

Solvent proton relaxation of aqueous solutions of the serum proteins α_2 -macroglobulin, fibrinogen, and albumin

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ABSTRACT The longitudinal, transverse, and spin-locked rotating frame relaxation rates have been measured for water protons in aqueous solutions of the human serum proteins albumin, fibrinogen, and α_2 -macroglobulin in the physiological concentration range below 50 g/liter, corresponding to an

upper limit for molarity of 725, 147, and 69 μ M, respectively. The linear concentration dependence of all the relaxation rates measured at 100 MHz was used to provide the molar sensitivities of each relaxation process for each of the protein solutes. Both the solute dependence and the relaxation-process de-

pendence of the molar sensitivities have been analyzed in terms of a model that has emerged from previous R_1 dispersion measurements. This analysis demonstrates consistency between our data and that model for the active motions and their motional rates.

INTRODUCTION

The modification of water proton relaxation rates by soluble proteins in both model systems and tissue has been a problem of longstanding interest and debate (reviews 1–3 and references therein). It is a problem that is very relevant to the development of proton magnetic resonance imaging (MRI) as a quantitatively specific diagnostic modality. The development of specificity has been hindered by a least two factors, namely by an insufficient understanding of the mechanisms governing proton relaxation in tissues, of which protein solutions (4–11) are often used as the simplest of models, and by a self-imposed limitation of the imaging technique to the laboratory frame longitudinal (R_1) and transverse (R_2) relaxation rates. The inclusion of rotating frame relaxation measurements ($R_{1\rho}$) offers an additional means of interrogating the changes in water motion that take place during pathological change in tissue (12). This, together with a more complete body of understanding of the sensitivity characteristics of the various proton relaxation mechanisms to the concentration of individual serum proteins and their mixtures in aqueous solution, could improve our current understanding of water proton relaxation in tissue.

For both the aqueous protein solution model and for tissue, it is usually accepted that the two-site rapid exchange of water molecules, between aqueous solvent and the surface region of protein macromolecules, is an important determinant of the observed water proton relaxation rates. In the aqueous solvent compartment of either system, motional averaging is always taken to be

complete. However, the relaxation mechanisms operating at the sites of water molecules “associated” with protein surfaces have been interpreted differently. For macromolecular solutions, where the majority of the data has come from measurements of the field dependence of the longitudinal relaxation rate, (4, 9, 10), two successive motional processes have been invoked to explain the experimental results. At the higher frequencies of the motional power spectrum, anisotropic reorientation of the “associated” water molecules has been proposed (13) as an active relaxation mechanism but this motion acts as only a partial averager of the local fields at the water proton sites. Protein motion through the solvent has been proposed (14) as the slower motion that completes the averaging of these local fields before two-site exchange. In tissue experiments, on the other hand (15, 16), the lower frequency motion that completes the averaging started by anisotropic reorientation is assumed to be the two-site exchange process itself. If this difference in mechanism is indeed the case, even for the largest of serum proteins, then it limits the potential usefulness of protein solutions as a model of tissue in the experimental regime of transverse and rotating frame relaxation.

By taking an approach that is somewhat different from the R_1 dispersion method, namely the study of several proteins spanning a very wide range of size by means of relaxation measurements that are readily accessible for in vivo NMR purposes, it has been possible to demonstrate that even for the two very large proteins fibrinogen and α_2 -macroglobulin, protein motion and not two-site exchange completes the averaging of the local fields at “associated” water sites. Moreover, changes in the molar sensitivity between the different relaxation parameters

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are sufficiently different from those that would be expected from a simple single motion model, to enable us to establish a quantitative consistency between the data presented here and the two-motion model that has emerged from a substantial amount of previous R_1 dispersion work on aqueous macromolecular solutions. A preliminary report of this work was presented at the sixth Annual Meeting of the Society of Magnetic Resonance in Medicine (17).

