WATER IN AGAROSE GELS STUDIED BY NUCLEAR MAGNETIC RESONANCE RELAXATION IN THE ROTATING FRAME

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ABSTRACT The dependence of the spin-lattice relaxation time in the rotating frame ($T_{1\rho}$) on radio frequency (RF) field strength and temperature has been studied for agarose gels in order to investigate molecular motion. The results indicate the presence of slow motions with a correlation time of ca. $5 \cdot 10^{-6}$ s at room temperature. This interaction is responsible for the short spin-spin relaxation times (T_2) for water protons in agarose gels and is ascribed to firmly bound water. The fraction of bound water is estimated to about 0.003 for a 7.3% agarose gel. The motion of the more mobile protons in agarose-water systems can not be characterized by single correlation time. This fraction is presumably composed of water in different motional states and some of the agarose hydroxyl protons. Higher mobilities are the most common.

INTRODUCTION

Numerous nuclear magnetic resonance (NMR) studies have been undertaken to investigate the role of water in biological systems. In the majority of these investigations, the nuclear relaxation times, T_1 and T_2, have been measured. Agarose gels constitute one of the systems for which T_2 for the protons is much shorter than T_1. The observations of $T_2 < T_1$ in such systems is usually interpreted in terms of two or more motional states for the water. To obtain more detailed information concerning the distribution of motional states, the frequency dependence of T_1 for protons (1, 2) and deuterons (1) in agarose gels were measured. Since the frequency dependence of T_1 values determined on commercial spectrometers is sensitive to relatively rapid motions characterized by correlation times in the range 10^{-10}–10^{-8} s, the slower motions were estimated indirectly from the measurements of T_2.

In the present work, the proton spin-lattice relaxation time in the rotating frame ($T_{1\rho}$) for water in agarose gels has been studied as a function of temperature and RF field strength (H_1). The dependence of $T_{1\rho}$ on H_1 is sensitive to relaxation producing interactions possessing correlation times longer than $\simeq 10^{-7}$ s and can thus help in interpreting the factors influencing relaxation times for protons in biological systems. A similar study of the dispersion of $T_{1\rho}$ for protons in tissues has recently been reported (3).

MATERIALS AND METHODS

Preparation of Samples

The gelling component of agar, agarose, was obtained as a powder (electrophoresis grade) from BDH Chemicals (Poole, England) and used without further purification. Gels were prepared in glass tubes (6 mm OD) by adding deionized distilled water to agarose powder without degassing. After preparation, the sample tubes were sealed and homogeneity of the gel was achieved by placing the tubes in boiling water. The dispersion time varied with agarose concentration but was in all cases between 15 and 30 min. Samples were then left to age at room temperature for about 24 h to attain reproducible gel strengths.

Bovine serum albumin (BSA) was purchased from Sigma Chemical Co. (St. Louis, Mo.) (essentially fatty acid free) and used without further purification.

NMR Measurements

NMR measurements were carried out on a Bruker B-KR 322 s spectrometer (Bruker Scientific Inc., Elmsford, N.Y.). The proton spin-lattice relaxation times (T_1) were measured using a $180° - \tau - 90°$ pulse sequence. The T_2 relaxation times for ^1H were determined from a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with the spacing between the 180° pulses t_{cp} = 1 ms.

The values of $T_{1\rho}$ were measured on exact resonance from amplitudes of the free-induction decay following the second pulse in a conventional two pulse sequence (4). The radio frequency

