq 2 to the data. "Re" stands for "the real part of".

For $\beta = 2$, eq 2 reduces to a constant plus a Lorentzian dispersive term:

$$1/T_1 = (1/T_{1w}) + D + A/(1 + (\nu/\nu_c)^2) \quad (3)$$

The dispersive part of the Cole–Cole expression, like the Lorentzian, drops to half its maximum value of A at $\nu = \nu_c$, the frequency at which the curve inflects. However, for $\beta < 2$, the Cole–Cole expression has a slower variation with ν than the Lorentzian. A simple two-site model of water exchange between bulk solvent and protein (shown to be inadequate by Koenig et al., 1975) predicts a Lorentzian contribution to the relaxation (Koenig and Schillinger, 1969), whereas the experimental data are known to vary more slowly with ν (Fabry et al., 1970; Lindstrom and Koenig, 1974). The Cole–Cole expression should be regarded as a heuristic equation that, as will be seen, represents the relaxation dispersion data very well; it has no a priori validity.

Results

The presentation of the proton relaxation data is organized to show, first, the general features of the $1/T_1$ dispersion, and then to show that the numerical values of ν_c as well as its dependence on protein molecular weight, shape, and absolute temperature, are related directly to the orientational relaxation time of the *solute* macromolecules as indicated by eq 1. Analogous, though more limited, data on deuteron relaxation dispersion for lysozyme, hemoglobin, and hemocyanin solutions are also included.

Proton Relaxation in 100% ^1H_2O. Figure 1 shows proton relaxation dispersion data for a solution of yeast alcohol dehydrogenase at 5.9 °C. The dashed line is a fit of the Cole–Cole expression, eq 2, to the data points. The relative contributions of A, D, and $1/T_{1w}$ are indicated. Their values, and those for β, for this and all other samples, are collected in Table I. The solid line represents a Lorentzian dispersion with the same

TABLE I: Results of a Least-Squares Fit of the Cole-Cole Expression (Equation 2) to Solvent Proton Relaxation Dispersion Data for Various Macromolecular Solutions at 25 °C. τ_R Was Obtained Using Equation 1.

Sample	Mol Wt $\times 10^{-5}$	Concn (mM)	A (s^{-1})	D (s^{-1})	β	ν_c (MHz)	τ_R (μs)	$A/[C]$ s^{-1} M^{-1}	$D/[C]$ s^{-1} M^{-1}	$A\nu_c/[C]$ $s^{-1}M^{-1}$ $\times 10^{-6}$
Lysozyme	0.15	3.1	0.25	0.07	1.47	5.5	0.050	81	20	430
Trypsin	0.28	2.0	1.23	0.05	1.55	3.5	0.079	620	40	2100
Carbonic anhydrase B	0.30	1.6	0.48	0.03	1.68	6.4	0.043	310	20	1900
Transfer RNA	0.26	1.5	0.43	0.05	1.21	3.6	0.077	280	30	1000
Apoconcanavalin A	0.54	0.38	0.41	0.03	1.64	2.5	0.11	1100	80	2700
Hemoglobin A (CO)	0.64	1.70	1.36	0.15	1.55	2.7	0.10	810	90	2200
Alkaline phosphatase	0.80	0.75	2.18	0.22	1.71	2.0	0.14	2900	300	5900
Apotransferrin	0.84	0.60				1.0	0.28			
Ceruloplasmin	1.5					1.0	0.28			
Alcohol dehydrogenase	1.6	0.20	0.74	0.09	1.53	1.6	0.17	3600	450	5600
Immunoglobulin G	1.6	0.28	1.41	0.08	1.40	0.65	0.42	5000	300	3300
Hemocyanin (1/20)	4.5	0.024	0.67	0.02	1.35	0.73	0.38	2.8×10^4	1000	2.1×10^4
Hemocyanin (1/10) L	9	0.021	1.3	0.02	1.37	0.31	0.89	1.1×10^5	1300	3.3×10^4
Hemocyanin (1/10) C	9	0.021	1.8	0.05	1.44	0.17	1.6	1.4×10^5	4000	2.5×10^4
Hemocyanin (1/2)	45	0.0024	3.4	0.04	1.37	0.074	3.7	1.4×10^6	1.8×10^4	1.1×10^5
Hemocyanin (1/1)	90	0.0013	3.2	0.04	1.32	0.058	4.8	2.5×10^6	3.3×10^4	1.5×10^5