EXPERIMENTAL

Materials

Solutions of human α_2 -macroglobulin, human fibrinogen, and human serum albumin were made using isotonic saline buffers that consisted of doubly distilled, deionized water, which was made isotonic with human serum by dissolving reagent grade NaCl in appropriate amounts. To keep the pH of the protein solution physiological, and to facilitate dissolving large amounts of protein, Tris-HCl was used to adjust the solution pH between 7.2 and 8. Previous studies (4, 9, 10) suggest that there is little pH dependence of the relaxation rates R_1 and R_2 in this physiological pH range. The protein concentrations were also chosen to represent the range of physiologically expected values. Human fibrinogen (lyophilized, Sigma Chemical Co., St. Louis, MO) and human serum albumin (supplied as 100 g/liter in 0.9% NaCl, Sigma Chemical Co.) were studied in the range 0–50 g/liter (0–147 μ M and 0–725 μ M, respectively). Human α_2 -macroglobulin (lyophilized, Sigma Chemical Co.), on the other hand, was only studied in the range 0–16.67 g/liter (0–23 μ M) because of its poor solubility. The relevant properties of these proteins are shown in Table 1 (18, 19). All samples were stored at 4°C and used within 72 h of preparation, although repeatable results could be obtained over periods of as long as 3 wk.

Relaxation measurements

The proton relaxation rates were measured at 100 MHz with a model CXP spectrometer (Bruker Instruments, Inc., Billerica, MA). The line width obtained for a 100- μ l sample in the 40-cm bore magnet was typically 5 Hz. During the experiment, sample temperatures were kept at $37 \pm 2^\circ\text{C}$ by warm airflow. The coil and receiver electronics had a combined dead time of 40 μ s. This precluded accurate measurement of the "solid" signal originating from protons bound firmly in the protein structure. The transverse relaxation of the solvent was measured using a Carr-Purcell-Meiboom-Gill (CPMG) add/subtract sequence (20, 21), with an inter-echo spacing of 400 μ s. This choice of echo time was made to minimize the effects of diffusion on R_2 . The add/subtract sequence was repeated 32 times and the resulting signal was averaged. The

longitudinal magnetization was measured using the Freeman-Hill (22) modification of the inversion recovery sequence,

$$[180_y - \tau - 90_x - \text{AQ} - \text{TR} / 0 - \tau - 90_x - \text{AQ} - \text{TR}],$$

where TR is a fixed magnetization recovery interval. The τ values, ranging from 10 μ s to 7.9 s, were arranged to provide 10 data points in each decade of time. The advantage of using this particular sequence lay in the fact that the magnetization recovery curve is always positive, dropping from $2M_0$ to zero, with the initial magnetization M_0 being measured as often as the partly relaxed magnetization. Eight acquisitions of each add/subtract pair were averaged. The magnetization decay in the rotating frame was measured using the phase alternated sequence

$$[90_x - \tau(\text{lock})_y - \text{AQ} - \text{TR} / 90_x - \tau(\text{lock})_y - \text{AQ} - \text{TR}],$$

in which the free induction decay was collected after each spin-locking pulse. The length of the spin-locking pulse was varied over the same 60 τ values used for the inversion recovery experiment and the signal averaging was also equivalent to that of the inversion recovery experiment. Four different amplitudes, B_1 , of the spin-locking pulse were employed, namely, 3.07, 6.15, 9.22, and 18.44 G. B_1 was calculated from the length of a 90° pulse in a special pulse program and was known to $\pm 5\%$.

All relaxation rates were obtained with a recovery interval (TR) of 30 s and a 90° pulse length of 7.5 μ s. R_1 , R_2 , and $R_{1\rho}$ were obtained by means of a nonlinear least-squares fitting program capable of fitting up to four exponentially decaying components to a curve. The repeatability of a particular relaxation rate measurement on the same samples over a period of several weeks was $\pm 0.4\%$ for R_2 , $\pm 3\%$ for R_1 , and $\pm 5\%$ for $R_{1\rho}$. These uncertainties do not include all sources of random error, such as that in concentration for example, that contribute to the scatter in later figures.