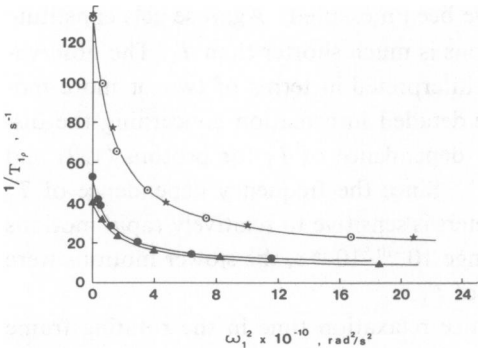

FIGURE 1

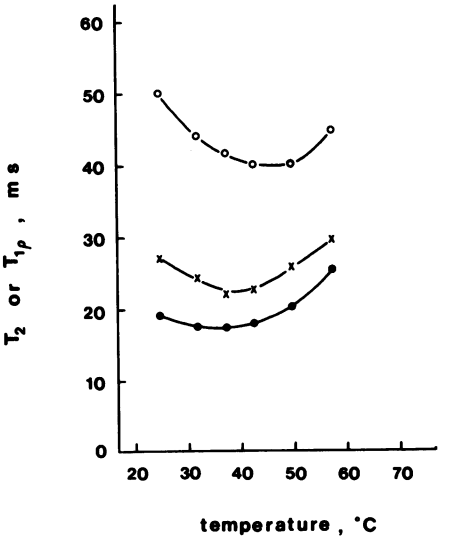

FIGURE 2

FIGURE 1 The proton $1/T_{1\rho}$ obtained at 61 MHz vs. ω_1^2 for 5.75% (- ▲ -), 7.3% (- ● -), and 19% (- ○ -) agarose gels. The lines have been calculated according to Eq. 4 with the parameters from Table I. Measurements performed at 39 MHz for a 19% agarose gel (- ⋆ -) are included in the figure.

FIGURE 2 The temperature dependence of T_2 (- ● -), $T_{1\rho}$ at $H_1 = 4$ G (- × -) and $T_{1\rho}$ at $H_1 = 8.1$ G (- ○ -) for a 7.3% agarose gel.

field H_1 was adjustable between 2.5 and 18 G and measured from the duration of pulses of length $n\pi$ for a number of integer values of n using the expression

$$\alpha = \gamma H_1 t_\alpha,$$

where t_α is the duration of pulse of length α and γ is the magnetogyric ratio. The uncertainty in the determination of the relaxation times from straight-line plots of magnetization decays was ca. 5%; additionally, approximately a 5% uncertainty in the RF field strengths exists.

The temperature of the samples was kept constant to ± 0.5°C. The resonance frequency was 61 MHz unless otherwise stated.

RESULTS

Fig. 1 shows the observed $1/T_{1\rho}$ for protons in gels containing 5.75, 7.3, and 19 wt % agarose in H_2O as a function of the angular frequency ω_1, ($\omega_1 = \gamma H_1$). The data at $\omega_1 = 0$ are the measured $1/T_2$ values for the same samples. In all systems investigated, the $1/T_{1\rho}$ values decrease with increasing ω_1 and are seen to approach $1/T_2$ as $\omega_1 \rightarrow 0$. Several additional values of $1/T_{1\rho}$ at lower resonance frequency (39 MHz) are for the most concentrated gel included in the figure.

Fig. 2 depicts the temperature dependence of T_2 and $T_{1\rho}$ for a 7.3% gel in the temperature range 25–60°C. The $T_{1\rho}$ was determined at two H_1 levels, 4 and 8.1 G. The measurements were carried out starting from the lowest temperature. It is seen from Fig. 2 that T_2 exhibits a minimum at $t \simeq 35$°C. $T_{1\rho}$ minima are shifted to higher temperatures, the temperature shift being larger at stronger H_1.

The effect of temperature on the frequency dependence of $1/T_{1\rho}$ in a gel consisting of 13% agarose in H_2O is shown in Fig. 3.

Fig. 4 depicts $1/T_{1\rho}$ for protons as a function of ω_1 for two samples, differing only

