A NUCLEAR MAGNETIC RESONANCE STUDY OF HYDRATED SYSTEMS USING THE FREQUENCY DEPENDENCE OF THE RELAXATION PROCESSES

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ABSTRACT A practical method is described for determining some characteristics of the spectrum of proton mobilities in a hydrated system from the frequency dependence of the nuclear magnetic resonance (NMR) relaxation processes. The technique is applied to water in association with agarose and gelatin. The results for agarose are consistent with the hypothesis that a fraction of the protons is distributed over states of reduced mobility and exchanges rapidly with the remaining fraction which is attributed to water in the normal state. No variation in the characteristics of the modified fraction could be detected for water concentrations in the range 1.2–50 g H_2O/g agarose. Within the modified fraction, higher mobilities are more common than low mobilities; at 1.2 g H_2O/g agarose, not more than 10% of the proton population has mobilities more than 100 times smaller than normal. The modified proton fraction is tentatively identified with agarose hydroxyl protons and possibly water molecules bound to the polymer. Proton states with mobilities intermediate between water and ice have also been detected in hydrated gelatin. As in agarose, higher mobilities are the most common. In contrast to agarose, the characteristics of the modified proton states are markedly dependent on water concentration. They are tentatively attributed to gelatin protons coupled for spin-lattice relaxation with those of the bulk phase by exchange and spin diffusion.

INTRODUCTION

A number of proton NMR studies of hydration have been made by varying the sample temperature while the frequency of the radio frequency (RF) signal has been maintained constant (1, 2). With living tissue or heavily hydrated specimens, the possible temperature range is somewhat limited.

It is also possible to determine the distribution of proton mobility and, hopefully, water mobility, by keeping the temperature constant and varying the resonant frequency (3). In this report a practical method is described for analyzing the NMR relaxation dispersion data in terms of a continuum of nuclear correlation times. The technique is applied to observations of proton relaxation rates at three frequencies

(2.3, 8.9, and 30.0 MHz) for a hydrated polysaccharide (agarose) and a hydrated protein (gelatin) at 25 and 2°C.

MATERIALS

Gels of gelatin and agarose were prepared by the addition of distilled water and application of heat. For the agarose samples in which there was less than 10 g water/g agarose, the water and agarose were mixed thoroughly but not heated.

The water contents of the substances employed were determined by drying specimens of each to constant mass at 105°C *in vacuo*. The agarose was obtained from Seravac Laboratories Pty. Ltd. (Maidenhead, Berkshire, England) and bore the batch no. 173.

METHODS

Proton spin-lattice and spin-spin relaxation times T_1 and T_2 were determined on a pulsed NMR spectrometer operating at 2.3, 8.9, and 30 MHz. For T_1, 90°–90° pulse pairs were used. T_2 was determined directly from the free induction decay (FID) after a 90° pulse or by using 90°–180° or $90°\text{–}n \times 180°$ (Carr-Purcell) pulse sequences. Measurements on the FID were always made at least 100 μs after the application of the sampling pulse.

The number of protons contributing to the observed NMR signal was determined by comparing the height of the FID after a 90° pulse, extrapolated back to the time of the pulse, with the output obtained when a calibrated RF current was injected into the sample coil circuit. This procedure was designed to eliminate errors caused by variations of the Q factor of the sample coil.

RESULTS

The results of the relaxation time measurements are presented in Table I. T_1 is the spin-lattice and T_2 the spin-spin relaxation time.

The heights of the signals from the agarose samples were found to be within ±10% of the values expected from the water protons alone. Solvent spin-spin and spin-lattice relaxation followed a single exponential decay. The fast decaying polymer signal was not examined because of the long receiver recovery time.

For gelatin, if the water content was greater than 75%, the observed relaxation followed a single exponential, and the FID height at $t = 0$ corresponded to the water content. When the water content was 50% or less, however, the signal was 20 ±10% larger than expected, and spin-spin relaxation was not exponential, 10–30% of protons appearing in a fast decaying fraction with $T_2 < 4$ ms. The remainder of the spin-spin relaxation in each sample was well described by a single, longer relaxation time. It is these longer times that are listed in Table I. Spin-lattice relaxation in the samples discussed here was always exponential within the accuracy of measurement.

The standard error of the T_1 data is ±5%. Operating conditions were such that high accuracy in T_2 was not obtained using the Carr-Purcell sequence. T_2 estimates were therefore made using 90°–180° pulse pairs or directly from the free induction decay. As noted by Hansen (4), these methods can lead to underestimation of T_2

TABLE I

PROTON RELAXATION TIMES (ms) IN HYDRATED AGAROSE AND GELATIN

stance	Sample no.	Water content ($g\ H_2O/g$ dry matter)	Relaxation times											
			25°C						2°C					
			2.3		8.9		30		2.3		8.9		30	
			T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
	A1	0.25	—	—	32	0.39	67	0.3	—	—	27	0.3	61	—
	A2	1.20	46	1.8	99	1.8	193	1.8	36	3	74	2.8	133	3.0
ose	A3	9.0	330	13	610	14	890	—	250	24	430	24	620	23
	A4	15.7	500	22	950	21	1250	—	365	36	560	38	780	—
	A5	50	1110	68	1600	66	1960	—	730	105	1030	109	1130	—
	G1	0.40	10	2.5	30	2.5	54	2.6	—	—	—	—	—	—
in	G2	1.00	67	21	116	22	138	19	—	—	—	—	—	—
	G3	4.13	460	200	640	250	870	—	260	170	345	163	420	—

because of diffusion in local magnetic field gradients. The following facts render it unlikely that such effects have introduced errors here.