RESULTS

The concentration dependence of R_1 and R_2 of the protein solutions are shown in Figs. 1 and 2. The concentration dependence of $R_{1\rho}$ for the same solutions is shown in Fig. 3 at a spin-locking field of 3.07 G. The buffer intercepts for albumin are different from those of fibrinogen and α_2 -macroglobulin because of the influence of the supplier's buffer in which the parent sample of albumin was dissolved. A crucial point in all three cases is that the magnetization recovery, or decay, was monoexponential over a change in amplitude of several decades. This is consistent with the results of other workers on the labora-

TABLE 1 Physical parameters of the human serum proteins

Protein	Molecular weight	Molecular radii*	Diffusion constant†	Plasma concentration
	<i>kD</i>	<i>A</i>	$\times 10^{-7} \text{cm}^2 \text{s}^{-1}$	<i>g/liter</i>
Albumin	69	70 × 25 × 25	6.7	40.0–50.0
Fibrinogen	340	450 × 15 × 15	1.9	2.0–5.0
α_2 -Macroglobulin‡	725	100 × 100 × 100	2.3	2.2–3.8

Based on reference 18 and references therein.

*Shapes modeled as prolate ellipsoids with symmetric semiminor axis. † $D_{20,w}$. ‡Reference 19.

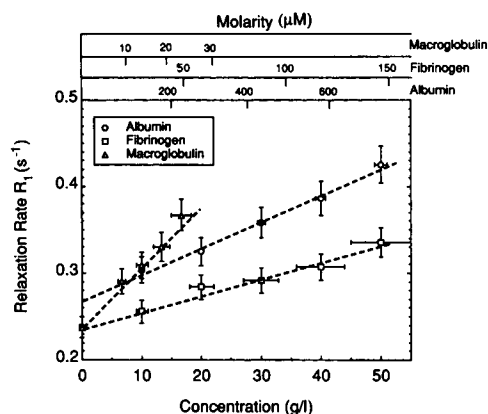


FIGURE 1 A comparison of the concentration dependence for the ^1H longitudinal relaxation rates obtained for the serum proteins albumin (O), fibrinogen (\square), and α_2 -macroglobulin (\triangle) in aqueous solution. Error bars represent the standard errors derived from measurements on four repeated occasions when a solution of the same concentration was manufactured. The fitted lines were calculated from a linear least-squares procedure. The slopes and intercepts thus calculated are shown in Table 2.

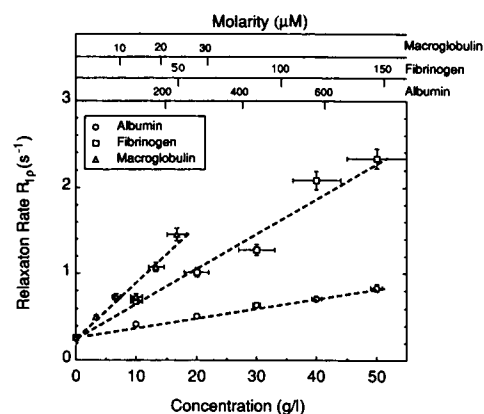


FIGURE 3 A comparison of the ^1H longitudinal relaxation rates in the rotating frame, observed at a spin-locking field of 3.07 G for the serum proteins albumin (O), fibrinogen (\square), and α_2 -macroglobulin (\triangle) in aqueous solution. Error bars represent the standard errors derived from measurements on four repeated occasions when a solution of the same concentration was manufactured. The lines were derived from a linear least-squares fit to the data and the slopes and intercepts are shown in Table 2. The relaxation rates observed at higher spin-locking fields are qualitatively similar to those shown in this figure.

tory frame relaxation in dilute solutions of fibrinogen (4) and albumin (10, 23, 24). The spin-locking field dependence of $R_{1\rho}$ is shown for selected concentrations in Fig. 4.

