

STUDY OF SPIN-LATTICE AND SPIN-SPIN RELAXATION TIMES OF ^1H , ^2H , AND ^{17}O IN MUSCLE WATER

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ABSTRACT Spin-lattice (T_1) and spin-spin (T_2) relaxation times of proton, deuteron, and oxygen-17 in muscle water have been measured at 9.21 MHz in the temperature range of 0°–40°C. The values of the apparent activation energy for the three nuclei are (in $\text{kJ}\cdot\text{mol}^{-1}$) 9.1, 19, and 18 for $1/T_1$, and -1.3 , 4.2 , and 14 for $1/T_2$, respectively. The relatively small values for T_2 for ^1H and ^2H and their low apparent activation energies are attributed to hydrogen exchange between water and proteins; this exchange does not affect the ^{17}O relaxation. Quantitative calculations on deuteron T_1 and oxygen-17 T_1 and T_2 have been made. The effect of surface-induced anisotropy on a minor fraction of water molecules is considered in some detail, and a new expression for its spectral density similar to that of liquid crystalline systems is applied in the calculation. It is suggested that water on the surfaces of macromolecules has a rotational correlation time of $\tau_c \sim 1 \times 10^{-9}\text{s}$, with a time constant of $\tau_x \sim 3 \times 10^{-7}\text{s}$, which is characteristic of the relaxation of the local structure.

INTRODUCTION

Water is the most abundant compound in all living organisms, and the nature of water in biological systems has been explored extensively in recent years by various techniques. Information on the motional behavior of water can be obtained from nuclear magnetic resonance studies. Magnetic relaxation of ^1H , ^2H , and ^{17}O in muscle water has been studied by a number of investigators, and this subject has been reviewed in two recent articles (1, 2).

Magnetic relaxation of water can be characterized by spin-lattice (T_1), spin-spin (T_2), and rotating frame spin-lattice ($T_{1\rho}$) relaxation times (3). Self-diffusion coefficients can also be obtained from spin echo studies (4). For water in muscle, T_1 , T_2 , and $T_{1\rho}$ of proton have been studied over wide ranges of frequency and temperature (5–15). T_1 of deuteron over a wide frequency range at 25°C and its temperature dependence at several frequencies has been reported (14, 16–18). For oxygen-17, T_1 at several temperatures at two frequencies has been measured (19–21). T_2 of ^2H and ^{17}O at room temperature has also been studied (21). However, we are not aware of any temperature-dependent study of T_2 of ^2H and ^{17}O in muscle water. To understand the nature of water in muscle we must be able to explain the frequency and temperature dependence of the relaxation data of all three nuclei. Quantitative calculations have been made by several groups including ourselves (5, 7–9, 18). However, we will argue in the Discussion that these calculations are no longer valid. We have made a systematic study of T_1 and T_2 and ^1H , and ^2H , and ^{17}O in muscle water at 9.21 MHz over the temperature range of 0°–40°C. The results and interpretations are given in this paper. Calculations based upon recent formulism in relaxation theories are presented.

EXPERIMENTAL

Mature female ARS HA (ICR), albino mice (Sprague-Dawley) weighing ~35 g each were used in this work. Each mouse was killed by cervical dislocation. Muscle from a hind leg was carefully dissected to remove attached fat. A muscle strip weighing ~0.12 g was then soaked at 25°C for 90 min in either 10 ml (for ^1H and ^2H) or 1 ml (for ^{17}O) of a modified Krebs solution (9) that was bubbled with a gentle stream of oxygen. D_2O was obtained from Stohler Isotope Chemicals Inc., Waltham, Mass. and water enriched with 20% H_2^{17}O was obtained from Merck, Sharp & Dohme, Canada, Ltd., Montreal, Quebec. The ^{17}O -enriched water was recovered by vacuum distillation and used repeatedly with small decreases in isotope enrichment.

All relaxation measurements were studied at 9.21 MHz using a CPS 2 spectrometer (Spin-Lock Ltd., Port Credit, Ontario, Canada) with a variable field, unshimmed high resolution magnet (Bruker Instruments, Inc., Billerica, Mass.) Signal accumulations were made with a 1072 Signal Averager (Nicolet Instrument Corp., Madison, Wisc.). For ^2H and ^{17}O resonances, a proton external lock unit (Schema Versatae, Berkeley, Calif.) operating at 60.0 and 67.94 MHz, respectively, was used. T_1 was measured by the $180^\circ\text{--}\tau\text{--}90^\circ$ method, T_2 by the Carr-Purcell-Meiboom-Gill sequence.