(*a*) Echo widths were always similar to those from water samples of the same volume. Where necessary T_2 values were corrected for diffusion by assuming the effect on the echo decay to be similar to that seen with water samples. Walter and Hope (5) have shown that the self-diffusion coefficient of water in the dilute gels to which this correction applies is very close to the bulk value. Under the conditions of these experiments, the correction to T_2 was never greater than 10%.

(*b*) After correction as described in *a* the echo decay was exponential.

(*c*) If the local field gradients arose from inhomogeneities in magnetic suscep- ibility in the sample, the apparent reduction in T_2 should have been greatest when he static magnetic field strength was largest, i.e., at the highest resonant frequency. o significant trend for T_2 to decrease with frequency was observed.

(*d*) T_2 measured by the Carr-Purcell technique was usually within 10% of the 0°–180° value corrected as in *a*.

In view of the above, the T_2 data have been assigned a probable uncertainty of ±10%.

DISCUSSION

Bloembergen et al. (6) have shown that if all the protons have the same correla- on time τ, and if proton-proton magnetic interactions dominate the relaxation rocess, then the relaxation rates are given by

$$T_1^{-1} = \frac{2\sigma_0^2}{3}\left[\frac{\tau}{1+\omega_0^2\tau^2} + \frac{4\tau}{1+4\omega_0^2\tau^2}\right], \tag{1}$$

$$T_2^{-1} = \sigma_0^2\left[\tau + \frac{5\tau}{3(1+\omega_0^2\tau^2)} + \frac{2\tau}{3(1+4\omega_0^2\tau^2)}\right] \quad \text{for} \quad \tau \ll (2/\pi\sigma_0^2)^{1/2},$$

$$= (2\sigma_0^2/\pi)^{1/2} \quad \text{for} \quad \tau \gg (2/\pi\sigma_0^2)^{1/2}, \tag{2}$$

where ω_0 is the resonant angular frequency; τ is the correlation time (assumed the same for rotation and translation); and σ_0^2 is the rigid lattice second moment, which equals $2.6 \times 10^{10}\ \text{s}^{-2}$ for ice (6) and $1.57 \times 10^{10}\ \text{s}^{-2}$ for isolated water molecules (7) and is thought to be about 1.6 times larger in bulk water (8). In structurally modified water, σ_0^2 is not likely to differ from the bulk water value by more than a factor of 2.

Fig. 1 illustrates the form of Eqs. 1 and 2. In free water, $\tau \sim 3.10^{-12}$ s (9) and $T_1 = T_2 \sim 3.5$ s. In ice, $\tau \sim 10^{-5}$ s, T_1 is large, and T_2 is of the order of microseconds.

The data in Table I ($T_1 \sim 500$ ms, $T_2 \sim 10$ ms) are inconsistent with the assumption of a single correlation time, since this would have to be near 10^{-6} s, for which value $T_2 \sim 10\ \mu$s. Therefore a distribution in correlation times, $p(\tau)$ must be considered (10) when,

$$T_1^{-1} = \frac{2\sigma_0^2}{3}\int p(\tau)\left(\frac{\tau}{1+\omega_0^2\tau^2} + \frac{4\tau}{1+4\omega_0^2\tau^2}\right)\mathrm{d}\tau, \tag{3}$$

$$T_2^{-1} = \frac{\sigma_0^2}{3}\int p(\tau)\left(3\tau + \frac{5\tau}{1+\omega_0^2\tau^2} + \frac{2\tau}{1+4\omega_0^2\tau^2}\right)\mathrm{d}\tau + R_2, \tag{4}$$

with

$$\int p(\tau)\,\mathrm{d}\tau = 1. \tag{5}$$

In Eqs. 3, 4, and 5 the integration is only over those states for which there is rapid exchange either physically or (for T_1 only) by spin diffusion with the bulk of the water proton population. The integration ranges for T_1 and T_2 need not coincide (11). The term R_2 in Eq. 4 represents a contribution to the observed spin-spin relaxation rate from transfer to states which are not in rapid exchange, i.e., states for which the residence time is not small compared with the corresponding relaxation time (Eq. 2). The corresponding term for spin-lattice relaxation is expected to be negligible and has been omitted from Eq. 3. The practical significance of R_2 will be assessed later.

