Deuteron Magnetic Relaxation Studies on the Solution Properties of Some Denaturing Agents and Surfactants

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Abstract: Deuteron relaxation times in D_2O solutions of urea, acetone, guanidine hydrochloride, dimethyl sulfoxide, *t*-butyl alcohol, sodium dodecyl sulfate, and several commercial surfactants are reported. The observed interaction of these with water at low concentration is analyzed and discussed. It is of the same order of magnitude as water–ion interactions. Properties of compound formation between dimethyl sulfoxide and water at high solute concentrations is also discussed. The results indicate $3H_2O$–DMSO as the formula of the complex and this is compared with the results of other methods.

Studies of the effects of denaturing agents on water structure have a long history. Various methods, both classical (*e.g.*, viscometry) and nonclassical (*e.g.* infrared spectral studies), have been utilized. A recent paper[1] contains several references to such work, and serves as an introduction to the literature.

In this paper the effects of the following list of molecules on the deuteron spin–lattice relaxation time, T_1, of their D_2O solutions are described. The molecules are urea, acetone, guanidine hydrochloride, dimethyl sulfoxide, *t*-butyl alcohol, sodium dodecyl sulfate, "Triton X-100" (octylphenoxypolyethoxyethanol), "CF-10" (a benzyl ether of octylphenol condensed with ethylene oxide), and "DF-16" (a terminated ethoxylate of linear primary alcohols). The latter three are commercial nonionic surfactants manufactured by the Rohm and Haas Co., Philadelphia, Pa. Similar studies have been performed on simple organic and inorganic electrolytes,[2,3] and on polyelectrolyte solutions.[4] The impetus behind such studies is that deuteron relaxation in these systems provides a sensitive measure of the magnitude of the interaction of water with the solute molecules and ions. The theory of this has been well laid out.[5] The basic experimental observation is that the binding, hydration, or association of water to a molecule or ion is reflected by a *decreasing* spin–lattice relaxation time for the deuterons in solution. Disruption of interaction of water with itself due to the dissolved species is reflected in an *increased* relaxation time. Studies on inorganic electrolytes such as NaCl, KCl, etc., indicate that the sequence of increasing and decreasing relaxation times follows the same sequence as "structure-breaking" or "structure-making" ions as defined from viscosity measurements and other classical experiments.

The experiments reported here were initiated in an effort to find out whether or not similar conclusions and interpretations are applicable to organic species which are known to have similar effects on bulk water properties, and which also have important biophysical effects. As discussed fully in a later section the answer is no.

Experimental Section

All materials used in these experiments were reagent grade. In the case of dimethyl sulfoxide freshly distilled material stored over a molecular sieve was used. The handling of this was under as anhydrous conditions as possible and proton magnetic resonance spectra showed no indication of water.

T_1 measurements were made using an adiabatic fast-passage apparatus[6] attached to a Varian DA-60EL spectrometer operating in the wide-line mode at 9.2 MHz (V-4210A transceiver). Measurements were made on samples having a volume of 4 ml at a temperature of 31 ± 1°. Each T_1 value shown in Figures 1–3 is the result of three independent null point experiments on separate photographs. Reproducibility between measurements averaged ±3%.

Results

Figure 1 shows deuteron relaxation *times* in the region of mole fraction solute, X_s, equal to 0.0 to 0.6. This covers the solubility range of urea and guanidine hydrochloride and even for the lowest mole fraction is far in excess of the critical micelle concentration of the surfactants. Figure 2 shows the behavior of $1/T_1$ in the region of low solute concentration for the surfactants. Relaxation *rate vs. molal concentration* is plotted in this figure for purposes of later discussion. It is clear that all the compounds studied *shorten* T_1 in the mole fraction region studied. The most common denaturing agent, urea, has the least effect up to the limit of solubility. On the other hand dimethyl sulfoxide, which is a denaturant only under rather severe conditions,[7] and in whose concentrated solutions at least some enzymes retain activity,[8] results in the greatest effect among the simpler molecules.