The temperature-dependent measurements were carried out by lowering the temperature of the sample gradually from ~37° to ~0°C. The temperature was later raised back to 25°C for several samples to check whether there was any hysteresis in T_1 or T_2 in this temperature range. It was found that the relaxation times at 25°C remained unchanged within experimental error.

RESULTS AND DISCUSSION

T_1 and T_2 for ^1H , ^2H , and ^{17}O in muscle water at 9.21 MHz all exhibit Arrhenius behavior in the temperature range of 0°–40°C. Their values at room temperature are similar to those reported for frog muscle at 8.21 MHz (21). They are listed in Table I together with the apparent activation energies for the relaxation rates. The characteristics of each nucleus are discussed separately.

Proton T_1

It was observed that the reduction in T_1 of muscle water vs. pure water is much larger for ^1H than for ^2H and ^{17}O (20). Using the T_1 values of these nuclei in liquid water (22, 23) and their T_1 values that we obtained, the ratio of T_1 (liquid water): T_1 (muscle water) has been calculated for the three nuclei from 0° to 40°C, and the data are plotted in Fig. 1. It is clear that over this temperature range the reduction factor for ^2H and ^{17}O is the same within experimental error, whereas that for ^1H is much larger. This is not due to the effect of bubbling O_2 in the soaking process, because T_1 of water protons in the soaked muscle was not any shorter than that of unsoaked muscle. It is due to different relaxations mechanisms

TABLE I
RELAXATION TIMES AT 9.21 MHz AND 298 K FOR ^1H , ^2H , AND ^{17}O IN MUSCLE WATER
AND THE ACTIVATION ENERGIES FOR THE CORRESPONDING RELAXATION RATES

	^1H		^2H		^{17}O	
	T	E_a	T	E_a	T	E_a
	ms	$\text{kJ} \cdot \text{mol}^{-1}$	ms	$\text{kJ} \cdot \text{mol}^{-1}$	ms	$\text{kJ} \cdot \text{mol}^{-1}$
Spin-lattice	400	9.1	130	19	1.8	18
Spin-Spin	66	-1.3	15	4.2	1.1	14

Error limits are ~5%.

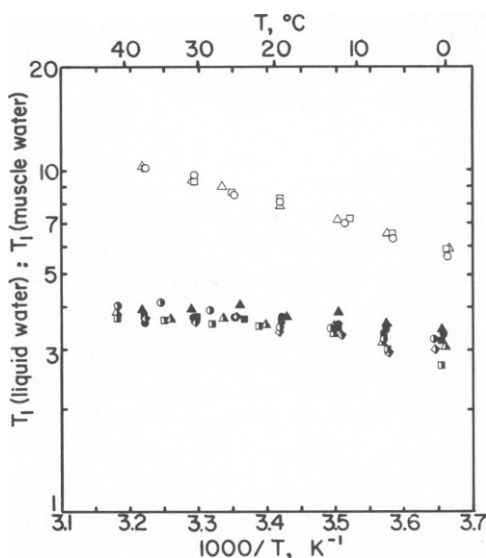


FIGURE 1

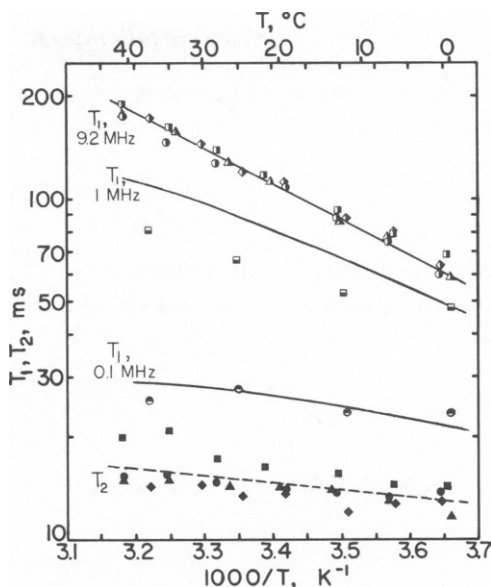


FIGURE 2

FIGURE 1 The ratio of T_1 between liquid water and muscle water at 9.21 MHz. Open symbols, proton; half-filled symbols, deuteron; closed symbols, oxygen-17.

