THE RELATIONSHIP BETWEEN THE TRANSVERSE AND LONGITUDINAL NUCLEAR MAGNETIC RESONANCE RELAXATION RATES OF MUSCLE WATER

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ABSTRACT Whole frog sartorius and gastrocnemius muscles were incubated in Ringer's solutions, either unenriched or enriched with $H_2{}^{17}O$ or ^2D_2O. Subsequently, the rates of transverse ($1/T_2$) and of longitudinal ($1/T_1$) nuclear magnetic relaxation were measured for ^{17}O, ^2D, and ^1H at room temperature and at 8.1 MHz. The ratio (T_1/T_2) for ^{17}O was measured to be approximately 1.5–2.0, close to the value roughly estimated from the Larmor frequency dependence of $1/T_1$ alone over the range 4.3–8.1 MHz. On the other hand (T_1/T_2) for ^2D and ^1H were both measured to lie in the range 9–11. Insofar as the entire ^{17}O signal was detected, the data indicate the presence of an exchange mechanism between the major fraction of intracellular water and a minor fraction characterized by enhanced rates of relaxation. Possible molecular mechanisms are presented.

INTRODUCTION

In simple protein solutions, the longitudinal rates of nuclear magnetic relaxation (NMR) are increased for the three nuclides of water, and exhibit a dispersion phenomenon when plotted as a function of the Larmor frequency (ω_0) (Hallenga and Koenig, 1976). The frequency at which the inflection point is observed correlates very well with the orientational correlation time of the protein in the test solution. In these simple protein solutions, the relative increases are similar for the three water nuclides over the entire range of frequencies studied; the relative increase is the same for ^{17}O and ^2D, and slightly higher (up to a factor of two) for the water protons.

A similar increase and frequency dependence of the rate of longitudinal relaxation have been noted for the water nuclides within the intracellular fluids of biological cells. In particular, it is striking that, for Larmor frequencies above 4 MHz, the relative increases for ^{17}O and ^2D are identical, while those for protons are about twice as large, mimicking the results obtained in protein solutions. Given the different axes of interaction for ^{17}O, ^2D, and ^1H underlying the relaxation mechanism, these results impose severe constraints on any model involving ordering with respect to the protein surface.

They cannot be accommodated by a simple polarization characterized by a single ordering parameter (Civan and Shporer, 1975).

Despite many striking similarities between the NMR properties of the water nuclides in protein solutions and in biological cells, certain differences have also been reported. In cell-free protein solutions, the rates of longitudinal ($1/T_1$) and transverse ($1/T_2$) relaxation can be fitted reasonably well over the full frequency range of observation by expressions based on a single dispersion effect occurring at several megahertz (Koenig et al., 1975). The same is true for simple cells such as human erythrocytes (Lindstrom and Koenig, 1974). However, this is far from the case in complex biological cells, such as striated muscle. Here, $1/T_2$ differs substantially from $1/T_1$, and a second frequency dispersion has been found at some 10's of kHz both for the $1/T_1$ of water protons (Thompson et al. 1973; Knispel et al., 1974; Finch and Homer, 1974) and for the $1/T_1$ of water deuterons (Fung, 1977).

These observations indicate the presence of a relaxation mechanism governed by a dynamic process much slower than the Larmor frequency of a typical NMR experiment. The rate of transverse relaxation is dominated essentially by this slow process. Therefore, comparison of the rates of transverse and longitudinal relaxation for the three nuclides of the present study permits certain conclusions concerning this relatively slow process.

THEORETICAL BACKGROUND

For a single population of water molecules dominated by a single correlation time, the rates of longitudinal and transverse relaxation of the three water nuclides can be expressed by:

$$1/T_1 = 0.2C\tau[(1 + \omega_0^2\tau_c^2)^{-1} + 4(1 + 4\omega_0^2\tau_c^2)^{-1}] \simeq C'\tau(1 + B\omega_0^2\tau_c^2)^{-1}, \quad (1)$$

$$1/T_2 = 0.1C\tau[3 + 5(1 + \omega_0^2\tau_c^2)^{-1} + 2(1 + 4\omega_0^2\tau_c^2)^{-1}] \quad (2)$$

(Abragam, 1961), where the parameters C and C' include the strength of the dipolar or quadrupolar interaction, and where the constant B has been best fit to 2.67 over the range $0.1 < \omega_0\tau_c < 2.0$ (Reuben and Luz, 1976).