In the following, σ_0^2 is taken to be $2.5 \times 10^{10}\ \text{s}^{-2}$. Should this value be inappropriate, quantitative but not qualitative errors will be introduced. We now wish to determine what distribution $p(\tau)$ is consistent with the data of Table I. A model frequently considered (5, 12, 13) is a two-state distribution:

$$p(\tau) = x\delta(\tau - \tau_1) + (1 - x)\delta(\tau - \tau_2), \tag{6}$$

in which there are two fractions in proportions x and $(1 - x)$ with correlation times τ_1 and τ_2, respectively. Normally one of these fractions corresponds to free water

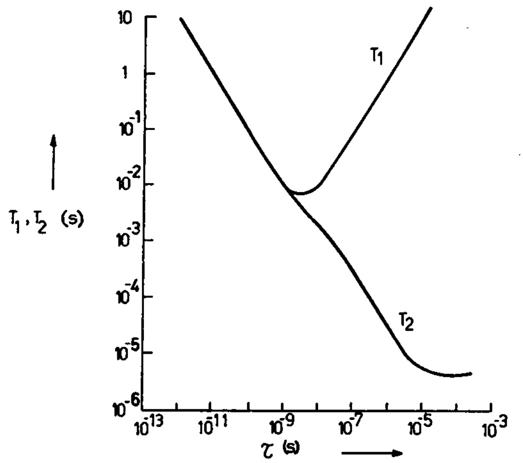


FIGURE 1

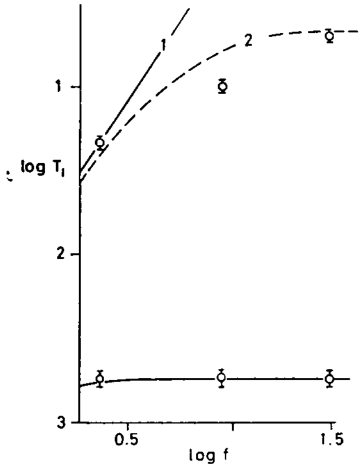


FIGURE 2

FIGURE 1 The relationship between relaxation time and nuclear correlation time τ described by Eq. 1. $\sigma_0^2 = 2.5 \times 10^{10}\ \mathrm{s^{-2}}$, $\omega_0 = 1.88 \times 10^8$ rad $\mathrm{s^{-1}}$.

FIGURE 2 The T_1 and T_2 data for sample A2 at 25°C fitted by a two-state distribution (Eq. 6) where $x = 0.019$, $\tau_1 = 10^{-6.4}$ s, and T_1 in the "free" fraction $= 2.5$ s (curve 1) or 0.25 s (curve 2). f is the resonant frequency (14Hz).

with $\tau \sim 10^{-11}$ s, but even if this restriction is relaxed, Eq. 6 is not consistent with the data in Table I. This is illustrated in Fig. 2, where the values of x, τ_1, and τ_2 have been chosen to fit the data for sample A2 at 2.3 and 30 MHz, at 25°C.

Alternatively, a continuous distribution in τ may be considered, satisfying the following requirements. (*a*) The nuclei are divided into two phases, a fraction x in a modified form and the remainder unmodified. (*b*) Nuclei in the free phase have a common correlation time τ_2, and corresponding relaxation times $T_{1(\mathrm{free})}$ and $T_{2(\mathrm{free})}$. (*c*) As a trial, the following form for $p(\tau)$ is assumed:

$$p(\tau) = x\frac{A}{\tau}\left(\log_{10}[\tau/\tau_2]\right)^a + (1-x)\delta(\tau-\tau_2), \qquad (7)$$

the two terms relating to the modified and free components, respectively. In the first term, A is a normalizing constant such that

$$A\int_{\tau'}^{\tau''}\left(\log[\tau/\tau_2]\right)^a\frac{d\tau}{\tau} = 1. \qquad (8)$$

τ' and τ'' are the limits of the τ distribution. $\tau_2 < \tau' < \tau''$. $p(\tau)$ is zero outside the range τ', τ'' except for the δ function at τ_2 for the unmodified component.

Since the first term in Eq. 7 corresponds to a power distribution in log τ, the distribution 7 will be referred to as the bimodal log power (BLP) τ distribution. In arriving at this form for $p(\tau)$ the following points were taken into consideration.

Because of the form of Eq. 1 (see Fig. 1) most of the information from T_1 measurements relates to τ values in the range $0.6/\omega_0\text{min}–0.6/\omega_0\text{max}$ ($3.4 \times 10^{-8}–3.1 \times 10^{-9}$ s in the present work). Similarly, the properties of Eq. 2 (see Fig. 1) and Eq. 12 below ensure that the most heavily weighted contributions to T_2^{-1} come from states near the fast exchange cutoff, which occurs at $\tau < 10^{-5}$ s. Thus, results obtained by the frequency scan method can be expected to reflect properties of $p(\tau)$ in the range $10^{-9} < \tau < 10^{-5}$ s. Since $\tau_2 \sim 10^{-11}$ s, the restriction $\tau > \tau_2$ should not impair the ability of the BLP distribution to fit the NMR data. Nevertheless, if this is achieved it must be remembered that relaxation is insensitive to τ values much less than $1/\omega_0\text{max}$ and does not rule out the existence of such states.