FIGURE 2 Deuteron relaxation times of muscle water at 9.21 MHz. T_1 at two other frequencies are also shown (data from reference 14). The solid lines for T_1 are calculated from Eqs. 1–3, 5, and 6. The dashed line for T_2 is an Arrhenius plot and is not calculated from relaxation equations. T_1 data are given by half-filled symbols, and T_2 data by closed symbols.

between dipolar and quadrupolar nuclei. For proton T_1 in muscle water, it has been demonstrated (14, 15, 20, 25) that intermolecular interaction between protons in macromolecules and protons of muscle water in the hydration layer is the dominant proton relaxation mechanism. In earlier calculations of proton T_1 , a log-Gaussian (5, 7, 9) or a bimodal log power (8) distribution function for the rotational correlation time (τ_c) of muscle water was used. Intermolecular interaction was not explicitly considered, and the physical significance of the parameters were often obscure. However, in view of recent evidences of the importance of cross relaxation for proton T_1 , calculations using formulae for rotational motions alone can no longer be justified. Because of the complexities of the formulism for cross relaxation (26) and translational motion (27), we will not attempt to calculate the proton T_1 for muscle water.

Deuteron T_1

For deuteron and oxygen-17, quadrupole relaxation dominates. Cross relaxation is probably not important, and consideration of rotational motion alone may be sufficient.

If two fractions of water are in exchange with each other in the liquid portion of a heterogeneous system, the spin-lattice relaxation rate in the limit of fast exchange is given by (28):

$$\frac{1}{T_1} = \frac{P_a}{T_{1a}} + \frac{\mathcal{F}^2 \cdot P_b}{T_{1b}}, \quad (1)$$

where P_a and P_b are fractions of water molecules in the bulk and surface layer, respectively, and \mathcal{F} is a parameter determined by the anisotropic orientational probability distribution of water molecules in the surface layer (28). The relaxation rate of each fraction for nuclei with nuclear spin $I = 1$ is (29):

$$\frac{1}{T_{1k}} = C \cdot [J_1(\omega_0) + 4J_2(2\omega_0)]_k, \quad (2)$$

where ω_0 is the Larmor frequency, the J 's are spectral densities, and C is a proportionation constant determined by the quadrupole coupling constant e^2qQ/h and asymmetry parameter η :

$$C = \frac{3\pi^2}{20} \cdot \left(\frac{e^2qQ}{h}\right)^2 \cdot \left(1 + \frac{\eta^2}{3}\right), \quad (3)$$

The spectral densities for molecules with T_a or higher symmetry is:

$$J(\omega_0) = \frac{2\tau_c}{1 + \omega_0^2\tau_c^2}, \quad (4)$$

where τ_c is the rotational correlation time.

For water in muscle and other heterogeneous systems, the effect of surface-induced anisotropy is very important in determining the relaxation of water molecules. In addition to the anisotropic parameter \mathcal{F} used in Eq. 7 (28), modifications in the spectral densities must also be considered. For a symmetric top, two correlation times and three dispersion terms should appear in the spectral density. For an asymmetric top, three correlation times and five dispersion terms are needed to describe the rotational motion. The water molecule is very small, and its three correlation times would be extremely close to each other. We found that the frequency dispersion of ^2H muscle water (11) cannot be explained by the presence of a small rotational anisotropy of water molecules in the surface layer. One alternative approach is to regard the surface water as having a wide distribution of rotational correlation times. A previous treatment using a log-Gaussian distribution, $P_b = 0.12$ and $\mathcal{F} = 1$ for deuterons in muscle water (18) yielded $\tau_{c,b} = 2.0 \times 10^{-13}$ s at 25°C, ~ 10 times shorter than that of liquid water. The distribution function would require 50% of the water molecules in the surface layer to rotate faster than liquid water. These are clearly unreasonable results. The use of a small value of \mathcal{F} or application of the Cole-Cole expression (30) may overcome these difficulties. However, we prefer to use a different approach which may give a better insight to the motion of the water molecules in muscle.