Of primary interest is the relative molar sensitivity of the relaxation rates R_i (R_1 , R_2 , or $R_{1\rho}$) to protein solute. These sensitivities, namely the gradients g_i (g_1 , g_2 , or $g_{1\rho}$), of the R_i vs. molarity are detailed in Table 2. For

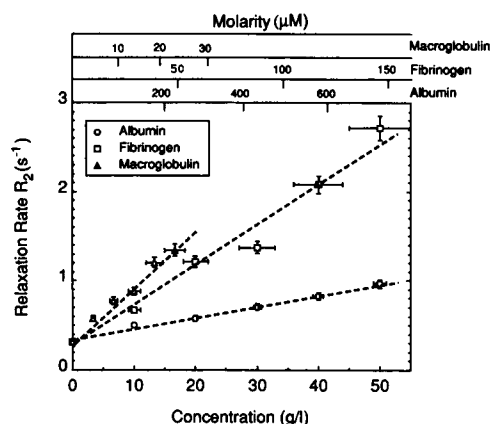


FIGURE 2 A comparison of the ^1H transverse relaxation rate concentration dependence for the serum proteins albumin (O), fibrinogen (\square), and α_2 -macroglobulin (\triangle) in aqueous solution. Error bars represent the standard errors derived from measurements on four repeated occasions when a solution of the same concentration was manufactured. The best-fit lines were derived from a linear least-squares procedure. The slopes and intercepts are shown in Table 2.

comparative purposes, the range of sensitivities g and g_2 derived from the work of various other workers is also shown. It should be borne in mind, however, that in addition to differences in frequency and temperature, these other workers employed different buffers, and in many cases, did not divulge their inter-echo spacing. That this is an important omission for protein solutions that exhibit substantial diffusion is exemplified by the fact that, with our magnet inhomogeneity and at 37°C , the apparent R_2 can be increased by a factor of two if one increases the inter-echo interval from $400\ \mu\text{s}$ to $40\ \text{ms}$.

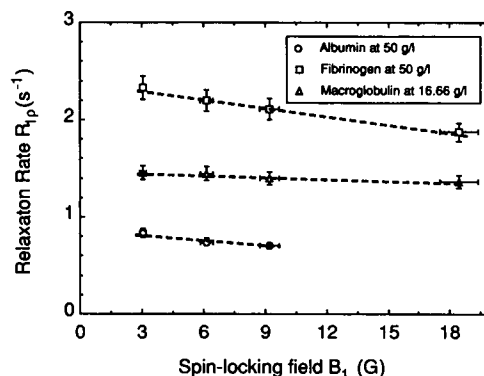


FIGURE 4 The ^1H longitudinal relaxation rate in the rotating frame as a function of the spin-locking field. Data are shown for the concentration of each protein indicated in the figure. The best-fit lines were obtained from a linear least-squares fitting routine.

TABLE 2 Molar sensitivities of R_1 , R_2 , and $R_{1\rho}$ to proteins in aqueous solution

Protein	g_1	R_{1b}	g_2	R_{2b}	$g_{1\rho}$ (@3 G)	$g_{1\rho}$ (@18 G)	$R_{1\rho b}$
	$\times 10^3 s^{-1} M^{-1}$	s^{-1}	$\times 10^3 s^{-1} M^{-1}$	s^{-1}	$\times 10^3 s^{-1} M^{-1}$	$\times 10^3 s^{-1} M^{-1}$	s^{-1}
Albumin	0.21	0.27	0.8	0.35	0.7	0.6 (@9 G)	0.27
Fibrinogen	0.63	0.24	15	0.30	14	11	0.26
α -Macroglobulin	5.0	0.24	46	0.30	46	44	0.26
Albumin (9,23,25,26)	0.1–0.75	—	1.0–2.5	—	—	—	—
Fibrinogen (4)	0.85	—	—	—	—	—	—

Uncertainty in $g_i \leq \pm 10\%$. Uncertainty in $R_i \leq \pm 5\%$.