Further indication of the nature of these results is shown in Figure 3 where the absolute value of the *excess* relaxation time for DMSO–D_2O mixtures is plotted *vs.* X_{DMSO}. The excess relaxation time is defined as the difference between the observed T_1 and that for the ideal mixture. The value of T_1 for $X_{DMSO} = 1.0$ is found by extrapolation. The excess dielectric constant at 3.5 MHz[9] for the same mixtures of DMSO–H_2O is plotted in the same figure.

There is a clear maximum for both the curves at $X_{DMSO} = 0.25$. The real part of the dielectric constant is

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[1] G. G. Hammes and J. C. Swann, *Biochemistry*, **6**, 1591 (1967).

[2] H. G. Hertz and M. D. Zeidler, *Ber. Bunsenges. Phys. Chem.*, **68**, 821 (1964).

[3] G. Engel and H. G. Hertz, *ibid.*, 72, 808 (1968).

[4] J. A. Glasel, *Nature*, 220, 1124 (1968).

[5] H. G. Hertz, *Ber. Bunsenges. Phys. Chem.*, 71, 979 (1967).

[6] J. A. Glasel, *J. Sci. Instrum., Series 2*, **1**, 963 (1968).

[7] J. A. Glasel, S. Hendler, and P. R. Srinivasan, *Proc. Nat. Acad. Sci. U. S.*, **60**, 1038 (1968).

[8] S. M. Vratsanos, M. Bier, and F. F. Nord, *Arch. Biochem. Biophys.*, 77, 216 (1958).

[9] Y. Doucet, F. Calmes-Perrault, and M.-T. Durand, *Compt. Rend.*, **260**, 1878 (1965).

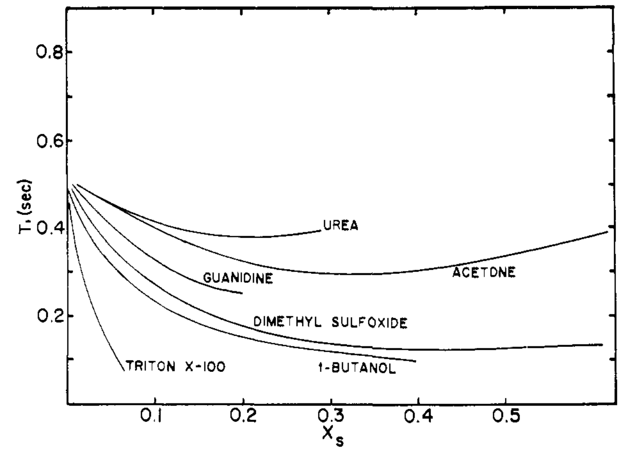

Figure 1. Relaxation times *vs.* mole fraction of solute, X_s, for several molecules in D_2O solutions.

found to be frequency independent over the range 100 KHz to 10 MHz in the study referenced above. This indicates that there is no absorption due to reorienting molecular dipoles below 10 MHz. Emphasis must be placed on the presence of inflection points in these curves. This is not the usual behavior in deviations from ideality.

In order to quantify some of these results and to compare them with similar work on ionic solutions it is helpful to define the quantity

$$B' = \frac{(\mathrm{d}[1/T_1]/\mathrm{d}C)_0}{(1/T_1)_0} \tag{1}$$

where $(\mathrm{d}[1/T_1]\mathrm{d}C)_0$ is the slope of a plot of relaxation rate $1/T_1$ *vs.* molal concentration, C, at low concentrations. $(1/T_1)_0$ is the relaxation rate for the pure solvent. Values derived from the data shown in Figures 1 and 2 are given in Table I along with some values from studies on simple electrolytes.[1,2]