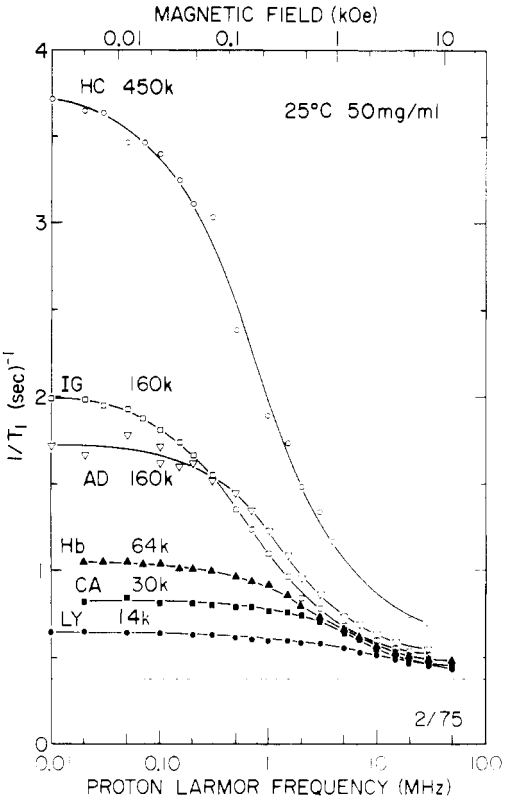

FIGURE 2: Dispersion of the solvent water proton magnetic relaxation rate $1/T_1$ for 50 mg/ml solutions of proteins with a range of molecular weights, at 25 °C. The abbreviations are: HC, hemocyanin from *Helix pomatia;* IG, nonspecific human γ-immunoglobulin; AD, yeast alcohol dehydrogenase; Hb, human adult carbonmonoxyhemoglobin; CA, human erythrocyte carbonic anhydrase B; and LY, hen egg-white lysozyme. The relaxation rate of the protein-free solvent is indicated by the dashed line.

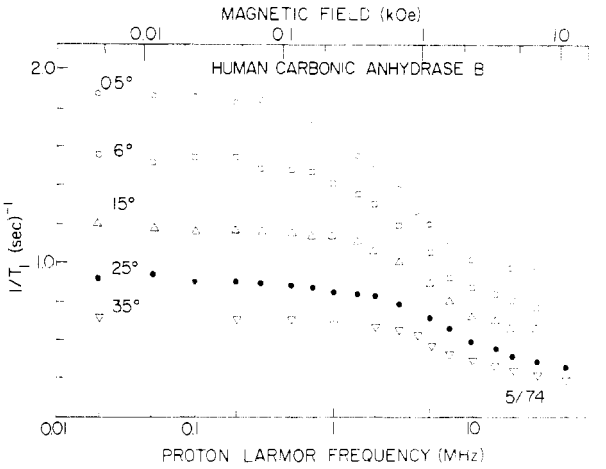

FIGURE 3: Temperature dependence of solvent water proton magnetic relaxation dispersion for a 48 mg/ml solution of human carbonic anhydrase B.

values for A, D, and $1/T_{1w}$ as the dashed line; it is clearly an inadequate representation of the data.

Figure 2 shows representative proton dispersion results for aqeuous solutions of proteins ranging in molecular weight from roughly 10^4 to 10^6. The results, all at 25 °C, were normalized to a protein concentration of 50 mg/ml by linearly scaling the observed data since the samples in most cases did not have this precise concentration. It is known from the present and previous work (Koenig and Schillinger, 1969; Lindstrom and Koenig, 1974) that $1/T_1$ corrected for the buffer contribution is reasonably linear in protein concentration in the range used (Table I). Figure 2 is meant to illustrate the dependence of the dispersion phenomena on molecular weight. There are several points to note: with increasing molecular weight, the low field limiting value of $1/T_1$ increases, and ν_c decreases, for fixed molecular weight, comparing the immunoglobulin and alcohol dehydrogenase data, the more anisotropic protein behaves as though it were the heavier, and it has a somewhat broader dispersion profile. The solid lines through the data points are least-squares fits of eq 2 to the respective data.

Figure 3 shows the temperature variation of the dispersion of $1/T_1$ for a solution of carbonic anhydrase. It is seen that with decreasing temperature (as with increasing molecular weight at fixed temperature) the low field limiting value of $1/T_1$ increases and ν_c decreases. This is in agreement with results for

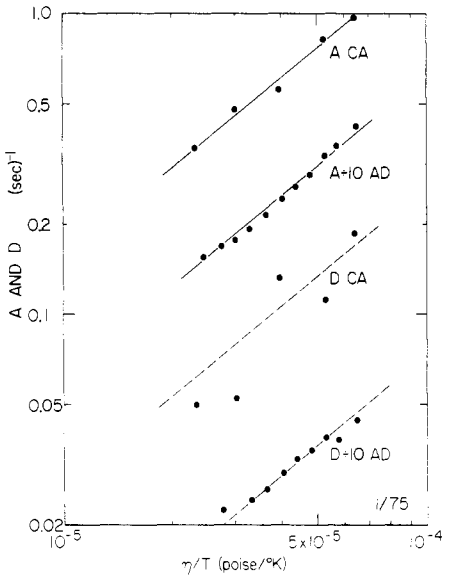

FIGURE 4: Temperature dependence of the parameters A and D, eq 2, for samples of human carbonic anhydrase B and yeast alcohol dehydrogenase. A is the amplitude of the main dispersive contribution to the relaxation rate, and D is the amplitude of the residual high frequency contribution. The data are plotted against the ratio of solvent viscosity η to absolute temperature T. The dashed solid lines are the best fits to the data of lines with unity slope, which would indicate a linear dependence of A and D on η/T.