Our choice of the BLP distribution was also influenced by the further points. (*a*) In heavily hydrated systems it is reasonable to expect the τ distribution to be skewed in favor of free water. (*b*) The BLP distribution is consistent with a model in which the activation energy for rearrangement of water molecules is assumed to follow a power law of distance from perturbing centers. (*c*) The number of degrees of freedom of $p(\tau)$ should be as large as possible consistent with a manageable curve-fitting procedure. Properties of $p(\tau)$ determined by the data are then less likely to be confused with those imposed by the definition.

A Systematic Technique for Fitting the BLP Distribution to NMR Data

In the distribution, Eq. 7, after τ_2 has been assigned experimentally, four adjustable parameters x, a, τ', and τ'' remain. We assume that $a \leq 0$ and that $\tau' \leq 10^{-8}$ s. These are not severe restrictions since if $a > 0$ or $\tau' > 10^{-8}$ s, the behavior of the model approaches that of the two-phase model for $f > 2$ MHz. It is found that T_1 is effectively independent of τ''. Hence since T_2 varies only slightly with frequency, τ'' may be adjusted to fit the T_2 values after the other parameters have been fitted to the T_1 data.

Optimization of the four parameters is performed graphically. The procedure is outlined here. As indicated above, T_1 is determined by three of the parameters, (a, τ', x). Since

$$\frac{1}{T_1} = \frac{x}{T_1\,(\text{mod})} + \frac{(1-x)}{T_1\,(\text{free})}, \tag{9}$$

then

$$\frac{1}{T_1} - \frac{1}{T_1\,(\text{free})} = x\left[\frac{1}{T_1\,(\text{mod})} - \frac{1}{T_1\,(\text{free})}\right]. \tag{10}$$

Thus by taking the ratio r of the values on the left-hand side of Eq. 10 at 30 and 2.3 MHz, we have a variable which depends on only two of the parameters (a and τ'). Fig. 3 shows plots of r as a function of a and τ'.

From the observed value of r, a pair (a, τ') is determined and to this set the corresponding value of x is added from the value of T_1 at 2.3 MHz (Eq. 9). The set is

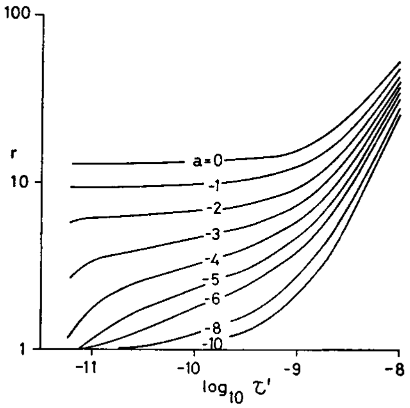


FIGURE 3 r (Eq. 9) as a function of τ' and a (Eq. 7) $\tau_2 = 10^{-11.32}$ s and $\tau'' = 10^{-5.5}$ s.

discarded if $x > 1$. This is repeated for a number of sets (a, τ', x). Each set retained gives the correct value of T_1 at 2.3 and 30 MHz, by definition. The choice between the sets is made by selecting that one which gives the best fit at one or more intermediate frequencies. Finally τ'' is found from the T_2 data.

The BLP Distribution Applied to Hydrated Agarose

T_1 was measured in nondegassed water and τ_2 was set equal to the most appropriate values for which curve-fitting diagrams were available: $10^{-11.8}$ s at 25°C and $10^{-11.1}$ s at 2°C. The corresponding values of T_1 are 2.5 and 1.6 s compared with the measured values 2.6 ±0.3 and 1.3 ±0.3 s. A more accurate procedure for selecting τ_2 is not warranted; any consequent error is completely submerged in the uncertainty relating to the number of states with $\tau \leq 10^{-9}$ s.

Relaxation times for the more dilute agarose samples can be accurately predicted from those of sample A2 by supposing the additional water is in the free state, as seen in Fig. 4. This confirms the suitability of the chosen values and implies that the curve-fitting procedure need only be applied to samples A1 and A2. Within the experimental accuracy, the population of modified proton states per gram of agarose is constant in samples A2–A5. Fig. 5 shows a representative group of curves fitted to the T_1 data for sample A2. The likely limits for the set (a, τ', x) at 25°C are $(-2, 10^{-11.2}, 1.0)$ to $(-6, 10^{-8.9}, 0.05)$ and at 2°C are $(-2, 10^{-11}, 1.0)$ to $(-6, 10^{-8.9}, 0.07)$.