In the study of deuteron relaxation of small molecules in liquid crystalline solutions it was found that Eq. 4 cannot describe the frequency dependence of T_1 . It was suggested that the motion of the solvent also affects the relaxation of the small molecules (31, 32), and the spectral densities would have the form (33):

$$J(\omega) = \frac{2\tau_c}{1 + \omega^2\tau_c^2} + S^2 \frac{2\tau_x}{1 + \omega^2\tau_x^2}, \quad (5)$$

where S is a local order parameter of the small molecule and τ_x is a time constant characteristic of the structure of the liquid crystalline phase. Apparently, spectral densities of

this form are not limited to small solute molecules in thermotropic liquid crystalline solvents. The characteristics of proton and deuteron in a lyotropic liquid crystalline solution of poly- γ -benzyl-L-glutamate in CDCl_3 and CD_2Cl_2 also cannot be explained by Eq. 4 (34,35). For deuteron in the solvent, $T_1 > T_2$ was observed even for $\omega_0\tau_c \ll 1$, and Eq. 5 would probably be capable of describing the effect of the polypeptide motion on the relaxation of the solvent molecules. By analogy, let us consider that the motion of water near the surface of either a macromolecule or a solid can be described by two time constants, one for its rapid tumbling described by Eq. 4 and another characteristic of the slowly relaxing local structure due to its interaction with the surface. Eq. 5 can now be used to describe the spectral density of water molecules on a surface as well.

To study the temperature dependence of T_1 , the correlation times τ_c and τ_x are regarded to have Arrhenius behavior:

$$\tau = \tau_0 \cdot \exp(E/RT). \quad (6)$$

Then, one should be able to use Eqs. 1–3, 5, and 6 to describe the frequency and temperature dependence of deuteron T_1 in muscle water. In our approach, fraction a is identified with the major fraction of muscle water that can be frozen, with $P_a \sim 0.9$ (9, 18). Small changes in P_a do not appreciably affect the calculated results. T_{1a} is regarded as having the same value for liquid water, which has no frequency dependence. The values of T_{1a} at different temperatures have been determined (23). Fraction b is identified with surface water, and its relaxation behavior is to be determined. The values of e^2qQ/h and η would not differ much from those of liquid water, namely, 258 kHz and 0.1, respectively (23). P_b and \mathcal{F} are not independent of each other, and cannot be determined separately. Some of the six parameters ($P_b \cdot \mathcal{F}^2$, S , τ_{oc} , E_c , τ_{ox} , and E_x) are tightly coupled, and we were not able to determine all of them simultaneously by least square calculations. Therefore, those parameters were determined in the following way. A single calculation was first made to find out the values of $P_b \cdot \mathcal{F}^2$, S , τ_c , and τ_x at room temperature from the frequency dispersion data. Since τ_x was found to be 3×10^{-7} s, and S^2 to be of the order of 10^{-2} , the contribution of the terms containing τ_x to $1/T_1$ at high frequencies is small (<3% at 9.21 MHz). Therefore, the value of τ_x was not further varied and was regarded as having no temperature dependence. Then, the frequency dispersion data at room temperature and the temperature dependence of $1/T_1$ at 9.21 MHz were simultaneously fitted by changing sets of τ_{c0} and E_c values and regarding $P_b \cdot \mathcal{F}^2$ and S as undetermined parameters. The parameters were chosen to give the best general fit to the data at all frequencies and temperatures. The parameters obtained are: $P_b \cdot \mathcal{F}^2 = 6.1 \times 10^{-3}$, $S^2 = 1.8 \times 10^{-2}$, $\tau_c(298 \text{ K}) = 1.0 \times 10^{-9}$ s, $E_c = 20 \text{ kJ} \cdot \text{mol}^{-1}$, $\tau_x(298 \text{ K}) = 3.0 \times 10^{-7}$ s, and $E_x = 0$. The calculated values are plotted as solid lines in Figs. 2 and 3, respectively. Although there is $\sim 30\%$ deviation between the calculated values and the experimental data at intermediate frequencies, the general agreement over wide ranges of frequency and temperature is quite satisfactory. A better fit to the room-temperature frequency dispersion data could be obtained with a larger value of τ_c , but it would not account for the temperature dependence at various frequencies.

An important feature in the proton and deuteron T_1 data for muscle water is their smaller temperature dependence at lower frequencies (9, 18) (Fig. 2). This is opposite to the trend of a simple liquid, but can be well accounted for in the present approach by considering the correlation time of the slow motion to have a very small activation energy ($E_x \sim 0$).