ANALYSIS AND DISCUSSION

Linear concentration dependence and two-site rapid exchange

It is clear from the data presented in the Results section that, like several other workers on protein solutions (4, 23, 24), we find that each of the relaxation rates displays a linear concentration dependence over the concentration range to 50 g/liter. Such behavior is consistent with a two-site rapid-exchange model of relaxation (25), and even though this model is not universally accepted (11), it has been invoked (4, 9, 14, 23, 24, 26–30) to explain the observed relaxation of solvent nuclei in many dilute macromolecular solutions. We therefore choose to analyze our results according to this model. Workers using macromolecular concentrations much higher than those found physiologically have suggested an extension of this model to three or more exchanging fractions (3, 10, 30–33). These extra fractions, generally thought to consist of very tightly bound water, are only expected to be discernible when the overall proportion of water is very low and not at physiological concentrations.

For a two-site rapid-exchange model in which the motion at each site completely averages the local magnetic fields, the observed solvent relaxation rate, R_1 , is given by

$$R_1 = p_a R_{1a} + p_b R_{1b}, \quad (1)$$

where R_{1a} is the relaxation rate of the “associated” water protons, R_{1b} is that of the bulk water protons, and p_a and p_b represent their relative fractions, i.e., $p_a + p_b = 1$. The relaxation efficiency at each site, namely, R_{1a} and R_{1b} , is then dependent upon the magnitude of the local field being averaged and on the rate at which it is being modulated. Rewriting the relative fractions of the water species, in terms of the protein concentration, c , R_1 becomes,

$$R_1 = R_{1b} + c[0.018 h_a (R_{1a} - R_{1b})/M], \quad (2)$$

where h_a is the average number of water molecules “associated” with each protein macromolecule and M is the protein molecular weight (9). The linear dependence of R_1 on c shown in Figs. 1–3 reflects the form of Eq. 2 and is consistent with a concentration independence of h_a and R_{1a} at the low physiological concentrations studied. These two variables define a molar sensitivity, g_1 , where

$$g_1 = 0.018 h_a (R_{1a} - R_{1b}), \quad (3)$$

which is tabulated in Table 2. It will be noticed that although the gradients of Figs. 1–3 do not increase monotonically with increasing solute molecular size, the molar sensitivities do.

The dependence of g_1 on protein solute could arise through either h_a or R_{1a} or both. Assuming that h_a is proportional to the surface area, A_s , of the model molecular shapes whose dimensions are given in Table 1, any remaining dependence on protein solute that might arise through R_{1a} can be detected from the variation of g_1/A_s , listed in Table 3. For the transverse and rotating frame relaxation, $g_{1\rho}/A_s$ retain a strong dependence on protein solute. In fact they are in a very similar ratio, 1:5:8, to that for the solute molecular mass, namely, 1:5:10, suggesting that the mechanisms governing R_{2a} and $R_{1\rho a}$ depend on protein motion within the solvent. In contrast, g_1/A_s shows much less solute dependence, being essentially unchanged between the ellipsoidal molecules albumin and fibrinogen. One might therefore speculate that for albumin and fibrinogen at least, and to a lesser extent for α_2 -macroglobulin, the longitudinal relaxation rate R_{1a} is governed by a mechanism that is not too sensitive to overall molecular size but more to the local environment of the “associated” water molecules. The protein dependence of the molar sensitivities is therefore suggestive of the same model as that derived from R_1 dispersion measurements on a variety of protein solutes (4, 10, 11), namely that the lower frequency motional spectral densities that determine transverse and rotating frame relaxation efficiencies arise primarily from overall solute motion, whereas local motion on the surface of the macromolecule is apparently much more important in

determining the high frequency motional spectral densities that govern the longitudinal relaxation efficiency at 100 MHz. One mechanism previously suggested for this local motion is the anisotropic reorientation of the water molecules associated with the protein surface (13). Although such reorientation is an effective relaxation agent, it is not capable of fully averaging the local dipolar fields and therefore it leaves the opportunity for the slower protein motion to complete the averaging, before two-site exchange becomes effective at averaging the local fields at "associated" sites of water molecules.