Table I. B' Values for H_2O or D_2O Solutions of Various Species

Solute	B′	Mol wt	Source of data
$(C_3H_7)_4NBr$	1.80	186	*a*
$(C_2H_5)_4NBr$	1.03	130	*a*
$(CH_3)_4NBr$	0.69	74	*a*
DMSO	1.17	78	This work
t-BuOH	1.55	74	This work
Urea	0.33	60	This work
Acetone	0.33	58	This work
	Surfactants		
CF-10	0.50	1000[b]	This work
DF-16	0.50	600[b]	This work
Triton X-100	0.50	628[b]	This work
Mg^{2+}	0.50		*a*
CO_3^{2-}	0.25		*a*
$Hg(CN)_2$	0.13		*a*

[a] See ref 2. [b] Before micelle formation.

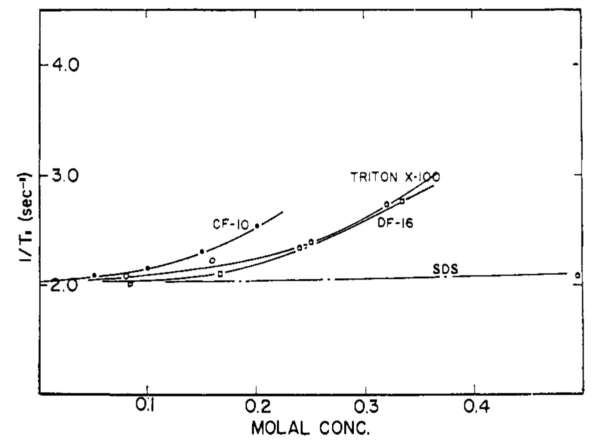

Figure 2. Relaxation rates *vs.* molal concentration for several surfactants in the low concentration range (D_2O solutions).

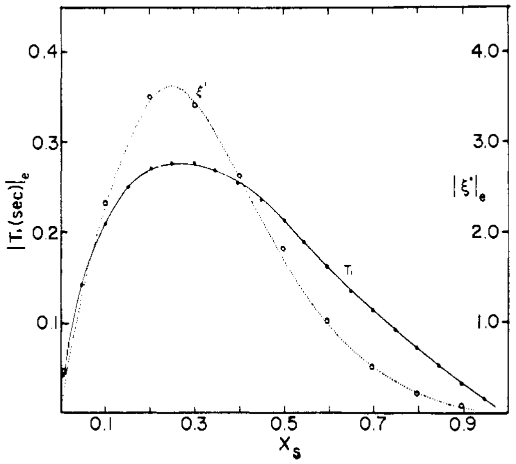

Figure 3. Absolute values of the excess relaxation time and real part of the dielectric constant *vs.* mole fraction of solute for DMSO–water solutions.

Discussion

One fact stands out from this and previous studies. In *no* case of a measurement on a solution of an organic molecule or cation has a relaxation time *longer* than for pure water been found. Hence, from a naive interpretation of these techniques there would result the conclusion that all such compounds "order" water. In fact, however, the form of the curves of T_1 *vs.* concentration does not fit, except in the very dilute range, the two-phase equation.[5,10,11] The equation assumes a rapid exchange between bulk D_2O molecules and a phase identified with the hydration layer on the solutes. This results in a general two-phase equation of the form[5]

$$(1/T_1)_{\mathrm{obsd}} = (1/T_1)_{\mathrm{bulk}}\left[1 - \frac{n_{\mathrm{H}}C}{50}\right] + \frac{3}{8}\left(\frac{e^2qQ}{\hbar}\right)^2 \frac{n_{\mathrm{H}}\tau_{\mathrm{c}}C}{50} \tag{2}$$

where n_{H} is the number of associated water molecules per molecule of solute, $e^2qQ/\hbar$ is the quadrupole coupling constant of the deuteron in the associated phase (assumed to be numerically equal to that in the bulk phase), and τ_{c} is the rotational reorientation time of water in the associated phase. The limiting case when $n_{\mathrm{H}}C/50 \ll 1$ is

$$(1/T_1)_{\mathrm{obsd}} = (1/T_1)_{\mathrm{bulk}} + \frac{3}{8}\left(\frac{e^2qQ}{\hbar}\right)^2 \frac{n_{\mathrm{H}}\tau_{\mathrm{c}}C}{50} \tag{3}$$

(10) J. Clifford, B. A. Pethica, and E. G. Smith, NATO Symposium on Membranes, Frascati, 1967.