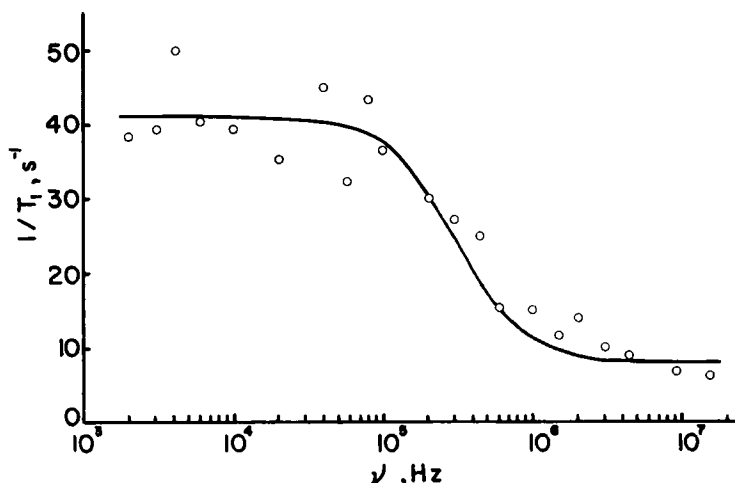


FIGURE 3 Dispersion of deuteron relaxation rate of muscle water at 298 K. The experimental data are quoted from reference 14, and the solid line is calculated from Eqs. 1-3 and 5.

We must note that the two-site exchange model (Eq. 1) is a great simplification of the actual situation of water in muscle, which is structurally very complicated. The surfaces of the macromolecules are not uniform and the motional behavior of the minor fraction would be rather complicated. Actually, the application of Eq. 5 to muscle water must be regarded as a postulation with certain justifications rather than a rigorous theoretical expression with a priori proof. Even for small solute molecules in liquid crystalline solutions, it was warned that it would be unwise to attach much quantitative significance to the parameters in view of the simplified model leading to Eq. 5 (33).

Proton and Deuteron T_2

For the rotational motion of a system with spin = 1 or a pair of spin $1/2$, the spin-spin relaxation rate of each liquid fraction is (29):

$$\frac{1}{T_{2k}} = C \cdot \left[\frac{3}{2} J_0(\omega_0) + \frac{5}{2} J_1(\omega_0) + J_2(\omega_0) \right]_k. \quad (7)$$

The corresponding observed relaxation rate would have an expression similar to Eq. 1 for fast exchange between the two fractions. Using these expressions and the parameters listed above, one can calculate $1/T_2$ for ^2H in muscle water at 9.21 MHz as a function of temperature. However, the values thus obtained (ranging from 65 ms at 40°C to 37 ms at 0°C) are much larger than those observed experimentally (ranging from 15 to 12 ms in the same temperature range) and have a much stronger temperature dependence. In fact, the observed values of T_2 for both protons and deuterons are unusually small, as can be seen by comparing the ratio T_1/T_2 for ^1H , ^2H , and ^{17}O in muscle water (20) (Fig. 4).

Let us first ask whether a limited lifetime for the *b* fraction might be the reason for the reduction of T_2 for protons and deuterons. A simple calculation based upon equations accounting for the effect of limited lifetime in exchange (36-38) showed that this would make the ratio $T_1:T_2$ decrease, opposite to what was observed.

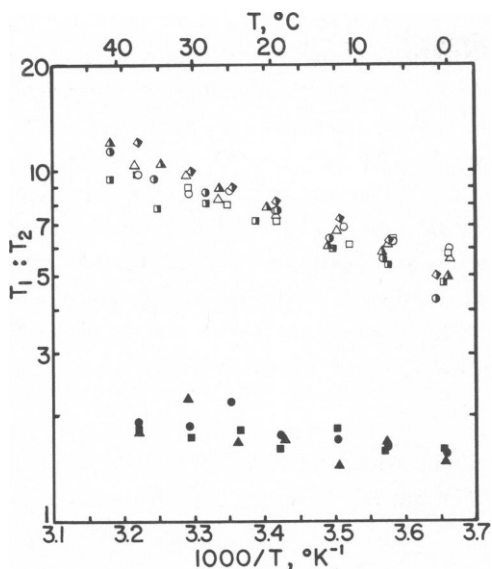


FIGURE 4

FIGURE 4 The ratio $T_1:T_2$ for muscle water at 9.21 MHz as a function of temperature. Open symbols, proton; half-filled symbols, deuteron; closed symbols, oxygen-17.