This view is not, however, consistent with the interpretation of results from tissue, a system that one might wish to model using protein solutions. Burnell et al. (15) and Diegel and Pintar (16) have explained the marked dispersion of their $R_{1\rho}$ data from tissue by using the two-site exchange process itself as the mechanism that completes the averaging of local dipolar fields. The $R_{1\rho}$ data presented here, on the other hand, display a much smaller frequency dependence than that observed in tissue, even for large molecules such as fibrinogen and α_2 -macroglobulin, and moreover, $g_{1\rho}/A_s$ is strongly solute dependent. We therefore assume protein motion to be the ultimate averager of the local fields at the sites of water molecules "associated" with these protein surfaces, before two-site exchange with bulk water molecules, and write

$$R_{ia} = R_{iar} + R_{ipm}, \quad (4)$$

where R_{iar} and R_{ipm} correspond respectively to the contributions to the relaxation efficiency of the "associated" water molecules from their anisotropic reorientation and from protein motion. The tissue data can be reconciled with this if one supposes that in tissue the majority of the macromolecular surface results from the nonmobile cellular architecture and not from mobile proteins.

Having made this assumption, the consistency between the solute and concentration dependence data presented here and that of earlier workers using R_1 dispersion methods can be carried further, because the relative magnitudes of the different g_i for a single protein solute are indicative of the relative strengths and the frequency ranges of influence of these two relaxation mechanisms. For example, in very simple circumstances where all three relaxation processes are governed by the same single motion, it is possible, by taking the ratios g_i/g_j , to relate all three sensitivities in terms of a single variable, the correlation time of that motion τ_c . Such relationships are arrived at by making the standard assumptions about dipolar relaxation (34, 35), and by taking the ratio of the sensitivities one is able to eliminate the unknown parameter, h_s . Solution of the ratio equations in this simplest of cases will then give consistent values for τ_c , irrespective of

which ratio of experimental sensitivities is chosen. For our data on albumin and fibrinogen $g_2/g_{1\rho}$ and g_2/g_1 predict values of τ_c that are different by two to three orders of magnitude, differences that are well beyond the uncertainties carried through from the measurement error. The assumption embodied in Eq. 4 enables us to reconcile these differences and at the same time demonstrate the agreement between the two experimental methods.

The ratios of the molar sensitivities, g_i , for a single protein species give rise to an expression in R_{iar} and R_{ipm} alone, i.e., one that eliminates the need to estimate the unknown parameter h_s , namely,

$$g_i/g_j = [(R_{iar} + R_{ipm})/(R_{jar} + R_{jpm})] \cdot [1 - R_{ib}/(R_{iar} + R_{ipm}) + R_{jb}/(R_{jar} + R_{jpm})], \quad (5)$$

where the terms in R_{ib} and R_{jb} are small and can usually be neglected. For instance, from the data presented in the results section, it is clear that terms containing R_{ib} and R_{jb} are of the order of 5×10^{-3} for R_2 and $R_{1\rho}$, and $\sim 5 \times 10^{-2}$ for R_1 . We have therefore neglected them in what follows.

If the motions that govern R_{iar} and R_{ipm} can be characterized by correlation times τ_{ar} and τ_{pm} , respectively, and if the corresponding dipolar interaction strength factors can be represented by S_{ar} and S_{pm} , Eq. 5 can be rewritten

$$g_i/g_j \approx [S_{ar}\zeta_i(\tau_{ar}) + S_{pm}\zeta_i(\tau_{pm})] / [S_{ar}\zeta_j(\tau_{ar}) + S_{pm}\zeta_j(\tau_{pm})], \quad (6)$$

where ζ_i is the sum of Debye spectral density functions governing the relaxation rate R_i (34, 35).