Eqs. 1 and 2 are rigorously correct for the intramolecular proton-proton dipolar interaction and closely approximate relaxation behavior based on dipolar interactions in general. The equations can also be used to describe exactly the rates of magnetic relaxation determined by the nuclear quadrupolar interaction with ^{2}D, whose spin number (I) is one.

The relaxation behavior characterizing nuclides with $I > 1$ is more complex, since the observed rates consist of several components. For nuclides such as ^{17}O, with a spin of $\frac{5}{2}$, three components contribute to the relaxation rates; an exact solution is not available in closed form. In this case, Eqs. 1 and 2 describe the relaxation behavior of the weighted average (McLachlan, 1964). For $1/T_1$, Rubinstein et al. (1971) have demonstrated by numerical analysis that only one component is dominant. Thus, Eq. 1 is a close approximation to the longitudinal relaxation behavior of ^{17}O.

Analysis is more complicated for the transverse relaxation behavior of ^{17}O. In this case, all three components can be significant, depending upon the value of $(\omega_0 \tau_c)$.

Eqs. 1 and 2 must be modified if the observed relaxation rates reflect two different populations of water molecules in rapid exchange with each other. In the absence of a chemical shift, and if the relative mole fraction (P_b) of the minor species is much less than that (P_a) of the major species,

$$1/T_2 = 1/T_{2a} + P_b(T_{2b} + \tau_b)^{-1}, \quad (3)$$

(Woessner and Zimmerman, 1963), where τ_b is the mean lifetime of the nuclide in the "*b*" form.

METHODS

Samples

As previously described (Civan and Shporer, 1972), sartorius and gastrocnemius muscles were excised intact from doubly pithed frogs, *Rana esculenta*, and aerated initially for 5–10 min in a standard Ringer's solution (Civan and Podolsky, 1966) (NaCl, 115.5 mM; Kcl, 2.5 mM; $CaCl_2$, 1.8 mM; Na_2HPO_4, 2.5 mM; NaH_2PO_4, 0.5 mM; and *d*-turbocurarine chloride, 9 mg/liter). Subsequently, the muscles were transferred to a final incubation medium consisting of fresh Ringer's solution, Ringer's solution enriched with deuterium, or Ringer's solution enriched with $H_2{}^{17}O$. After incubations of some 2½–4 h, during which time oxygen or air was bubbled into the solution, the isotope compositions of the water within the muscles and within the bathing media were identical (Civan and Shporer, 1972); the final concentrations of deuterium and $H_2{}^{17}O$ were 34–43% and 7–8%, respectively. At that time the muscles were removed, blotted dry on filter paper, and gently packed into tared thin-walled test tubes, of 10 mm OD. The tubes were reweighed before the NMR measurements began; where appropriate, a sample of incubation medium, approximately equal in weight to the wet weight of the muscle sample, was introduced into a similar tube as a control. The dry weights of the muscles were measured after at least 24 h of drying at about 100°C.

Although the muscles were studied under anerobic conditions, previous observations have indicated that such preparations continue to contract in response to electrical stimuli after even more prolonged periods of investigation (Civan et al., 1976).

NMR Techniques

All measurements were performed with a Bruker pulsed NMR B-KR spectrometer (Bruker Physik AG, Karlsruhe, Germany) at a frequency of 8.1 MHz; the strength (H_0) of the steady magnetic field, provided by a 12-inch V 4012 A-HR electromagnet system of a Varian DP-60 NMR spectrometer (Varian Associates, Palo Alto, Calif.), was adjusted appropriately.

Both T_1 and T_2 were measured at 20–24°C by standard techniques of paired pulses. T_1 was calculated from measurements of the intensity of the free induction decay after the second of paired pulses of 180° and 90°. T_2 was calculated from measurements of the intensity of the signal after paired pulses of 90° and 180°; the pulses were separated by a variable time interval τ (in milliseconds), and the measurements performed at 2τ milliseconds after the initial 90° pulse, at the expected peak of the spin echo. Signals were time-averaged to enhance the signal-to-noise ratio. The duration of the 90° pulses for ^{17}O and ^2D was 40–45 μs, and for protons was 4–5 μs.