At the lower temperature, the minimum value of x is slightly larger, but otherwise the results at 25° and 2°C are very similar. The minimum value of x is higher than the estimate of bound protons obtained by Woessner and Snowden (14) for agar gels, but their calculation was based on the assumption that T_1 was determined by bound protons with a single correlation time. The distributions $p(\tau)$ fitted to the 25°C data are illustrated in Fig. 6. As expected there are considerable variations

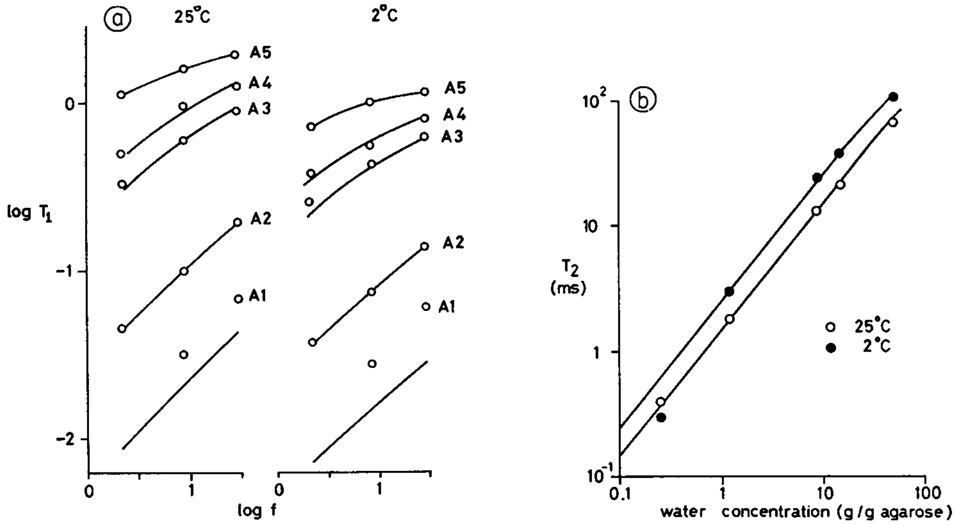
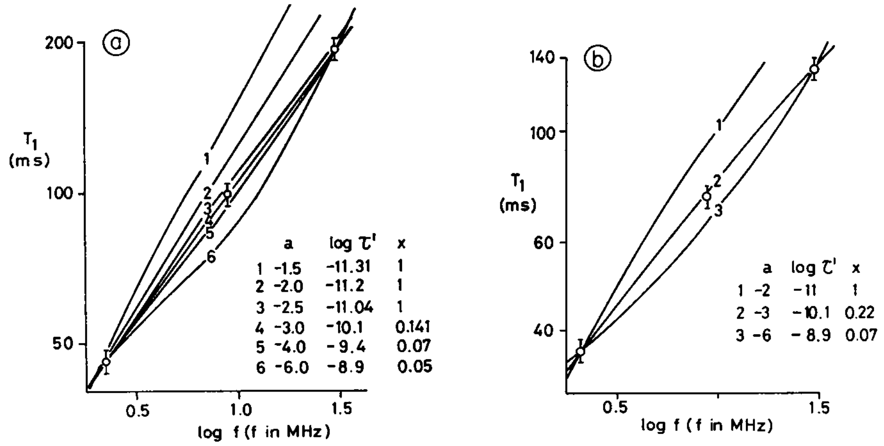


Figure 4 Proton relaxation times in hydrated agarose. The theoretical lines were calculated from the data of sample A2 by assuming that water added or removed was in a normal state with $T_1 = 2.5$ s at 25°C and 1.4 s at 2°C.



	a	log τ'	x
1	-1.5	-11.31	1
2	-2.0	-11.2	1
3	-2.5	-11.04	1
4	-3.0	-10.1	0.141
5	-4.0	-9.4	0.07
6	-6.0	-8.9	0.05

	a	log τ'	x
1	-2	-11	1
2	-3	-10.1	0.22
3	-6	-8.9	0.07

Figure 5 The T_1 data for sample A2 fitted by the BLP distribution (Eq. 7). (*a*) 25°C; (*b*) 2°C.

among $p(\tau)$'s that are consistent with the T_1 data, but there is a general trend for $\log p(\tau)$ to increase as $\log \tau$ goes from -7.5 to -9 and the increase may persist past $\log \tau = -10$. Unless there is a significant number of states with $\tau \ll \tau_2$, no more than 6% of proton states have $\tau > 10^{-9}$ s.