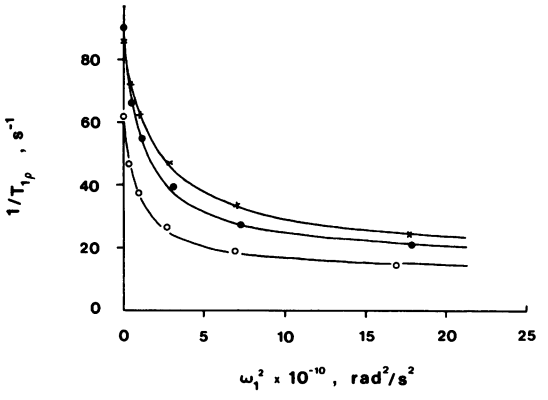

FIGURE 3

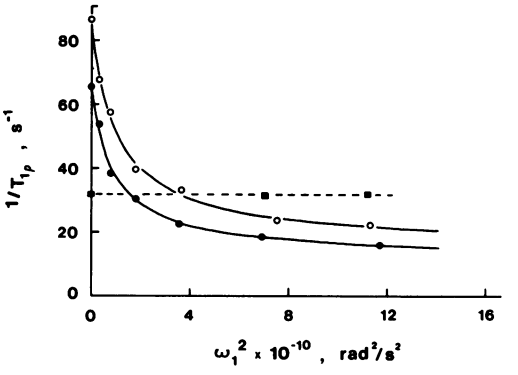

FIGURE 4

FIGURE 3 The $1/T_{1\rho}$ vs. ω_1^2 for protons in a 13% agarose gel at 11°C (- o -), 32°C (- • -), and 45.5°C (- × -). The lines have been calculated from Eq. 4 using the parameters in Table II.

FIGURE 4 The proton $1/T_{1\rho}$ vs. ω_1^2 for a 12% agarose in H_2O (- o -), 50% H_2O + 50% D_2O (- • -) and for a 25% BSA in H_2O (- ■ -) at 25°C.

in that water in one sample was isotopically diluted with D_2O to ca. 50. Fig. 4 likewise shows the values of $1/T_2$ and $1/T_{1\rho}$ for the water protons in a 25% aqueous solution of BSA at room temperature. Within experimental error, $T_{1\rho}$ is equal to T_2. Here, as in Figs. 1 and 3, the data at $\omega_1 = 0$ are the measured $1/T_2$ values for corresponding samples.

DISCUSSION

Theory

For the case of two equivalent protons the relaxation rates caused by dipolar interaction between these spins are given by the following expressions (5):

$$\frac{1}{T_1} = K\left(\frac{\tau_c}{1 + \omega_o^2\tau_c^2} + \frac{4\tau_c}{1 + 4\omega_o^2\tau_c^2}\right),$$

$$\frac{1}{T_2} = K\left(1.5\tau_c + \frac{2.5\tau_c}{1 + \omega_o^2\tau_c^2} + \frac{\tau_c}{1 + 4\omega_o^2\tau_c^2}\right), \tag{1}$$

where ω_o ($= \gamma H_o$) is the angular resonance frequency, τ_c is the correlation time characterizing the motion of the nuclei, and K is the interaction constant, determining the magnitude of the relaxation.

The expression for the spin-lattice relaxation rate in the rotating frame employing a weak collision approach (6) is:

$$\frac{1}{T_{1\rho}} = K\left(\frac{1.5\tau_c}{1 + 4\omega_1^2\tau_c^2} + \frac{2.5\tau_c}{1 + \omega_o^2\tau_c^2} + \frac{\tau_c}{1 + 4\omega_o^2\tau_c^2}\right), \tag{2}$$

where ω_1 ($= \gamma H_1$) is the angular frequency of nuclear spins in the rotating frame. It follows from Eqs. 1 and 2 that for $\omega_1^2\tau_c^2 \ll 1$ the values of $T_{1\rho}$ and T_2 are equal whereas in the case $\omega_1^2\tau_c^2 \ll 1$ and $\omega_o^2\tau_c^2 \ll 1$ all three relaxation times are equal.

For systems in which hydrogen nuclei undergo rapid exchange between states differing in correlation times, the observed ^{1}H relaxation will be exponential with the relaxation rate being a sample weighted average,

$$\frac{1}{T_j} = \sum_i \frac{P_i}{T_{ji}}, \tag{3}$$

where P_i is the fraction of protons in state i with the relaxation times T_{ji} ($j = 1, 2$ and 1ρ) given by Eqs. 1 and 2. An underlying assumption in Eq. 3 is that the lifetime of exchangeable protons in different states is small compared with the corresponding relaxation times. Furthermore, the contribution to $1/T_2$ and $1/T_{1\rho}$ due to the difference in chemical shift between the nucleus at different environments is considered negligible.

Calculations

We have noted single exponentials for each of the relaxation rates in all the studied agarose gels; this confirms the previous findings (1, 7) of rapid exchange of water protons between different environments.