Parenthetically, it should be noted at this point that the dipolar interaction that is modulated by anisotropic reorientation and which leads to R_{iar} also facilitates the exchange of spin energy between protein protons and "associated" water protons (36). The consequence of the spin energy exchange will be to reduce the effective R_{iar} , because this pathway to the thermal bath will now also be used to transfer Zeeman energy from the protein protons to that bath (37). Estimates of the number of "associated" water molecules from hydration fractions (3), suggests that in a 20 g/liter solution the protein protons and the "associated" water protons have similar thermal capacities. As a result spin energy exchange could have a significant effect on the strength S_{ar} of R_{iar} .

If we assume that R_2 and $R_{1\rho}$ are both dominated by the slower motion characterized by τ_{pm} , Eq. 6 for the case of these two relaxation rates reduces to

$$g_2/g_{1\rho} \approx \zeta_2(\tau_{pm})/\zeta_{1\rho}(\tau_{pm}) \approx \tau_{pm}/\zeta_{1\rho}(\tau_{pm}), \quad (7)$$

from which an estimate of τ_{pm} can be obtained. This low-frequency motion hypothesis is supported by the observation of a weak RF field dependence in the $R_{1\rho}$

measurements and gives rise to the estimates for τ_{pm} given in Table 3 that are consistent with R_1 dispersion data at low fields from several different macromolecules (4, 10, 11). R_1 , on the other hand, depends only upon spectral densities at ω_0 and $2\omega_0$ (where $\omega_0/2\pi = 100$ MHz) and will therefore be influenced more by the faster of the two motions. For R_2 and R_1 , Eq. 6 will approximate to

$$g_2/g_1 \approx S_{pm} \zeta_2(\tau_{pm})/S_{ar} \zeta_1(\tau_{ar}). \quad (8)$$

Using the τ_{pm} from Table 3 enables us to obtain estimates for the product $S_{pm}/S_{ar} \zeta_1(\tau_{ar})$.

To relate S_{ar} and S_{pm} , earlier workers (4, 15) took advantage of the fact that both of the motions under discussion were modulating the local field at the same proton site. By writing the static value of this local field as S , the strengths S_{ar} and S_{pm} can be related (15) in terms of an order parameter χ , such that

$$S_{ar} = (1 - \chi^2) S, \quad \text{and} \quad S_{pm} = \chi^2 S.$$

g_2/g_1 therefore gives rise to a value for the quantity $\chi^2/(1 - \chi^2) \cdot \zeta_1(\tau_{ar})$. To separate χ from τ_{ar} in this quantity, it is necessary to invoke additional information. For example, an upper limit for $\chi^2/(1 - \chi^2)$ may be obtained by utilizing in Eq. 8 the maximum value of $\zeta_1(\tau_{ar})$. At a Larmor frequency of 100 MHz, this maximum is 2.268×10^{-9} and it occurs when $\omega_0 \tau_{ar} = 0.62$ at the so-called BPP maximum in R_1 . The values of $\chi^2/(1 - \chi^2)$ in this limiting case are given in Table 3. For fibrinogen the limit is consistent with the value obtained from R_1 dispersion measurements (4), suggesting that $\tau_{ar} \approx 10^{-9}$ s (also consistent with the results of references 4 and 11). For albumin the lower limit for $\chi^2/(1 - \chi^2)$ is an order of magnitude smaller (consistent with R_1 dispersion measurements of reference 10). It should be noted that the quantity $\chi^2/(1 - \chi^2)$ is analogous to the ratio $A\tau_{ar}/D\tau_{pm}$ (where A and D are defined in reference 4) introduced by workers using the modified Cole-Cole expression to explain R_1 dispersion measurements (4, 6, 7,

10, 11). The values of $\chi^2/(1 - \chi^2)$ obtained here are very similar to their values of $A\tau_{ar}/D\tau_{pm}$.