(11) J. R. Zimmerman and W. E. Brittin, *J. Phys. Chem.*, **61**, 1328 (1957).

Therefore, the initial slope of a plot of $(1/T_1)_{\text{obsd}}$ *vs.* C has a slope

$$\frac{3}{8}\left(\frac{e^2qQ}{\hbar}\right)^2 \frac{n_{\text{H}}\tau_{\text{c}}}{50} = \frac{\text{d}(1/T_1)_{\text{obsd}}}{\text{d}C} \equiv B'(1/T_1)_{\text{bulk}} \quad (4)$$

From Figures 1 and 2 it is seen that this equation holds at low concentrations, but breaks down badly at higher concentrations. The breakdown at higher concentrations may be formally accounted for by a power expansion of τ_{c} as

$$\tau_{\text{c}} = (1 + \alpha C + \beta C^2 + \cdots)\tau_{\text{c}}^0 \quad (5)$$

giving

$$(1/T_1)_{\text{obsd}} = (1/T_1)_{\text{bulk}}\left(1 - \frac{n_{\text{H}}C}{50}\right) + \frac{3}{8}\frac{n_{\text{H}}C}{50}\left(\frac{e^2qQ}{\hbar}\right)^2 \tau_{\text{c}}^0 + \frac{\alpha 3 n_{\text{H}}C^2}{8\ \ 50}\left(\frac{e^2qQ}{\hbar}\right)^2 \tau_{\text{c}}^0 + \cdots \quad (6)$$

The exact physical meaning of the constants α, β . . . is not immediately clear. However, essentially the nonlinear terms represent the interaction between associated phases on each solute molecule. That is, deviations from linearity toward shorter relaxation times represent cooperative "ordering" of the solvent system, and *vice versa.*

From Table I it appears that the B' values for a given series of molecules or ions are roughly proportional to their molecular weight. This is to be expected from a Debye–Stokes–Einstein (DSE) treatment of the solute molecules where *their* reorientation times are given by

$$\tau_{\text{s}} = \frac{\eta_0}{RT}\frac{MW}{\langle P\rangle_{\text{m}}} \quad (7)$$

where η_0 is the viscosity of the solvent, MW is the molecular weight of the solute, and $\langle P\rangle_{\text{m}}$ is the average density of a solute molecule. This observation indicates that the associated molecules are in the vicinity of the solute molecules for many rotational reorientations of the latter. Thus, the water molecules assume the reorientation times of the solute molecules upon association.

It is concluded that at low concentrations the main effect observed is due to the fact that the organic solutes reorient more slowly than the smaller ions, and carry the associated water molecules along with them. At low concentrations, urea, for instance, has a very low B' value and hence a small $n_{\text{H}}\tau_{\text{c}}$ product. For relatively large τ_{c} this indicates that the number of waters hydrated is small and hence the interaction with water is not a long range phenomenon. For urea, the interaction at *any* concentration is weaker than for any of the other molecules studied, and is the same order of magnitude as for simple electrolytes Therefore, explanations of the denaturing properties of urea must lie elsewhere than in a change of the structure of the solvent. That is, an explanation in terms of the stability of denaturant–polymer–hydration complexes must be sought.