The ω_1 dependence of $1/T_{1\rho}$ for agarose-water systems in Figs. 1, 3, and 4 exhibits similar behavior for different samples. The values of $1/T_{1\rho}$ approach $1/T_2$ as $\omega_1 \rightarrow 0$, decreasing continuously with increasing ω_1 and reaching limiting values at high ω_1. Thus, in order to analyze the experimental data, Eqs. 1–3 were combined giving

$$\frac{1}{T_{1\rho}} = \left(\frac{1}{T_2} - A\right)\left(\frac{1}{1 + 4\omega_1^2\tau_{c1}^2}\right) + A, \tag{4}$$

where τ_{c1} is the effective correlation time for the slowest motion and A is the contribution to the observed relaxation rate from the protons characterized by shorter correlation times (i.e. $\omega_1^2\tau_c^2 \ll 1$).

Eq. 4 was used to fit the experimental dependence of $T_{1\rho}$ on ω_1. The values of A and τ_{c1} calculated in this manner from the data in Fig. 1 are listed in Table 1. It is obvious that while $1/T_1$, $1/T_2$ and A are approximately proportional to agarose/water ratio in the samples, τ_{c1} remains essentially unchanged (the agreement within 20% is good with respect to the accuracy of the measurements and variation is sample homogeneities). This result indicates that the fraction of exchangeable protons in states with restricted motion increases with agarose concentration while the distribution of the correlation times is unaltered in the concentration range investigated.

The $1/T_2$ and $1/T_{1\rho}$ in 19% agarose gel determined at lower resonance frequency (39 MHz) were found the same as the corresponding values at 61 MHz (Fig. 1). Furthermore, the experimental T_2 was not observed to increase when the spacing between 180° pulses in the CPMG pulse sequence was reduced to 40 μs. These observations indicate that the contribution to $1/T_2$ and $1/T_{1\rho}$ due to the chemical shift difference between hydrogen nuclei at different environments (8) is negligible.

On the basis of their proton and deuteron relaxation studied in agar-water systems,

TABLE I

THE OBSERVED $1/T_1$ AND $1/T_2$ AND CALCULATED PARAMETERS FROM EQ. 4 FOR AGAROSE GELS AT 25°C

Agarose concentration	τ_{c1}	A	$\frac{1}{T_1}$	$\frac{1}{T_2}$
wt %	s	s^{-1}	s^{-1}	s^{-1}
5.75	$4 \cdot 10^{-6}$	6.9	0.50	40
7.3	$4.9 \cdot 10^{-6}$	9	0.55	52.9
19	$4.4 \cdot 10^{-6}$	16	1.12	131.9

Woessner and Snowden (1) concluded that only a very small fraction of water was firmly bound to agar chains and might provide the links through which intra- or interchain bonding can occur in the gel state. This type of water was suggested as being responsible for the observed short T_2 which shows great sensitivity to molecular configurations. The correlation time τ_{c1} which we have calculated describes, presumably, firmly bound water whose mobility is much slower than that of normal water ($\simeq 10^{-11}$ s). The fact that τ_{c1} is longer than the value estimated by Woessner and Snowden (1) implies that the fraction of water firmly bound to agarose is even smaller than the value of 0.008 proposed for a 7.3% gel (a more appropriate estimation is of the order 0.003).

The A's calculated using Eq. 4 represent the contributions to the relaxation rates ($1/T_2$ and $1/T_{1\rho}$) from hydrogen nuclei with the correlation times shorter than about 10^{-7} s. These values can be considered as $1/T_2$ ($= 1/T_{1\rho}$) for the samples in the absence of the slowest motion characterized by τ_{c1}. An analysis of the ratio between A and $1/T_1$ according to Eq. 1 reveals that the data are not consistent with the assumption of a single correlation time, τ_{c2}. This correlation time would be approximately $1\text{–}2 \cdot 10^{-8}$ s in disagreement with the value $6 \cdot 10^{-9}$ s obtained from the dependence of proton and deuteron spin-lattice relaxation times on ω_o (1). Therefore, a distribution in correlation times of the more mobile hydrogens in agarose-water systems must be considered. This conclusion is supported by a second NMR study (2). Our investigations of the NMR relaxation for sodium ions in agarose gels (9) gave an effective correlation time of $1.3 \cdot 10^{-8}$ s for the "bound" ions. Thus, as suggested previously (1), the fraction of relatively mobile protons includes presumably some of the agarose hydroxyl groups.