In the analysis presented here, we have interpreted the data in terms of two motional processes, each having a unique correlation time. The unique correlation time is likely to be an oversimplification of reality, particularly for the motion of anisotropic molecules such as fibrinogen. However, it is our view that the nature of the data presented does not justify the inclusion of other adjustable parameters in its interpretation, and we have therefore limited ourselves to the simplest of models (with three adjustable parameters τ_{pm} , τ_{ar} , and χ) that is capable of establishing the consistency of our data with that from a variety of aqueous and macromolecular solutions using R_1 dispersion techniques.

SUMMARY

We have investigated aqueous solutions of two very large serum proteins, namely fibrinogen (340 kD and markedly anisotropic) and α_2 -macroglobulin (725 kD and isotropic), in conjunction with measurements on solutions of albumin (69 kD and anisotropic). We have, moreover, employed a physiological range of concentrations of these proteins together with proton relaxation measurements that are readily accessible for in vivo NMR purposes namely, R_1 , R_2 , and $R_{1\rho}$, to obtain the molar sensitivities of each of these relaxation processes for each protein solute.

The solute dependence of the molar sensitivities of both transverse and rotating frame relaxation shows that the low-frequency motional power spectrum which governs the dipolar relaxation of water protons is dependent on protein mass in a way which suggests that protein motion through the solvent, and not two-site exchange, is responsible for completion of the averaging of the local fields at the water sites "associated" with macromolecular surfaces. This conclusion is reinforced by the weak RF field and strong solute dependence of $R_{1\rho}$. Even with very large

TABLE 3 Calculated parameters derived from molar sensitivities

Protein	g_1/A_s	g_2/A_s	$g_{1\rho}/A_s$	τ_{pm}	$S_{pm}/S_{ar}\zeta_1(\tau_{ar})$	$(\chi^2/1 - \chi^2)_{min}$
	$s^{-1}M^{-1}A^{-2}$	$s^{-1}M^{-1}A^{-2}$	$s^{-1}M^{-1}A^{-2}$	s^{-1}		
Albumin	0.012	0.045	0.034 (@9 G)	$(2.3 \pm 0.7) \times 10^{-6}$	1×10^6	0.003
Fibrinogen	0.009	0.23	0.16 (@18 G)	$(1.6 \pm 1.4) \times 10^{-6}$	1×10^7	0.02
α_2 -Macroglobulin	0.040	0.37	0.35 (@18 G)	$(1 \pm 1) \times 10^{-6}$	5×10^6	0.01

The uncertainties in g_i/A_s are at least 10% due to the uncertainty in g_i . The uncertainty in estimates $S_{pm}/S_{ar} \zeta_1(\tau_{ar})$ are of the order of 100%. $(\chi^2/1 - \chi^2)_{min}$ is a lower limit.

proteins, therefore, protein solutions are not an appropriate model for R_2 and $R_{1\rho}$ in tissue. The longitudinal relaxation rate at 100 MHz does not exhibit the same behavior.

In extracting our conclusions, we have used only three parameters, namely, τ_{pm} , τ_{ar} , and χ , in addition to the assumptions of the conventional models of dipolar relaxation. We have estimated a value for one, τ_{pm} , from our data; assumed a range for another, τ_{ar} , from previously published R_1 dispersion information; and estimated a limiting value for a third, χ . The models used to interpret R_1 dispersion data are often more complex and typically use four fitting parameters to interpret the data in terms of two different motions of the protons at the water sites associated with the macromolecule, namely, anisotropic motion on the macromolecular surface and overall macromolecular motion. Nevertheless, the relative magnitudes of g_1 , g_2 and $g_{1\rho}$ for a single protein are consistent not only with the correlation times derived from R_1 dispersion data, but also with the relative strengths, previously reported, for the two interactions that are modulated by these motions.

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