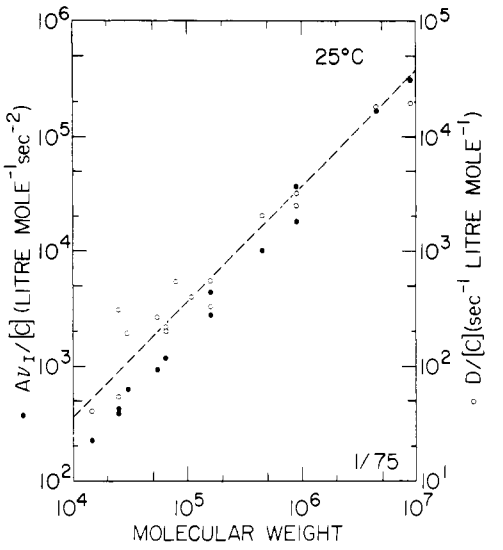

FIGURE 5: Dependence of $A\nu_c/[C]$ (open circles) and $D/[C]$ (solid circles) on molecular weight at 25 °C. Combining parameters in this fashion should remove the explicit dependence of A and D on protein concentration and molecular weight, and indicate their intrinsic dependence on protein molecular weight. The dashed line is a line of unity slope drawn through the points.

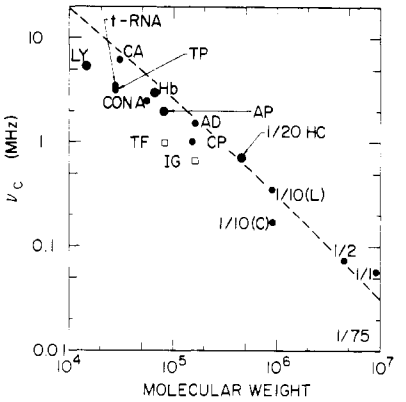

FIGURE 6: The variation of inflection frequency ν_c, eq 2, of solvent proton relaxation dispersion with molecular weight of solute macromolecules at 25 °C. The abbreviations are: LY, hen egg-white lysozyme; CON A, demetallized concanavalin A; TP, demetallized porcine trypsin; tRNA, nonspecific yeast ribonucleic acid; CA, human erythrocyte carbonic anhydrase B; Hb, human adult carbonmonoxyhemoglobin; AP, *E. coli* alkaline phosphatase; TF, demetallized human transferrin; IG, human nonspecific γ-immunoglobulin; AD, yeast alcohol dehydrogenase; CP, human ceruloplasmin; HC 1/20, 1/10(L), 1/10(C), 1/2, 1/1, various states of association of *Helix pomatia* hemocyanin (see text). The dashed line was calculated using eq 1 with *no adjustable parameters*, using the viscosity of pure water for η. The proteins were assumed spherical, and a 3.5-Å hydration layer was included in computing the hydrodynamic radii.

other proteins (Koenig and Schillinger, 1969; Fabry et al., 1970).

In Figure 4, the temperature variations of A and D for both carbonic anhydrase and alcohol dehydrogenase solutions are shown. A and D are plotted on a log–log scale against η/T. The relative error of D is much larger than that of A because the relaxation rates at the fields at which D is determined are, at most, only twice those of the protein-free buffers. Variations in dissolved O_2 and small buffer effects contribute to scatter for the values for D. The solid lines through the points for A and the dashed lines through those for D are best fits of lines with unity slope, indicating that A and D both vary linearly with η/T, as anticipated from earlier work on aprotransferrin (Koenig and Schillinger, 1969).

Both A and D must depend on protein concentration [C], and, in the limit of low concentration, the dependence is linear (Koenig and Schillinger, 1969). After normalization for concentration, both A and D are measures of the interaction of the individual protein molecules with solvent, and thus should depend on protein size. In Figure 5, $A\nu_c/[C]$ and $D/[C]$ are plotted against molecular weight. A is multiplied by ν_c to remove the explicit dependence on τ_R expected for A (cf. Discussion). Since D is the high field limiting value of $1/T_1$, it should be independent of the rotational motion of the protein; thus no normalization of D by τ_R is necessary even though both A and D vary with T in a similar fashion (Figure 4). The scatter of the points for $A\nu_c/[C]$ is large for two reasons: they are a combination of two experimental quantities, A and ν_c, with the concomitant cumulative errors; but more important, A is somewhat dependent upon pH and ionic strength for solutions of a given protein (Koenig and Schillinger, 1969; Lindstrom and Koenig, 1974). Nonetheless the points in Figure 5 fall reasonably well along the dashed line, drawn with unity slope. Thus, both A and D have an explicit (approximately) linear dependence on molecular weight, or volume, of the protein molecules, and not on their surface area.