Data Reduction

The data obtained were fitted to a single exponential ± SE with a standard multiexponential optimization program (available at the Medical School Computer Facility). In those cases where the results deviated from a single exponential decay, only the initial rate of decay (constituting a weighted average of the multiple components) was evaluated for the purposes of the present study.

RESULTS

Fig. 1 presents the longitudinal and transverse relaxation behavior of ^1H from the muscle water of the same preparation. The longitudinal relaxation can be well fit with a single exponential. However, as previously described (Hazlewood et al., 1974), the transverse relaxation of water protons consists of two or more exponential compo-

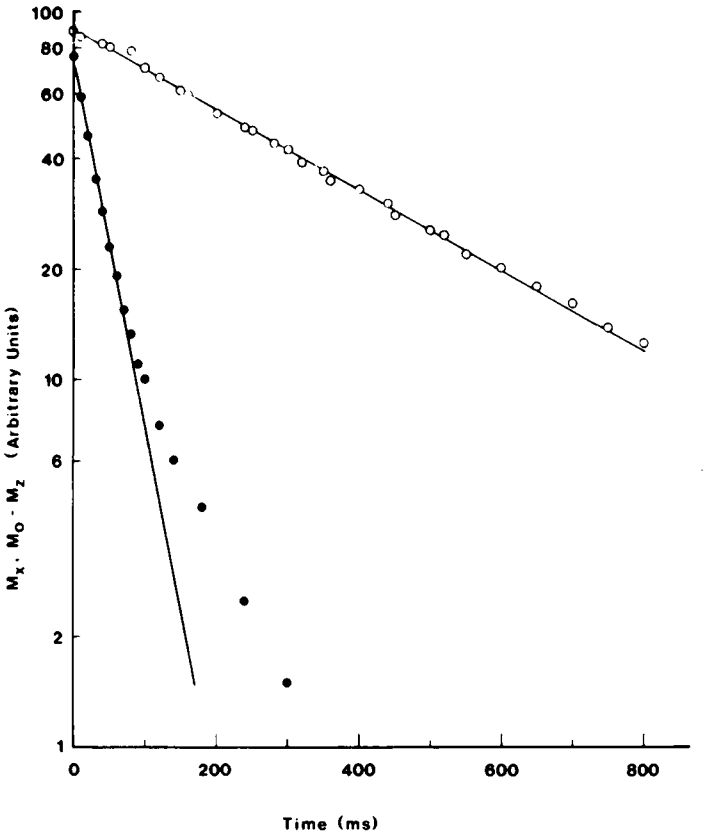

FIGURE 1 Longitudinal and transverse relaxations of water protons in frog striated muscle. The upper set of data points (open circles) in Figs. 1–3 presents the $\log_{10}$ of the difference (in arbitrary units) between the full magnetization (M_0) and the magnetization (M_z) as a function of time after a pulse of 180°. The lower set of data points (closed circles) in Fig.s 1–3 presents the transverse magnetization (M_x) as a function of the time after a pulse of 90°. Each solid line of Figs. 1–3 traces the initial relaxation behavior.

nents. For the purposes of the present study, we have been concerned with the initial slope only, which constitutes a weighted average of the several components.

As illustrated in Fig. 2, during the decay to 10% of the initial intensity no outstanding deviations from simple exponential behavior were noted either for the transverse or longitudinal relaxations of the water deuterons.

Fig. 3 presents representative measurements of the longitudinal and transverse relaxation behaviors of ^{17}O for the muscle water of a single sample. As previously noted (Civan and Shporer, 1974), the longitudinal relaxation of ^{17}O from fresh muscle appeared to deviate from single exponential behavior. A similar tendency was noted for the transverse relaxation of ^{17}O. Once again, we are concerned specifically with the initial slope of the nuclear magnetic relaxations.