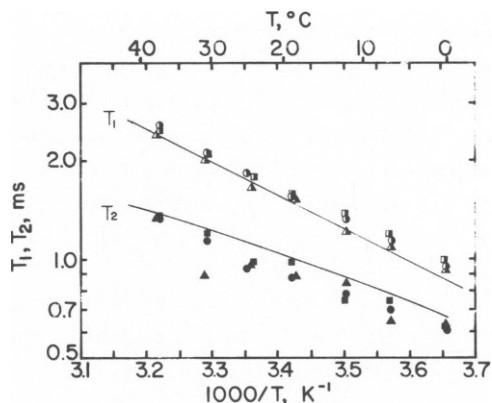


FIGURE 5

FIGURE 5 Oxygen-17 relaxation times of muscle water at 9.21 MHz. The solid lines are calculated from parameters obtained from deuteron relaxation, as described in the text.

A possible cause for the difference in the $T_1:T_2$ ratio for ^1H , ^2H , and ^{17}O is a relaxation mechanism that affects the relaxation rates of ^1H and ^2H , but not those of ^{17}O . Hydrogen exchange between water and labile protons in the proteins may be such a mechanism (21). For protein solutions, arguments have been made that the lifetime of the b fraction is the lifetime of whole water molecules and not that of exchangeable hydrogens in the proteins (39, 40). Muscle differs from protein solutions in two important aspects. First, the ratio of protein to water is much higher in muscle. Second, the contractile proteins in muscle, which make up 70% of all proteins in muscle (41), have much less motional freedom than soluble proteins. Therefore, the amino, hydroxyl, and sulfhydryl groups in these proteins would have much longer correlation times than those of water in the hydration layer or in the bulk. The effect of hydrogen exchange can be accounted for by adding other terms to the expressions of $1/T_i$ ($i = 1, 2$) for ^1H and ^2H , but not for ^{17}O :

$$\frac{1}{T_i} = \frac{1}{T_{ia}} + \frac{\mathcal{F}^2 \cdot P_b}{T_{ib}} + \sum \frac{P_{ej}}{T_{iej} + \tau_{ej}}, \quad (8)$$

where P_{ej} is the fraction of each type of labile hydrogen in the proteins with respect to all exchangeable hydrogens (in water and in proteins), T_{iej} is its relaxation time, and τ_{iej} is its lifetime. For the protons in immobile proteins, $T_{iej} \sim 10^{-1}$ s and $T_{2ej} \sim 10^{-5}$ s (25, 42), and the corresponding values for deuterons would be one order of magnitude smaller. For various types of exchangeable hydrogens, τ_{ej} ranges from 10^{-3} to 10^{-5} s (39, 40); therefore, $T_{1ej} > \tau_{ej} > T_{2ej}$ for both proton and deuteron. Since P_{ej} 's are necessarily very small, the exchange terms would be unimportant for $1/T_1$, but may have significant contributions to $1/T_2$. It is to be

noted that the apparent activation energy for deuteron $1/T_2$ is very small and that for proton $1/T_2$ is negative (Table I). Since τ_{2e} would have an opposite temperature dependence compared with T_{2a} , T_{2b} , and T_{2c} , the data in Table I serve as a strong indication that hydrogen exchange is important in determining $1/T_2$ for both protons and deuterons. Furthermore, the ratio $T_1:T_2$ is similar for ^1H and ^2H , but much smaller for ^{17}O (Fig. 4). This can also be explained by the importance of hydrogen exchange in $1/T_2$ for proton and deuteron in muscle water. Actually, the motions of the $-\text{NH}_2$, $-\text{OH}$, and $-\text{SH}$ groups in the muscle proteins would be rather anisotropic and may exhibit nonzero static dipolar or quadrupolar splittings ($\Delta\omega'$). Then, a factor containing $\Delta\omega'$ should be multiplied to the terms under the summation sign in Eq. 12. Because of this complication, the large number of unknowns, and the effect of cross relaxation for ^1H , we did not attempt to calculate T_2 values for either ^1H or ^2H .