Figure 6 summarizes the results for ν_c at 25 °C as a function of (unhydrated) molecular weight. The point for apotrypsin (TP) was obtained from data of Koenig and Epstein (unpublished), for apotransferrin (TF) from Koenig and Schillinger (1969), and for ceruloplasmin (CP) from Koenig and Brown (1973). The results for apotransferrin and immunoglobulin, which fall below the main sequence of points, are indicated with a separate symbol. These proteins, because of their large shape anisotropy, would have an averaged hydrodynamic behavior corresponding to τ_c greater than that of spherical proteins of the equivalent masses, in qualitative accord with the contention that $1/\nu_c$ is proportional to the orientational relaxation time of the protein molecules.

TABLE II: Comparison of the Orientational Relaxation Time τ_R of Several Proteins Obtained by Proton Relaxation Dispersion Measurements with Values Obtained by Other Experimental Methods.

Protein	Temp (°C)	τ_R (ns) Magnetic Relaxation	τ_R (ns) Other
Lysozyme	25	50	30[a]
Carbonic anhydrase B	25	43	34[b]
Concanavalin A	25	110	93[c]
Hemoglobin	1	166	210,[d] 257[e]
	6	173	175,[d] 221[e]
	25	102	97,[d] 129[e]
Immunoglobulin	25	420	500, 100[b]
Apotransferrin	25	280	430, 141[f]

[a] Light scattering (Dubin et al., 1971) at 20 °C. [b] Fluorescence depolarization (Yguerabide et al., 1970). [c] Fluorescence depolarization (Yang et al., 1974). [d] Dielectric relaxation (Grant et al., 1971). [e] Dielectric relaxation (Schlecht et al., 1969). [f] Dielectric relaxation (Rosseneu-Motreff et al., 1971, 1973; Lamote and Peeters, 1975).

The dashed line is obtained from eq 1; τ_R was calculated using for η the viscosity of pure water at 25 °C, and the value 0.73 for the partial specific volume needed to relate protein volume to protein mass. The protein molecules are assumed spherical, but corrections for hydration *are* included. It is assumed, as found for lysozyme (Dubin et al., 1971) that a hydration layer 3.5 Å thick should be included in calculating the hydrodynamic radii of all proteins. This adds about 10% to the radius of lysozyme, the lightest protein used, and a negligible amount to the heaviest. Other than this, *no adjustable parameters* are involved in computing the position of the dashed line.

The data points for the low molecular weight proteins fall systematically below the dashed line, Figure 6. It is not clear as yet if this behavior represents a fundamental deviation from the concepts underlying the dashed line. The effect may simply be due to a concentration dependence of ν_c. The total protein in the samples of low molecular weight protein is somewhat greater than for the other samples because of the small magnitude of A in this limit, and it is known that (in particular) lysozyme, concanavalin, and tRNA tend to polymerize.

The information contained in Figure 6 is the major result of the present work. Taken together with the data on temperature dependence, Figures 3 and 4, it demonstrates conclusively that the orientational relaxation time of solute macromolecules (which results from rotational Brownian motion) is conveyed to the solvent water molecules, and is detectable in their averaged behavior. (The nature of the mechanism that couples nuclear relaxation with the dynamical behavior of the water molecules is considered in the Discussion.)

The excellent agreement of the values of τ_R, as measured by relaxation dispersion and as computed from Stoke's law notwithstanding, it is of interest to compare the experimental results with values of τ_R obtained using other experimental techniques. Table II shows such a comparison for six different proteins. For the more spherical proteins such as hemoglobin and carbonic anhydrase, the agreement is excellent. For lysozyme, the higher protein concentration has caused τ_R obtained from magnetic relaxation dispersion measurements to increase by at least 20%; for the highly anisotropic immunoglobulin and apotransferrin, the magnetic relaxation measurements give a (weighted) average τ_R close to the longer τ_R values obtained from other methods.

A Comparison of Proton and Deuteron Relaxation. The strictly exponential decay of the nonequilibrium proton magnetization observed during a measurement of $1/T_1$, together with the magnitudes of the echos which indicate that all solvent protons (within about 5% uncertainty) contribute to the observed magnetization, show that all solvent protons have similar histories when averaged over a time of order T_1. One question that remains is whether the relaxation of solvent protons in protein solutions is due, as in pure water, to magnetic dipolar interactions among solvent protons, the influence of the protein being solely through its effect on the motion of the solvent water, or whether a substantial contribution to the solvent proton relaxation arises from interactions of solvent protons with protein protons.

Koenig and Schillinger (1969) compared proton relaxation in two solutions of apotransferrin, one 100% ^1H_2O solvent, the other an equal mix of protons and deuterons. The results suggest that protons are relaxed by both solvent and nonexchanging protein protons. Kimmich and Noack (1971) reached similar conclusions from a comparison of the $1/T_1$ dispersion with the $1/T_2$ (spin–spin relaxation) dispersion. In the present work, we compare proton and deuteron dispersions in partially deuterated solvent to monitor possible changes in the dynamics of the protein molecules due to the deuterated solvent, and then compare the dispersion behavior of deuterons and protons. The data are for lysozyme, carbonmonoxyhemoglobin, and hemocyanin solutions.