Effect of Temperature

Eq. 2 predicts that when ω_1 and ω_o are kept constant and τ_c is allowed to vary, $T_{1\rho}$ exhibits a minimum at $\omega_1 \tau_c \simeq 0.5$. In agarose-water systems $T_{1\rho}$ is dominated by the correlation time τ_{c1}. Since τ_{c1} is expected to decrease with temperature, the minimum in $T_{1\rho}$ should appear at higher temperatures for measurements performed at higher ω_1. In agreement with this consideration the temperature dependence of $T_{1\rho}$ measured at two different ω_1 (Fig. 2) gives minima at different temperatures. However, the quantitative analysis of the data in Fig. 2 is impossible as the fraction of hydrogen nuclei with $\tau_c = \tau_{c1}$ presumably varies with temperature. Moreover, a minimum in T_2 influences the temperature dependence of $T_{1\rho}$. The T_2 minimum has been observed previously (1, 7, 10) and indicates that the T_2 for bound water is comparable with the lifetime of a water molecule in the bound state. Thus, Eq. 3 should read

$$\frac{1}{T_j} = \frac{P_B}{T_{jB} + \tau_B} + \sum_{i \neq B} \frac{P_i}{T_{ji}}, \tag{5}$$

where the index B refers to the bound H_2O and τ_B is the lifetime mentioned above.

It appears from Fig. 2 that the observed $T_{1\rho}$'s approach the corresponding T_2 values

TABLE II

EFFECT OF TEMPERATURE ON THE NMR RELAXATION IN 13% AQUEOUS AGAROSE

Temperature	τ_{c1}	A	$\frac{1}{T_1}$	$\frac{1}{T_2}$
°C	s	s^{-1}	s^{-1}	s^{-1}
11	$5.3 \cdot 10^{-6}$	11.4	0.99	62.0
32	$4.6 \cdot 10^{-6}$	15.3	0.71	90.3
45.5	$3.5 \cdot 10^{-6}$	16.2	0.60	85.8

as the temperature increases, indicating that the correlation time, τ_{c1}, is a decreasing function of temperature. To obtain τ_{c1} for different temperatures, the data in Fig. 3 have been analyzed via Eq. 4. The calculated parameters, A and τ_{c1}, are given in Table II. As expected, the calculated τ_{c1} decreases with increasing temperature. The values of the correlation time obtained at lower temperatures might possibly be underestimated as the lifetime τ_B which appears in Eq. 5 has been neglected in the calculations.

It is interesting to note that A, unlike the experimental $1/T_1$, has been found to increase with temperature. For $\tau_{c2} = 6 \cdot 10^{-9}$ s (1) or longer, Eq. 1 gives $1/T_1$ which would increase with temperature. Thus, the data in Table II once again confirm the suggested distribution in correlation times for more mobile hydrogens. In this fraction higher mobilities are more represented than lower mobilities, starting possibly from those for ordinary water. The increase in A suggests an increase in population of hydrogen nuclei with correlation times not much shorter than ca. $3 \cdot 10^{-7}$ s. A gradual release of the bound water with increasing temperature and/or changes in the arrangement of the agarose macromolecules or water molecules attached to them might be responsible for this phenomenon.

Isotopic Dilution

The observed ^1H NMR relaxation rates are generally dependent on both the intra- and intermolecular dipole-dipole interactions. It is possible to separate the contribution of latent magnetic dipoles (e.g. nonexchangeable protons in the agarose) and of exchangeable protons in other water-molecules to the total relaxation rate by isotopic dilution of H_2O with D_2O. Woessner and Snowden (1) showed that in agar gels the latter relaxation contribution is much greater than that of latent dipoles. Our measurements in Fig. 4 are consistent with this conclusion. When a 1:1 mixture of H_2O and D_2O was employed for preparation of a 12% agarose gel, the observed relaxation rates decreased considerably. On the other hand, the calculated τ_{c1} was the same for both systems. The results obtained from the ω_1 dependence of $1/T_{1\rho}$ (Fig. 4) are listed in Table III.