Oxygen-17, T_1 , and T_2

For nuclei with spin > 1 , an exact solution for the relaxation equation is not available (29). Fortunately, it was shown by numerical analysis (43) that Eq. 2 is a good approximation for $I = 5/2$, with:

$$C = \frac{12\pi^2}{1,250} \left(\frac{e^2 q Q}{h} \right)^2 \cdot \left(1 + \frac{\eta^2}{3} \right). \quad (9)$$

However, Eq. 7 is a good approximation for $I = 5/2$ only when $\omega_0\tau \leq 0.5$ (43). For $\omega_0\tau > 0.5$ the magnetization decay is the sum of three components and T_2 (as defined by the time required for the magnetization to decline to $1/e$ of its initial value) has to be calculated numerically. Civan, Shporer, and Achlama studied T_1 of ^{17}O in muscle water at room temperature at two frequencies (20) and T_2 at 8.1 MHz (21). They suggest that the exchange between two fractions of water has a limited rate, and the minor fraction does not contribute to the ^{17}O signal. They estimated that the term $(\omega_0\tau_c)^2$ for the major fraction lies within the approximate range 0.1–0.2 (according to Dr. Shporer, the square sign was missing in the $\omega_0\tau_c$ term in reference due to a misprint). This does give a frequency dependence and a $T_1:T_2$ ratio reasonably close to experimental values. However, it does not yield correct values for the relaxation rates. If the experimental values of $e^2 q Q/h = 7.8 \times 10^6$ Hz and $\eta \sim 0.9$ for ^{17}O in liquid water (44) are substituted into Eqs. 2 and 7 for $(\omega_0\tau_c)^2 = 0.1$ –0.2 for the major fraction only, one obtains $1/T_1 \sim 3.4 \times 10^5$ – 3.9×10^5 s $^{-1}$ and $1/T_2 \sim 4.1 \times 10^5$ – 5.3×10^5 s $^{-1}$, three orders of magnitude larger than the relaxation rates at room temperature. Therefore, we proceed with the two-state model (Eq. 1), assuming that the exchange rate is fast enough so that both fractions contribute to the relaxation rates of all three nuclei.

T_1 of oxygen-17 in liquid water has been determined as a function of temperature (23), which is taken to be the same as T_{1a} in Eq. 1. To calculate the contribution of the minor fraction, parameters obtained from deuteron T_1 were used. The results calculated for T_1 of ^{17}O in muscle water at 9.2 MHz are shown as a solid line in Fig. 5. It is interesting to note that even though the parameters \mathcal{F} and S do not have to be the same for ^2H and ^{17}O , good agreement between experimental and calculated data was obtained by using the same parameters for the two nuclei. Since hydrogen exchange does not affect the relaxation rates of ^{17}O , $1/T_2$ can be obtained from Eq. 8 by setting the terms under the summation sign equal to zero. It was shown that for liquid water, T_{2a} is smaller than T_{1a} because of ^1H - ^{17}O spin-spin splitting (44). However, we found that for water enriched with 20% ^{17}O , $T_1 = T_2 = T_{1p}$ for ^{17}O

within experimental error. This is probably due to a large deuterium content in our sample, and our measurement of T_2 by pulse method with signal accumulation is probably more accurate than T_2 determination by line-width measurement (45). Therefore, T_{2a} was taken to be equal to T_{1a} for ^{17}O , and the values determined for liquid water (22) were used in the calculation. Even if the reduction of T_{2a} is taken into account, the calculated values of T_2 would only be reduced slightly ($\sim 20\%$ at room temperature), which would not affect the overall quality of the calculation. For the b fraction, the contribution of τ_c to $1/T_{2b}$ was evaluated by using Eq. 7, and the contribution τ_x was evaluated numerically by slightly extrapolating the data in Fig. 2 of reference 43. The results are plotted as a solid line in Fig. 5. Since T_1 and T_2 of ^{17}O in muscle water were calculated from relaxation equations with no adjustable parameters, the agreement between calculated and experimental values as shown in Fig. 5 is rather gratifying.

In summary, we conclude that the relaxation behavior of muscle water can be described reasonably well by a two-state, fast exchange model. The major fraction behaves like liquid water, which has a rotational correlation time of 2.3×10^{-12} s at room temperature. The minor fraction is identified with water on the surfaces of the macromolecules in muscle. Recently developed relaxation equations (Eq. 1, reference 28; and Eq. 5, reference 33) have been applied to describe its motion. By fitting deuterium T_1 data to the relaxation equations, it is estimated that the minor fraction of water in muscle has a rotational correlation time of $\sim 1 \times 10^{-9}$ s at room temperature, and it is further restricted by a surface orientation potential with a characteristic time constant of $\sim 3 \times 10^{-7}$ s. In addition, T_2 of proton and deuterium in muscle water may be affected by hydrogen exchange between water and labile hydrogens in the macromolecules.

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