A further point emerges when the T_2 data are considered: at 25°C, the observed relaxation time is smaller than predicted. The smallest value of T_2 consistent with the BLP distributions fitted to the T_1 data can be calculated as follows. The integral

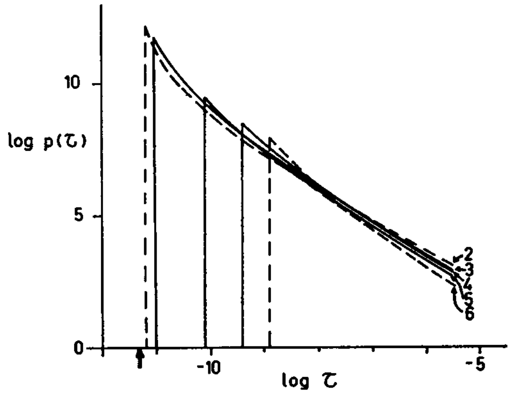


Figure 6 The distributions of nuclear correlation times in the modified fractions of the BLP distributions fitted to the T_1 data for sample A2 at 25°C. Curves numbered as in Fig. 5 *a*. The free fractions would be represented by delta functions at $\tau = 10^{-11.32}$ s (indicated by the arrow).

term in Eq. 4 accurately summates relaxation contributions coming from states where the residence time τ_{res} is much less than the corresponding T_2, as given by Eq. 2. If applied to states with longer τ_{res}, however, it overestimates the relaxation rate, since exchange rate limitations are neglected.

A more accurate expression in the case of a state b with long τ_{res} is (adapted from reference 15, Eq. 12):

$$R_2 = p_b[T_{2b} + \tau_{\text{res}}]^{-1} \text{ (assuming } p_b \ll 1), \tag{11}$$

where p_b is the population of state n expressed as a fraction of the total rapidly exchanging proton population, and T_{2b} is the relaxation time, obtained from Eq. 2 by inserting the appropriate nuclear correlation time τ_b. On equating residence time (τ_{res}) and nuclear correlation time (τ), Eq. 11 leads to a more general expression for the resultant spin-spin relaxation rate. The terms on the right-hand side of Eq. 4 are replaced by a single integral:

$$T_2^{-1} = \int_0^{\infty} p(\tau)[T_2(\tau) + \tau]^{-1}\,\mathrm{d}\tau, \tag{12}$$

where $\int p(\tau)\,\mathrm{d}\tau = 1$ (integration over rapidly exchanging states) and $\int_0^{\infty} p(\tau)\,\mathrm{d}\tau$ is not much greater than 1, and $T_2(\tau)$ is defined by Eq. 2. Because of the approximation $\tau_{\text{res}} = \tau$, and the employment of Eq. 2 for $T_2(\tau)$ in the transition region $\tau \sim \sigma_0^{-1}$, Eq. 12 tends to underestimate T_2.

The minimum values of T_2 consistent with the BLP distributions fitted to the agarose T_1 data can be calculated from Eq. 12. At 2°C they are comparable with the observed values, but at 25°C they are too large (by factors of $\gtrsim 2$ for the three best fitting curves in Fig. 5). As noted earlier, Eq. 12 gives heaviest weight to states with

$\tau \sim \sigma_0^{-1} \approx 10^{-5.2}$ s. Thus at 25°C the population of states with $\tau \sim 10^{-5}$ s is probably higher than the BLP model predicts.

Consistent with this is the fact that T_2 was observed to be longer at 2°C than at 25°C. This suggests that at 25°C there is a local peak in $p(\tau)$ at $\tau \sim 10^{-5.2}$ s which at 2°C has shifted to longer τ and consequently has a much diminished influence on $T_{2\text{obs}}$.

Identification of the Modified Proton States in Hydrated Agarose

The measured spin-lattice relaxation times are determined by the water protons and any other protons coupled to them by rapid exchange or by spin diffusion (16). We believe that the contribution from spin diffusion is negligible for the following reasons:

(*a*) As shown in Table II, T_1 for deuterons in deuterated water/agarose samples s much shorter than in free D_2O. A similar result was found by Child et al. (17). Spin diffusion would not be effective for deuterons on account of their small magnetic moment, so this indicates the existence of some other relaxation mechanism for water deuterons and hence for water protons.

(*b*) Consideration of the short spin-spin relaxation times in nonexchanging agarose protons (mostly <100 μs) and the composition of agarose (18) suggests that it contains few rotationally mobile but not rapidly exchanging protons which could act as relaxing centers in the way that methyl protons do (16).

(*c*) The relaxation times are directly related to the concentration of water protons (Fig. 4) and not of water plus polymer protons, suggesting that the bulk of the polymer proton spins are not strongly coupled to the water proton spins.

Since spin diffusion appears not to be the dominant process, the modified proton

TABLE II

PROTON AND DEUTERON SPIN LATTICE RELAXATION TIMES (ms) IN PARTIALLY DEUTERATED AGAROSE AND GELATIN SAMPLES AT 25°C

Sample	% D_2O* in added water	Water g/g polymer	^{2}H	^{1}H	
			2.3 MHz	8.9 MHz	30 MHz
D_2O	98	—	450	—	—
Agarose	98	3.16	27	—	—
	98	1.07	—	103	225
	75	7.0	—	500	770
Gelatin	98	0.98	14	~70‡	~85‡
	75	3.03	108	360‡	420‡

* %D_2O in water of prepared samples would be lower, because of exchange with some polymer protons.