TABLE III

EFFECT OF ISOTOPIC DILUTION ON THE PROTON RELAXATION TIMES AND THE PARAMETERS OBTAINED FROM DATA IN FIG. 4

Water composition	τ_{cl}	A	$\frac{1}{T_1}$	$\frac{1}{T_2}$
	s	s^{-1}	s^{-1}	s^{-1}
100 % H_2O	$5.0 \cdot 10^{-6}$	16.2	0.87	86.7
50 % H_2O + 50 % D_2O	$5.2 \cdot 10^{-6}$	12.1	0.735	65.5

BSA in Water

Unlike the observations for agarose gels, the values of ^1H $T_{1\rho}$ measured in a 25% aqueous solution of BSA were found equal to the corresponding T_2 (Fig. 4). This indicates the absence of motions with correlation times greater than ca. $3–5 \cdot 10^{-7}$ s.

Using the Stokes-Einstein formulae (ref. 5, p. 298) the correlation time for the tumbling motion of the protein can be estimated. The value of $2 \cdot 10^{-8}$ s calculated in this manner is considerably shorter than the limit value mentioned above, thus confirming our suggestion.

CONCLUSION

From the studies of proton NMR relaxation times $T_{1\rho}$, T_1 and T_2 in agarose gels, the following conclusions can be drawn:

(*a*) The results are not consistent with the assumption of two or even three proton fractions with different correlation times.

(*b*) The fraction of protons with the correlation time $5 \cdot 10^{-6}$ s at room temperature dominates the spin-spin relaxation and has been ascribed to a firmly bound water. This fraction is of the order 0.003 for a 7.3% gel.

(*c*) The more mobile protons in agarose gels cannot be characterized by a single correlation time. This fraction has been identified with exchangeable hydroxyl protons in the agarose and with water molecules in different motional states. High mobilities are the most common.

(*d*) There is a fast exchange between the proton populations mentioned above. However, for bound water the lifetime in the bound state is comparable with the corresponding T_2 values.

(*e*) As the temperature is increased, the motion of the bound water increases. A fraction of this type of water is simultaneously released or at least becomes described by much shorter correlation times ($< 3 \cdot 10^{-7}$ s).

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REFERENCES

1. WOESSNER, D. E., and B. S. SNOWDEN. 1970. Pulsed NMR study of water in agar gels. *J. Colloid Interface Sci.* **34**:290.
2. OUTHRED, R. K., and E. P. GEORGE. 1973. A nuclear magnetic resonance study of hydrated systems using the frequency dependence of the relaxation processes. *Biophys. J.* **13**:83.
3. KNISPEL, R. R., R. T. THOMPSON, and M. M. PINTAR. 1974. Dispersion of proton spin-lattice relaxation in tissues. *J. Magn. Res.* **14**:44.
4. FARRAR, T. C., and E. D. BECKER. 1971. Pulse and Fourier Transform NMR. Academic Press, New York. 91.
5. ABRAGAM, A. 1961. The Principles of Nuclear Magnetism. Oxford University Press, London. 290.
6. JONES, G. P. 1966. Spin-lattice relaxation in the rotating frame: weak-collision case. *Phys. Rev.* **148**: 332.
7. CHILD, T. F., and N. G. PRYCE. 1972. Steady-state and pulsed NMR studies of gelation in aqueous agarose. *Biopolymers.* **11**:409.
8. SWIFT, T. J., and R. E. CONNICK. 1962. NMR relaxation mechanism of ^{17}O in aqueous solution of paramagnetic cations and the lifetime of water molecules in the first coordination sphere. *J. Chem. Phys.* **37**:307.
9. ANDRASKO, J. 1974. Nonexponential relaxation of $^{23}Na^{+}$ in agarose gels. *J. Magn. Res.* **16**:502.
10. AIZAWA, M., S. SUZUKI, T. SUZUKI, and H. TOYAMA. 1973. Properties of water in macromolecular gels. V. Anomalous temperature dependence of the nuclear magnetic resonance line-width of water in macromolecular gels. *Bull. Chem. Soc. Jpn.* **46**:116.