The results of all five experiments are summarized in Table I. Each entry consists of the mean of two to four experimental determinations. The values of the ratio (T_1/T_2) were similar for ^1H and ^2D of muscle water. (T_1/T_2) was 9.3 ± 0.3 for ^1H, and was 9.8 ± 0.2 and 11.3 ± 0.3 for the ^2D of two different muscle samples from

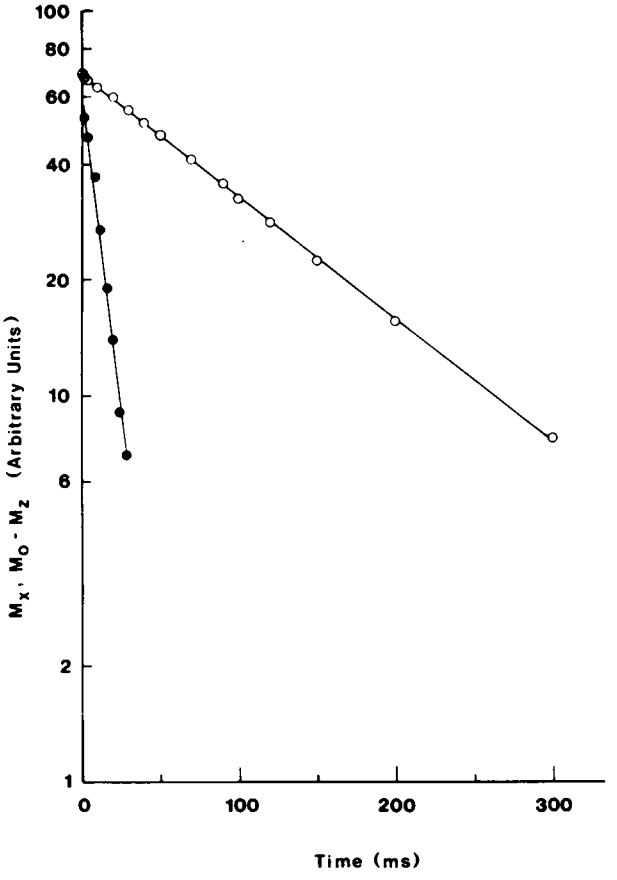

FIGURE 2 Longitudinal and transverse relaxations of water deuterons in frog striated muscle.

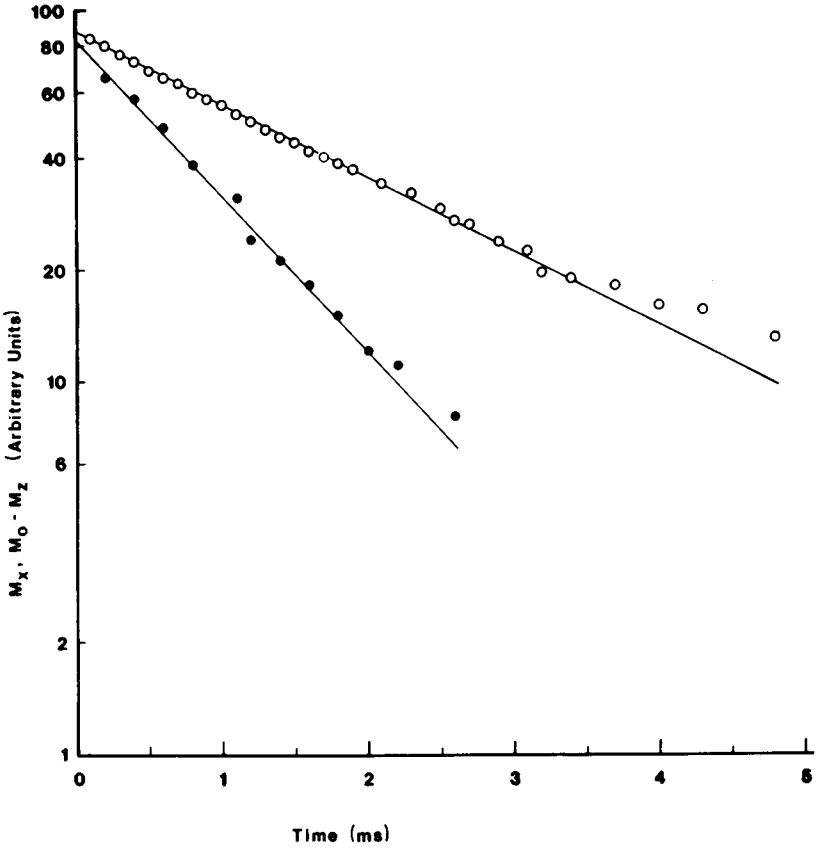

FIGURE 3 Longitudinal and transverse relaxations of ^{17}O from water in frog striated muscle.

different frogs. The data are insufficiently precise to conclude whether or not the slightly higher ratio for ^2D is significant.