At high concentrations Figure 3 may be interpreted more easily on the basis of compound formation between DMSO and D_2O on the basis of the distinctive deviation from ideality[12] rather than the formal treatment given by eq 5 and 6. The molecular formula indicated is DMSO–$3D_2O$. This is in agreement with the dielectric constant data, but in disagreement with excess heat of mixing, density, and viscosity data[13] where the results indicate a DMSO–$2H_2O$ complex. This apparent disagreement may be resolved by reference to: (1) the thermodynamic phase diagram of DMSO–H_2O mixtures[14] and (2) the characteristic time domain of the various measurements. From (1) it is clear that there are two eutectic points. One is at DMSO–$3H_2O$ and the other is at DMSO–$2H_2O$. That is, the two compounds DMSO–nH_2O with $n = 2, 3$ are thermodynamically defined in the solid state. The solution measurements indicate that compound formation persists in the liquid phase. The excess value functions for all the measurements, including the present ones, have shapes which are too flat and asymmetric to resolve a mixture of the two compounds in solution. Reference to (2) means the following. The classical methods reflect the properties of the solution on a weighted-average basis over very long times compared to molecular processes in solution. It is proposed that the correct interpretation of all the experiments is that they are a result of a mixture of the complexes DMSO–$3H_2O$ and DMSO–$2H_2O$. The dielectric constant measurements carried out in the frequency range 10^6–10^7 sec^{-1} and the nmr experiments carried out at 10^7 sec^{-1} simply show that dominant rotational reorientation of the D_2O in the structural complexes in solution takes place with $\tau_{\text{c}} < 10^{-8}$ sec. However, they cannot exclude the existence of two or more complexes because the dominant reorientation time is the longest. Thus, in the extreme narrowing approximation, $\omega_0^2/\tau_{\text{c},i}^2 \ll 1$, the weighted contributions to the relaxation rate from species i are

$$(1/T_1)_{\text{obsd}} \propto \sum_i \alpha_i \tau_{\text{c},i} \quad (8)$$

If, for instance, the 3:1 complex has $\tau_{\text{c1}} = 10^{-9}$ sec, and the 2:1 complex has $\tau_{\text{c2}} = 10^{-11}$ sec, then for a 50:50 mixture of the two there is no 2:1 complex effect on the measured rate. However, there will be an effect on the classical properties since the system has the steady-state properties of a mixture. The proportions in the mixture cannot be determined directly from the magnetic resonance experiment since the $\tau_{\text{c}i}$ are not known.

The situation appears to be a classic example of the importance of taking into account the characteristic measurement time when interpreting experimental data. This is particularly true when comparing microscopic events, such as angular reorientation, to macroscopic measurements.

Final conclusions are therefore: (1) organic molecules and cations can best be described in solution by association of water molecules with their surfaces, and not by long-range effects on water structure.

(2) The association of water molecules with organic solutes of the type studied here is not especially strong compared with ionic effects at the same concentration. The observed effects on magnetic resonance relaxation in water are dominated by the longer times of reorientation of the larger solute molecules. Thus, the magnitude of the observed effect is very large in micelle suspensions of surfactants. Further papers will deal with detailed investigations of these latter effects.

(3) DMSO–H_2O solutions are composed of mixtures of molecular complexes dominated in the proper

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(12) J. A. Geddes, *J. Amer. Chem. Soc.*, **55**, 4832 (1933).

(13) J. M. G. Cowie and P. M. Toporowski, *Can. J. Chem.*, **39**, 2240 (1961).

(14) D. H. Rasmussen and A. P. Mackenzie, *Nature*, **220**, 1315 (1968).

concentration range by 1:2 and 1:3 complexes. The different physical measurements used in detecting their presence are, however, not equally sensitive to both species because of the characteristic times of the various methods.

(4) The biophysical activity of these compounds in promoting denaturation depends upon competing interactions with polymers. The competitions proposed are

$$\begin{gathered} H_2O + B^{+0-} \rightleftharpoons H_2O\cdot B^{+0-} \\ M^+,X^- + B^{+0-} \rightleftharpoons M^+,X^-\cdot B^0 \\ A + B^{+0-} \rightleftharpoons A\cdot B^{+0-} \end{gathered} \qquad (9)$$

where B^{+0-} represents the polymer in positively charged, uncharged, and negatively charged states, M^+, X^- are counterions, and A represents the solute which interacts with the polymer and with water. The assumption is that the conformation of B^{+0-} is stabilized or destabilized depending upon the proportions and nature of A.