Values for the parameters of the Cole–Cole expression are given in Table III. (The raw dispersion data for some of the lysozyme and hemocyanin samples have been published elsewhere (Koenig et al., 1975).) There is reason to believe that the deuteration procedure may have introduced small amounts of paramagnetic impurities into the 100% deuterated stock samples, and thus a residual paramagnetic contribution to the proton relaxation, proportional to the partial molar fraction of deuterons, in the samples of mixed solvent. This is based on our measurements of carbonmonoxyhemoglobin for a wide range of solvent deuteron to proton ratio, as well as the experiences of Pearson et al. (1975). The impurities, if present, would not affect the deuterium relaxation data, since the magnetic coupling of a deuteron to any spin is much less than that of a proton, and the intrinsic deuteron relaxation rate is much greater. The impurity effects on the protons would also be small for those samples in partially deuterated solvent listed in Table III, and only affect the D term. Data are not included for the more highly deuterated samples because of the present uncertainty.

There are several points to note in Table III: (1) the values of ν_c obtained from deuteron and proton dispersion data obtained from the *same samples* are the same; (2) partial deuteration of the lysozyme and hemoglobin samples decreases ν_c more than the ~10% change in viscosity can account for; (3) the intrinsic magnitude of the dispersion, i.e., the A term normalized by concentration, the water relaxation rate, and ν_c (last column, Table III), is about twice as large for protons as for deuterons; and (4) except for the hemocyanin solution which may not be completely monodisperse, the shape of the deuteron dispersion curves is distinctly closer to a Lorentzian than the proton data, as indicated by the values of β.

Discussion

We have verified that the correlation frequency ν_c derived from the dispersion of solvent proton relaxation rates in protein

TABLE III: Comparison of Results Derived from Proton and Deuteron Relaxation Dispersion Data for Solutions of Three Different Proteins. The Last Column Gives the Basic Interaction A in Units of the Solvent Relaxation Rate, Normalized for Concentration and the Explicit Dependence on ν_c.

Protein	Temp (°C)	[C] (mM)	Nucleus	A (s^{-1})	D (s^{-1})	$1/T_{1w}$ (s^{-1})	β	ν_c (MHz)	$AT_{1w}\nu_c/[C]$ (s^{-1} M^{-1}) $\times 10^{-9}$
Lysozyme[a]	4	13.8	100% ^1H	5.1	0.34	0.50	1.4	1.6	1.2
	4	13.8	28% ^1H	3.8	0.53	0.19	1.4	0.9	1.3
	4	13.8	72% ^2H	35	6.7	4.7	1.9	1.0	0.54
	22	13.8	100% ^1H	2.0	0.34	0.30	1.6	3.8	1.8
	22	13.8	28% ^1H	1.5	0.34	0.11	1.4	2.5	2.5
	22	13.8	72% ^2H	12	3.7	2.5	1.8	2.6	0.91
Carbonmonoxyhemoglobin	25	1.75	100% ^1H	1.38	0.19	0.28	1.5	2.6	7.3
	25	1.75	40% ^1H	0.98	0.24	0.15	1.6	2.0	7.5
	25	1.75	60% ^2H	9.7	1.4	2.4	1.9	1.9	4.4
	25	1.75	100% ^2H	9.4	0.9	2.4	1.6	2.1	4.7
Hemocyanin (1/10)	25	0.012	100% ^1H	1.3	1.0	0.28	1.4	0.31	120
	25	0.029	26% ^1H	1.6	2.9	0.12	1.3	0.30	130
	25	0.029	74% ^2H	14	2.5	2.4	1.4	0.36	60

[a] The results for this sample, particularly for ν_c, differ from the lysozyme results in Table I because the protein concentration is much greater here. The rotational relaxation time increases significantly with concentration (cf. Figure 4 of Lindstrom and Koenig, 1974).

solutions is directly and simply related to the orientational relaxation time of the protein molecules. Indeed, measurement of ν_c is perhaps the best general experimental procedure for obtaining orientational relaxation times of macromolecules in solution. The validity of the method has been demonstrated for a three-decade range of molecular weights. Magnetic relaxation dispersion measurements are not limited by the need for low (electrically) conducting samples, as are dielectric dispersion and electrically induced optical birefringence experiments, and there are no restrictions on buffer composition, pH, and ionic strength other than those set by the protein. Moreover, the method is applicable to solutions of proteins that are optically isotropic, in contrast to optical methods that depend on depolarization of scattered light, or which require labeling of the protein with a dye marker to induce optical anisotropy. The major limitation of the method is the requirement of a fairly high protein concentration, typically greater than 1 wt %. On the other hand, one may readily study the dependence of ν_c on concentration at higher concentrations to gain information about protein–protein interactions. A particular advantage of magnetic relaxation measurements is that they also provide information about the orientational relaxation of protein molecules in heterogeneous systems. Thus, ν_c for hemoglobin molecules *within* erythrocytes (red blood cells) has been determined from measurements of $1/T_1$ of protons of *extracellular* water molecules, which of course exchange with the cell interior. This has been done for suspensions of erythrocytes containing normal adult hemoglobin (Lindstrom and Koenig, 1974) as well as SS (sickle-cell) hemoglobin (Lindstrom et al., 1976), to study intermolecular interactions within cells.