On the other hand, the value for (T_1/T_2) of ^{17}O from muscle water was strikingly different from those of the other two nuclides. In two different animals, (T_1/T_2) was 1.6 ± 0.1 and 1.8 ± 0.1.

Control measurements were also made of samples taken from the final incubation media bathing the muscles studied, using the same techniques. The rates of relaxation for ^2D and ^{17}O were $2.5 \pm 0.1\ s^{-1}$ and $150 \pm 26\ s^{-1}$, respectively. In the case of ^{17}O, comparison was also made of the extrapolated intensities to zero time of the free induction decay for water within muscle and within an equal weight of incubation medium. Considering that $19.2 \pm 0.7\%$ of the muscle weight was found to consist of dry matter, the signal intensities were not significantly different. Thus, if a fraction of the ^{17}O from the intracellular water was NMR-invisible, it could have mounted to only a few percent of the total.

TABLE 1

LONGITUDINAL AND TRANSVERSE RELAXATION RATES FOR ^1H, ^2D, AND ^{17}O OF MUSCLE WATER

Nuclide	$1/T_1$	$1/T_2$	T_1/T_2
	s^{-1}		
^1H	2.56 ± 0.043	23.8 ± 0.72	9.3 ± 0.32
^2D	7.48 ± 0.075	73 ± 1.4	9.8 ± 0.21
	8.16 ± 0.096	92 ± 2.1	11.3 ± 0.29
^{17}O	480 ± 35	778 ± 37	1.6 ± 0.14
	484 ± 17	868 ± 40	1.8 ± 0.10

Each row presents the values of the mean ± SE measured for a separate muscle sample.

DISCUSSION

The results of the present study demonstrate that the values of $1/T_2$ and $1/T_1$ characterizing ^1H and ^2D of water within frog striated muscle differ by an order of magnitude. These results are consistent with previous studies of the values of $(1/T_2)$ (Hazlewood et al., 1974) and of $1/T_{1\rho}$ (Thompson et al., 1973; Knispel et al., 1974; Finch and Homer, 1974) characterizing the water protons of tissue water; $1/T_{1\rho}$ may be considered the equivalent of $1/T_1$ at low values of H_0. The results are also consistent with Fung's (1977) report of the strong frequency dependence of the $1/T_1$ of tissue water deuterons and protons at low magnetic field.

On the other hand, the results also demonstrate that the values of $1/T_2$ and $1/T_1$ of ^{17}O from muscle water are similar, differing by less than a factor of two. These data are also in accord with the values of $1/T_2$ for the ^{17}O of muscle water estimated from the line width of the continuous wave NMR signal (Civan and Shporer, 1972; Swift and Barr, 1973). A small difference between $1/T_2$ and $1/T_1$ is to be anticipated on the basis of the following approximate analysis based on the simplification that a single correlation time characterized the ^{17}O of the muscle water.

Previous measurements of $1/T_1$ of ^{17}O from muscle water at room temperature have demonstrated that the longitudinal relaxation rate is greater at 8.1 MHz than at 4.3 MHz, by a factor of 1.27 ± 0.02 (Civan and Shporer, 1975). From this ratio and Eq. 1, we may estimate that $(\omega_0\tau_c)$ lies within the approximate range 0.1–0.2 under the conditions of the present study. Within this range, only one of the three components of $1/T_2$ provides a significant contribution to the observed transverse relaxation (Levanon et al., 1970). Thus, Eq. 2, describing a single exponential process, is appropriate to the present conditions.

From Eqs. 1 and 2 and the estimated value for $\omega_0\tau_c$, the ratio (T_1/T_2) may be roughly estimated at 1.3. This value is close to the experimentally observed ratio of 1.6–1.8 (Table I). It should be noted that even in simple protein solution, the dispersion curve deviates from the simplified Lorentzian shape predicted by Eq. 1. The

estimated correlation time is much longer than $(1/\omega_0)$ for the lowest Larmor frequency of 4.3 MHz, on which the estimate is based. In this case, the nature of the deviation from Lorentzian behavior would lead to an underestimate of the correlation time. An underestimate of two to threefold is entirely likely, and would well account for the discrepancy in the ratio. Thus, the data presented are consistent with the concept that the same mechanisms determine both the longitudinal and transverse relaxation rates for ^{17}O of muscle water.