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Participation of Water in Conformational Changes of Biopolymers as Studied by Deuteron Magnetic Relaxation

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Abstract: Deuteron magnetic spin-lattice relaxation experiments performed on D_2O solutions of various macromolecules are described and discussed. This technique allows the interaction of water with various conformations of biopolymers to be measured quantitatively. The molecules studied are: poly(L-glutamic acid), poly(L-lysine), poly(adenylic acid), poly(uridylic acid), poly(methacrylic acid), poly(vinyloxazolidinone methyl), and poly(vinylpyrrolidone). General classes of interactions are proposed on the basis of the results, and the effect of geometrical fluctuations of polymer conformation upon polymer–water interactions are discussed. It is concluded that the competing effects of counterion–polymer interaction (electrostatic) and polymer–water interaction (hydrogen bond) are of vital importance in mediating polymer conformation.

The phenomena associated with conformational transitions in biopolymers, such as polypeptides and polynucleotides containing ionizable groups, have been intensively studied. This is especially true of those such as poly(L-glutamic acid), poly(L-lysine), and poly(adenylic acid) which undergo helix–coil transitions in aqueous solutions. Various qualitative and quantitative explanations of these phenomena have been proposed[1] although most, for lack of information, neglect effects of solvent–polymer interaction.

In this paper experiments on D_2O solutions of the above-mentioned polymers and polymers I, II, III, and VII (see Figure 1), using the method of deuteron magnetic spin-lattice relaxation, are described.[2] The purpose of this is to provide information on the role of water structure in the stabilization or destabilization of the various polymeric tertiary structures encountered. This role has often been invoked to explain the properties of the polymers in solution. Unfortunately, it has been little investigated due to the scarcity or inapplicability of experimental methods. As previously described, nuclear magnetic resonance (nmr) relaxation methods in systems where quadrupole induced relaxation is dominant provide a powerful tool for such investigations.[3,4] The fundamental theory is based upon the fact that the spin–lattice relaxation time, T_1, for deuterons in D_2O is governed completely by the rotational reorientation time, τ, of the individual D_2O molecules (intramolecular relaxation). This is not true of proton relaxation. T_1 for pure D_2O is of the order of 0.5 sec at room temperature. The results in the following discussion are given in terms of the reciprocal of this, *i.e.*, $1/T_1$, which is the relaxation rate and is directly proportional to τ. For bulk D_2O it is therefore equal to about

$$1/T_1 = K\tau \qquad (1)$$

2 sec^{-1} at room temperature. When another species such as an ion, polymer, enzyme, or colloid is present in a solution of D_2O the molecules of bulk water collide with the species, spend an average lifetime, t_b, associated with it, and then return to the bulk phase. During their association with the dissolved species the rotational reorientation time of the associated D_2O molecules is no longer that of bulk water. It may be either longer or shorter than for bulk water. In the former case we say that the water interacting with the species is more "structured" than in bulk water, and in the latter case, "less structured." This is in analogy to the relative reorientation times in liquid water and in ice.

Special note should be taken of the difference between this and previous work on relaxation rates of protons, deuterons, and oxygen-17 in aqueous solutions of

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(1) T. M. Birshstein and O. B. Ptitsyn, "Conformations of Macromolecules," Interscience Publishers, New York, N. Y., 1966.

(2) J. A. Glasel, *Nature*, **220**, 1124 (1968).

(3) J. A. Glasel, *Proc. Nat. Acad. Sci. U. S.*, **58**, 27 (1967).

(4) J. A. Glasel, *Nature*, **218**, 953 (1968).