The problem that remains is to determine the physical mechanism underlying the observed phenomena. Without going into the quantitative details of relaxation theory, it is expedient to recall its general form (cf. Abragam, 1961):

$$1/T_{1i} \propto \int_0^\infty \langle I(t)I(t+\tau)\rangle \exp(-i\omega_i\tau)d\tau \qquad (4)$$

Here $1/T_{1i}$ is a contribution to the relaxation rate of a nucleus, and I is the interaction responsible for producing relaxation (the interaction of the deuteron nuclear quadrupole moment with the electric field gradient at the deuteron, or the magnetic dipolar interaction between two protons). The angular frequency ω_i is the energy (in frequency units) of the ith transition that contributes to $1/T_{1i}$. The bracket indicates that the product of the value of I at time t with its value at time τ later is to be averaged, statistically, over all values of t; this average is called the autocorrelation function of I. It is a rough measure of the time during which the sign or magnitude of I persists. For nuclear magnetic relaxation of solvent water molecules, this time is related to the rotational relaxation time of the water. In many situations, including the case of diffusive rotational motion, the autocorrelation function can be written as an exponential; generally, when the interaction is not known, the autocorrelation function is assumed exponential. Thus

$$\langle I(t)I(t+\tau)\rangle \equiv \langle I(t)I(t)\rangle \exp(-|\tau/\tau_c'|) \qquad (5)$$

Performing the integral with this assumption, eq 4, which is simply the Fourier transform of the autocorrelation function, gives

$$1/T_1 \propto \langle I^2\rangle \tau_c'/(1+\omega^2\tau_c'^2) \qquad (6)$$

For water nuclei, there are two transitions that contribute to the relaxation, corresponding to $\omega_i = 2\pi\nu$ and $4\pi\nu$, where ν is the nuclear Larmor frequency. The sum of their contributions is such that it can be very well approximated by a single value $\omega = \sqrt{3}(2\pi\nu)$, which leads directly to eq 1 (Koenig and Schillinger, 1969).

For the case of an exponential autocorrelation function, only one additional parameter, the correlation time τ_c', is needed to characterize the time dependence of the interaction responsible for relaxation. If the autocorrelation function is not exponential, the relaxation dispersion, eq 6, will not be Lorentzian. Note that for a fixed interaction strength, the magnitude of the relaxation at low fields ($\omega\tau_c' \ll 1$) increases as τ_c' lengthens, giving an intrinsic dependence of A, eq 2, on τ_c. If the autocorrelation is the sum of two exponentials of widely disparate τ_c' values, the relative contribution to $1/T_1$ of the term with the longer τ_c' will be greatly magnified compared with the other. In this way, an almost negligible contribution

to the autocorrelation which has, however, a long correlation time can become the dominant contribution to $1/T_1$. It is this fact which has implications for the present experimental results.

Two extreme types of models may be considered to explain the present data: (1) a two-site model in which a water molecule is either associated with protein or with the bulk solvent, with rapid exchange between the two types of sites; and (2) a model in which all solvent molecules are considered to be in continuous, long range, interaction with the macromolecules. In the first case, provided the waters on the protein were at least partially immobilized, $1/T_1$ would be the sum of two terms such as eq 4, weighted by the relative fractions of water molecules on the protein and in the solvent. Such a model explains much of the data (Koenig and Schillinger, 1969), but it has since been demonstrated, by a comparison of ^1H, ^2H, and ^{17}O relaxation, that models of this type are precluded (Koenig et al., 1975).

The second class of model seems more realistic. Although we have neither an exact theory, nor even an estimate resulting from an approximate theory, we believe the following considerations are relevant. Consider the rotational Brownian motion of a macromolecule for a period of the order of its rotational relaxation time. During this period the orientation of the molecule has changed by about 1 radian; though the motion has been a random walk, the net result is that the molecule has rotated about some axis in a well-defined time, and thus has had a definable mean angular momentum. In the long term, these rotations are random and this angular momentum averages to zero. But for this short period, there has been a fluctuation in the angular momentum of the macromolecule, which, since all collisions with solvent conserve angular momentum, must be equal and opposite to a fluctuation shared by the water molecules less than about half way to the next macromolecule. (The intermacromolecule distance is of order 100 Å, and water molecules within this radius mix in a time of the order of the protein relaxation time.) If the water is regarded as a continuum fluid, the angular momentum is taken up by hydrodynamic motion imparted to the water by the boundary conditions at the protein surface. This hydrodynamic angular momentum is then conveyed to the individual water molecules by their intermolecular collisions in the moving frame. (Alternatively, if the details of the protein–solvent collisions are looked at on a molecular basis, the transfer of angular momentum to the water molecules is direct. The two pictures must of course be consistent.) The net result is that superposed on the rapid rotational tumbling motion of a water molecule (with $\tau_R \sim 10^{-12}$ s) is a much slower motion, with $\tau_R \sim 10^{-7}$ s, that follows the Brownian motion of any protein molecule in its neighborhood. The effect is small, we have not estimated how small, but its contribution to the low field $1/T_1$ is magnified by a factor of order 10^5 as discussed above.