On the other hand, the deviations from 1.3 of the experimentally determined values of (T_1/T_2) for the protons and deuterons of muscle water (Table I) are too great to be accounted for by the distortion in the shape of the frequency dispersion.

It is reasonable to assume that basically the same physical interaction underlies all the nuclear relaxation behavior of water in protein solution and biological tissues. The longitudinal relaxation times for all three nuclides are decreased by a similar factor in protein solution and in muscle, in comparison to the values in pure water. The same is true for the transverse relaxation times of all three nuclides in protein solution, and for two of the nuclides (protons and deuterons) in the muscle samples. Coupled with the exceptional behavior of ^{17}O in muscle is the observation of a slow dynamic relaxation mechanism for both protons and deuterons. The effect of such a process is to reduce T_2 to low values. Since the relaxation times of ^{17}O are generally much shorter than those of ^1H and ^2D, the natural conclusion would be that such an effect arises from a limited rate of chemical exchange; the slow process would be restricted to a population of surface water not contributing to the observed ^{17}O resonance. Since the initial intensity of the ^{17}O-free induction decay approximately corresponds to the total amount of muscle water, the population of surface water must consist of only a small fraction of the total. This mechanism cannot involve chemical shifts since, at high Larmor frequencies, the $1/T_2$ of protons in muscle water is independent of ω_0 (Hazlewood et al., 1974).

The current measurements provide bounds for the rate $(1/\tau_b)$ of exchange; τ_b is the mean lifetime of water molecules within the minor fraction of muscle water. The major fraction of water molecules must be in a state of fast exchange with respect to the water proton; i.e., $(1/T_{2a})_H \ll (1/\tau_b)(P_b)$, where P_b is the mole fraction of the minor phase (Eq. 3). With respect to the water deuterons, the water molecules could be in a state either of fast or intermediate exchange; i.e., $(1/T_{2a})_D \lesssim (1/\tau_b)(P_b)$. Thus, $(1/\tau_b(P_b)$ must be no less than about 70 s^{-1}. If we assume that the entire water molecule participates in the exchange process without measurably affecting the transverse relaxation of ^{17}O, we can also estimate an upper bound for the rate of exchange; $(1/\tau_b)(P_b) \ll [(1/T_2)_O - (1/T_1)_O] \approx 400$ s^{-1}. Thus, if the entire water molecule participates in the exchange, $(1/\tau_b)(P_b)$ must lie within the range of some 70–200 s^{-1}.

The exchange process could reflect at least three molecular processes. First, the protons and deuterons of the muscle water could exchange with the nonwater exchangeable protons of the muscle. From a rough estimate of the intracellular concentration of exchangeable nonwater protons, $(1/\tau_b)$ must be no slower than some 10^4 s^{-1}.

This rate seems to be at the upper limit of previously described proton exchanges (Koenig and Schillinger, 1969).

The second possible mechanism could consist of an exchange of entire water molecules between a major fraction of free molecules and a minor fraction of rapidly relaxing water. This mechanism certainly cannot be excluded, but the constraints on the range of permissible rates of exchange are severe. As noted above, in this event, $(1/\tau_b)(P_b)$ is limited to the narrow range of 70–200 s^{-1}.

The third possible mechanism would be an exchange between the surface water on ordered macromolecules and the surrounding bulk phase water. The ordering of the interfacial water would result in line splittings $(\Delta\gamma)_H$, $(\Delta\gamma)_D$, and $(\Delta\gamma)_O$ for the ^1H, ^2D, and ^{17}O nuclides, respectively. For this splitting to be transmitted to the deuterons of the bulk water phase, $(1/\tau_b) \gtrsim (\Delta\gamma)_D$. On the other hand, the exchange rate would have to be sufficiently slow for the splitting not to be transmitted to the ^{17}O to the surrounding water; i.e., $(1/\tau_b) \ll (\Delta\gamma)_O$. Thus, an apparent ordered domain is formed with regard to protons and deuterons, but not with respect to ^{17}O. In addition, the transverse relaxation rates of the ^1H and ^2D from the muscle water could be further modulated by diffusion of the water molecules from surface-to-surface within the tissue. The concept that the low-frequency dependence of $1/T_1$ of water protons and deuterons could reflect both processes of exchange between interfacial and bulk water and diffusion between domains is similar in this respect to the model proposed by Berendsen and Edzes (1973) to describe the intracellular NMR behavior of ^{23}Na.

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