‡ Relaxation nonexponential. Tabulated value refers to slow component.

states would have to represent modified water or rapidly exchanging agarose protons. The water proton T_1 changed very little when 75% of the water was deuterated (Table II). A large increase should have been observed if the relaxation was caused principally by interactions between water protons. Water-water interactions have also been found to be unimportant for solvent proton spin-lattice relaxation in agar gels (14). Thus the observed spin-lattice relaxation cannot be attributed to the presence of bound water, unless perhaps the bound water was in intimate contact with nonexchanging polymer protons.

The majority of the modified proton states are not associated with the various acid groups which are present in agarose since these are present in agar in much higher concentration (18) whereas we have observed very similar spin-lattice relaxation rates both in agar and in agarose. This result is not unexpected. Even in agar only a few percent of the residues are acidified, mainly with sulphonate groups. Allowing two or three water molecules per sulphonate ion, there would be of the order of 10^{-2} g of electrostricted water/g agar. The present spin-lattice relaxation measurements would not have detected such a small difference between agar and agarose.

A reasonable, although not the only possible, interpretation of the data is that the T_1 dispersion analysis is qualitatively correct and that the inferred modified proton states are associated with rapidly exchanging agarose hydroxyl protons and possibly water molecules intimately bound to the polymer. This conforms with the suggestion of Woessner and Snowden (14) that T_1 relaxation in agar gels is dominated by exchanging hydroxyl protons.

It has been assumed throughout the foregoing that nuclear magnetic interactions dominate the relaxation process. In partial justification for this assumption, it may be noted that the reduction in T_1 for deuterons in agarose water is not consistent with causation by paramagnetic materials as these would have had a far greater effect on the proton relaxation than was observed.

Application of the BLP Distribution to Hydrated Gelatin

In contrast to agarose, the spectrum of modified proton states in hydrated gelatin was dependent on the water content. The samples were therefore analyzed individually.

Results of applying the curve-fitting procedure for the BLP distribution are illustrated in Fig. 7 and summarized in Table III. There is a trend for a to become more negative as the water content increases. From the T_2 data, $\tau'' \sim 10^{-5.5}$ s, consistent with fast exchange ceasing when $\tau \sim T_2$ in Eq. 4.

Identification of the Modified Proton States in the Gelatin Samples

T_2 decay in the gelatin samples was multiphasic and the height of the long T_2 component corresponded well with the water content. We attribute the fast component