We propose that the above hydrodynamic effect is the dominant mechanism responsible for relaxation dispersion of solvent water nuclei. It explains why the relaxation is relatively insensitive to the nature of the surface of the macromolecule (compare tRNA and protein) and its charge as determined by pH, ionic strength, isoelectric point, etc. It also explains an observation which two-site models cannot: the fact that data for the heaviest hemocyanin molecules, empirically, are consistent with data for lighter molecules. There is, in principle, an uncertainty concerning the applicability of the usual relaxation theory for samples containing high molecular weight proteins. The problem is that, for the usual theory of relaxation in liquids to apply (the limit of "motional narrowing" of the interaction responsible for relaxation, cf. Abragam (1961)), the correlation frequency must be much greater than, for protons, the magnetic dipolar interaction between the two protons of a water molecule, which is about 70 kHz. This restriction would be violated for the 1/1 hemocyanin samples for water molecules on the protein. The restriction is more severe by a factor of 3 for deuteron relaxation, and a factor of 50 for ^{17}O relaxation. Yet it is established that ^{17}O, deuteron, and proton relaxation dispersion data are quite similar for protein solutions (Koenig et al., 1975), which would not be expected if the motional narrowing condition were violated. However, on the hydrodynamic model, most of the interaction responsible for relaxation is averaged out by the rapid rotation of the water molecules, which reduces the effective interaction by several orders of magnitude, and the motional narrowing condition is not violated.

The preceding remarks refer to the dispersive term containing A, eq 2. The relaxation contribution represented by D has a different source. We have shown that the magnitude of D is approximately proportional to the protein molecular weight (it appears to vary a bit faster than the surface area of the protein), varies with temperature as η/T, and has no explicit dependence on the protein rotational relaxation time. Koenig and Schillinger (1969) indicated that the correlation frequency associated with the D term is about 150 MHz for apotransferrin solutions and suggested that the relaxation is associated with water molecules exchanging between the bulk and the "traditional" hydration shells of protein molecules. Gupta and Mildvan estimate roughly the same correlation frequency for oxyhemoglobin solutions. We have no reason to question this view, nor have we any additional confirmation of its validity, other than to note that the D contribution is common to all proteins investigated here. It should be noted, however, that the data for D are scant and not too accurate; at the fields used here there are uncertainties due to the tail of the main dispersion, and there are few data at higher fields.

The fact that the dispersion curves for protons and deuterons, when normalized to their respective relaxation rates in protein-free buffer, are so similar indicates that the dominant relaxation interaction cannot be between solvent nuclei and protein protons. Such an interaction could contribute to proton relaxation, as has been suggested by Kimmich and Noack (1970b, 1971) though discounted by Wennerstrom (1973), but any such interaction would be unobservably small for deuterons. The major contribution to the relaxation must be via an influence of proteins on the time-averaged dynamics of solvent water molecules. Nonetheless, the normalized proton dispersion has an amplitude that can be almost twice the deuteron amplitude. It is conceivable that this difference is related to solvent–proton interactions with nonexchangeable protons of the protein backbone and sidechains. We have tried to check this by measuring proton dispersion in solutions with progressively decreasing $^1H/^2H$ ratios, but, as mentioned above, it appears that paramagnetic impurities may have obscured the true behavior. The resolution of this point must be left to additional experiments. The point should be made, though, that the proposed hydrodynamic model allows for a difference in the normalized amplitudes of the proton and deuteron relaxation. The moments of inertia of ^1H_2O and ^2H_2O molecules are quite different, particularly about the water diad axis, and the averaging of the rotational motion that gives the autocorrelation function, eq 4, can produce different results for protons and deuterons.

Finally, a comment is in order regarding a recent paper in which data for $1/T_1$ of water protons are reported. Sloan et

al. (1973), in investigations of the hydration of yeast glyceraldehyde-3-phosphate dehydrogenase, derive a value for τ_c from measurements of $1/T_1$ at two fields, corresponding to 3.5 and 30 MHz; they remark that this value is an order of magnitude less than anticipated from the protein molecular weight. We note that their procedure is invalid; a fit, according to eq 2, requires that measurements be taken at, at least, four fields since four parameters must be determined. The procedure of Sloan et al. is equivalent to setting $\beta = 2$ and $D = 0$. But at 30 MHz, D is the dominant term. In addition, their 30-MHz data are highly uncertain since the contribution of the protein to the measured relaxation rate is only about 20%. This is somewhat less than the contribution of dissolved O_2 and, unless care is taken to maintain it constant, there can be large uncertainties introduced into the calculated value of the small protein contribution. Thus the results of Sloan et al. do not contradict our general findings; rather their data are insufficient for obtaining a value for τ_c.

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Conformational Properties of the Complexes Formed by Proteins and Sodium Dodecyl Sulfate†

Wayne L. Mattice,* James M. Riser, and Donald S. Clark

ABSTRACT: Circular dichroism spectra have been obtained for albumin, α-chymotrypsinogen, collagen, concanavalin A, elastase, hemoglobin, histone f_2b, α-lactalbumin, lactate dehydrogenase, β-lactoglobulin, lysozyme, myoglobin, papain, ribonuclease A, and thermolysin in the presence of sodium dodecyl sulfate and dithiothreitol. While all spectra have the shape anticipated for a mixture of random coil and α helix, the intensities differ markedly ($[\theta]_{222}$ ranges from −1400 to −15 000 deg cm^2/dmol). The variation in the circular dichroism can be quantitatively explained by a model which assumes that the arginyl, histidyl, and lysyl residues have an enhanced probability of propagating a helical segment in the presence of the detergent. The model also permits the computation of dimensional properties (unperturbed end-to-end distance and radius of gyration) for polypeptides of known amino acid sequence. Such computations have been performed for 67 proteins. The computed dimensions are compatible with experimental values and with the molecular weight dependence of the transport properties of the complexes. Furthermore, the model can account for the abnormal transport properties of the sodium dodecyl sulfate complexes formed by ribonuclease A, collagen fragments, and histones f_2a_1, f_2a_2, f_2b, and f_3. Even though some of the protein—sodium dodecyl sulfate complexes have helical contents as high as 50%, their overall conformation more closely approximates that of a random coil than a rod.

A variety of proteins with reduced disulfide bonds form complexes containing approximately 1.4 g of sodium dodecyl sulfate/g of protein when the detergent monomer concentration exceeds 0.0008 M (Pitt-Rivers and Impiombato, 1968; Reynolds and Tanford, 1970; Takagi et al., 1975). The electrophoretic mobility in cross-linked polyacrylamide gels (Shapiro et al., 1967; Weber and Osborn, 1969; Dunker and Rueckerts, 1969), logarithm of the intrinsic viscosity (Reynolds and Tanford, 1970), logarithm of the Stokes radius (Fish et al., 1970), and slow birefringence relaxation time (Wright et al., 1975) are proportional to the logarithm of the molecular weight (or degree of polymerization) of reduced proteins under these conditions. Electrophoretic mobilities on free-boundary electrophoresis are independent of the molecular weight of the protein used to form the protein–sodium dodecyl sulfate complex (Shirahama et al., 1974). The optical activity of protein–sodium dodecyl sulfate complexes suggests the presence of appreciable amounts of α helix (Meyer and Kauzmann, 1962; Jirgensons, 1966, 1967; Reynolds and Tanford, 1970; Visser and Blout, 1971). However, the nature of the protein examined affects both the optical activity of the complex and the direction of the change in the rotatory strength associated with the peptide n–π* transition upon the formation of the complex from the native protein (Jirgensons, 1966; Visser and Blout, 1971).

The effect of sodium dodecyl sulfate on several synthetic homopolypeptides has been examined. Sodium dodecyl sulfate has little effect on the helix–coil transition of poly(L-glutamic acid) (Fasman et al., 1964). In marked contrast, cationic homopolypeptides readily adopt ordered conformations in the presence of sodium dodecyl sulfate. Poly(L-ornithine) (Grourke and Gibbs, 1967; Satake and Yang, 1973) and poly(L-arginine) (McCord, Blakeney, and Mattice, submitted) form an α helix, poly(L-histidine) forms a β structure (McCord, Blakeney, and Mattice, submitted), and poly(L-lysine) forms either a β structure (Sarkar and Doty, 1966; Satake and Yang, 1973, 1975; Mattice and Harrison, 1976) or α helix (Satake and Yang, 1975), depending on the pH and temperature. The crucial role played by the cationic side chains is evident from the insensitivity of the helix–coil transition of poly(N^5-ω-hydroxypropyl-L-glutamine) (Lotan et al., 1965; Igou et al., 1974) and poly(N^5,N^5-di(ω-hydroxyethyl)-L-glutamine) (Igou et al., 1974) to the detergent. This consideration is in accord with the pH dependence of the interaction of sodium dodecyl sulfate with pentalysine (Mattice and Harrison, 1976). The different behavior of proteins toward cationic and anionic detergents also suggests an interaction between the detergent head group and the protein (Nozaki et al., 1974).

The hydrodynamic behavior of the protein–sodium dodecyl sulfate complexes has led to the proposal of three models for the complex: a rod-like particle (Reynolds and Tanford, 1970), a "necklace" model (Shirahama et al., 1974), and a deformable prolate ellipsoid model (Wright et al., 1975). None of these models can account for the various effects produced by sodium dodecyl sulfate on the optical activity of the proteins, nor do they explain why anomalous hydrodynamic behavior is exhibited by certain proteins, such as ribonuclease A (Shapiro et al., 1967), various histones (Panyim and Chalkley, 1971), and peptides derived from collagen (Furthmayr and Timpl,

† From the Department of Biochemistry, Louisiana State University, Baton Rouge, Louisiana 70803. *Received February 26, 1976.* Supported by National Science Foundation Grant BMS 72-02416 A01.