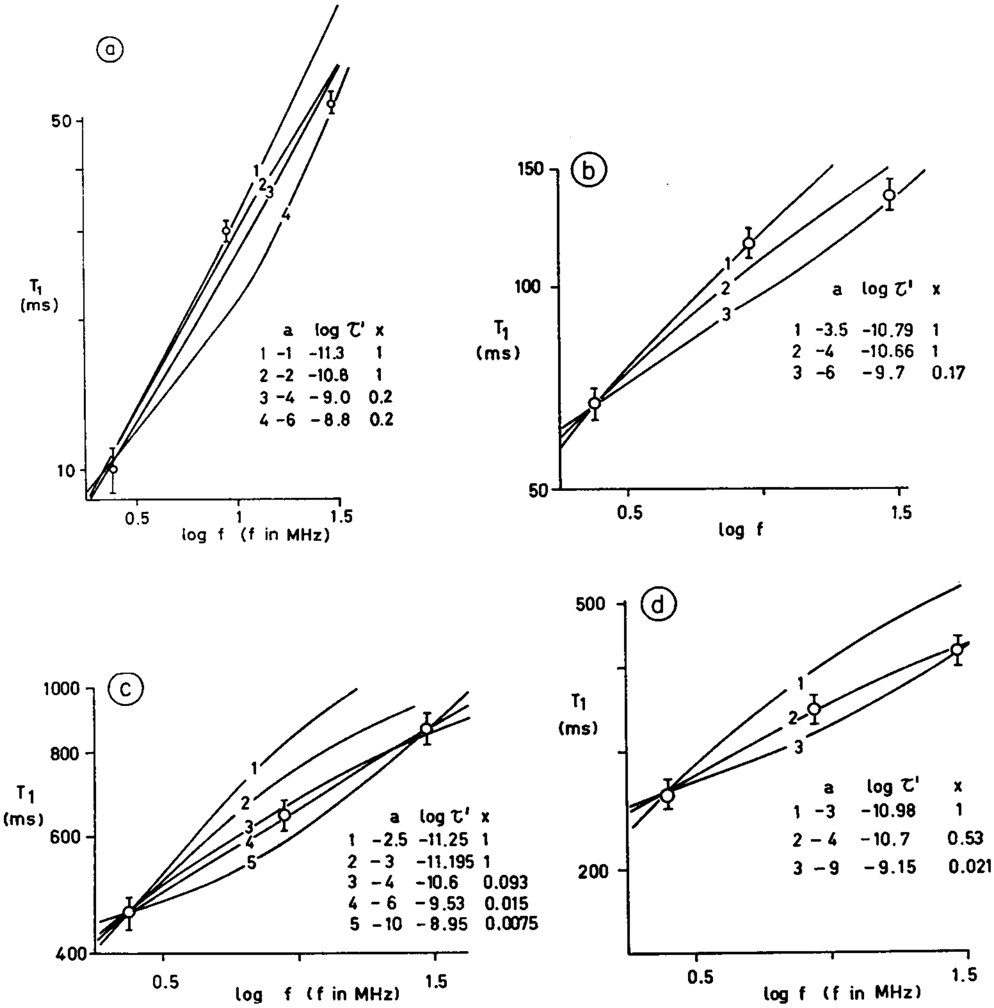


FIGURE 7 T_1 data for the gelatin samples fitted by BLP distributions (*a*) G1 at 25°C, (*b*) G2 at 25°C, (*c*) G3 at 25°C, (*d*) G3 at 2°C.

TABLE III

PARAMETERS (a, τ', x) FOR GELATIN SAMPLES AT 25°C

Sample no.	Water content g H_2O/g gelatin	a	log τ'	x
G1	0.4	−1 to −4	−11 to −9	0.2 to 1
G2	1.0	−4 to −6	−11 to −9.7	0.2 to 1
G3	4.13	−4 to −10	−11 to −9	0.008 to 0.1

to rotationally mobile gelatin protons which do not exchange rapidly with those of the bulk water phase.

Kimmich and Noack (3) assert that in gelatin gels protein and solvent protons share a common T_1 value unless the water is partially deuterated. In deuterated samples the proton spin-lattice relaxation is nonexponential. In nondeuterated samples, the results indicate that the protein and solvent protons are coupled by relaxation-limited spin diffusion. Kimmich and Noack interpret the nonexponential relaxation in the deuterated samples in terms of an impairment of the coupling process. We have also observed nonexponential proton spin-lattice relaxation in samples containing heavily deuterated water. The fast T_1 components are associated with the fast T_2 components already attributed to ratationally mobile protein protons. Longer T_1 components are attributed to solvent protons. Their relaxation times (Table II) are of the same order as in the nondeuterated samples, suggesting that water proton interactions do not dominate the relaxation.

T_1 for water deuterons is substantially reduced in the presence of gelatin (Table II) although to a lesser degree than the proton T_1. The small deuteron T_1 is probably due to exchange or water binding. All these data are consistent with the following model. Exchanging protein protons or possibly water molecules intimately bound to the polymer are couped by relaxation-limited spin diffusion to the remaining polymer protons.

Eq. 3 remains qualitatively valid if $p(\tau)$ includes the exchange and spin diffusion-coupled protons as well as the solvent protons. We identify a significant fraction of the modified proton states deduced from the T_1 dispersion analysis with the rotationally mobile protons responsible for the observed fast T_2 components. From Fig. 1 it is evident that states with τ between 10^{-8} and 10^{-9} s would give T_2 values in the required range (1–10 ms) if $\sigma_0^2 \sim 2.5 \times 10^{10}$ s^{-2}.

CONCLUSIONS

From the results of studies of the frequency dependence of proton NMR relaxation reported in this paper the following conclusions may be drawn.

(*a*) The results are not consistent with the assumption of a population of protons with a single correlation time, nor with two fractions with different correlation times.

(*b*) The population of protons in fast exchange with water protons in agarose hydrated with 1 g H_2O/g agarose apparently included a fraction which contains 10% or more of the protons and possesses a distribution of correlation times extending from $< 10^{-9}$ to $> 10^{-6}$ s. Not more than 10% of the protons are in states with correlation time greater than 10^{-9} s.

(*c*) The distribution of correlation times in the modified proton fraction is weighted towards low values.

(*d*) The properties of the modified proton states are, within the accuracy of measurement, independent of hydration level within the range 1.2 g H_2O to 50 g H_2O/g agarose.

(*e*) The modified proton fraction is not associated with the various acid groups which are present in agarose but in much lower concentrations than in agar.

(*f*) The majority of the modified proton states in the agarose samples are tentatively attributed to rapidly exchanging agarose hydroxyl protons and possibly water molecules bound to the polymer.

(*g*) The spectra of modified proton states in hydrated gelatin peak more sharply towards lower τ, as the water content is increased.

(*h*) The number of modified proton states detected per gram of gelatin is a maximum when the water content is near 1 g H_2O/g gelatin.

(*i*) Exchange with gelatin protons is likely to have contributed significantly to the modified proton states seen in the gelatin samples.

(*j*) The distribution $p(\tau)$ defined by Eq. 7 (BLP distribution) described the agarose data well and the gelatin data adequately. The number of degrees of freedom was sufficiently large, to permit meaningful estimations of the variability in $p(\tau)$ consistent with the data. It should be stressed however that the data do not *imply* that $p(\tau)$ was of the form of Eq. 7.

The authors are indebted to Mr. K. H. Marsden for several helpful discussions.

Received for publication 10 April 1972 and in revised form 19 July 1972.

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