PROTON AND DEUTERON RELAXATION OF MUSCLE WATER OVER WIDE RANGES OF RESONANCE FREQUENCIES

B. M. FUNG, *Department of Chemistry, University of Oklahoma, Norman, Oklahoma 73019*

ABSTRACT The spin-lattice relaxation time (T_1) of water protons in mouse muscle was studied from 10^4 to 10^8 Hz at several temperatures, and the deuteron T_1 of muscle water was studied from 2.0×10^3 to 1.54×10^7 Hz at several temperatures. Proton T_1's of muscle and brain water with different D_2O contents were measured at 25°C and 35 MHz. From the results of variable frequency and temperature measurements and the data of isotope substitution, it is concluded that the major relaxation mechanism for the protons in muscle water is the intermolecular dipolar interaction between the protons of the macromolecules and the protons of the water molecules in the hydration layer. It is also suggested that the relaxation of deuterons can be accounted for by a very small fraction of water molecules directly hydrogen-bonded to the macromolecules.

For the study of the state of water in biological systems, one of the most informative techniques is the measurement of magnetic relaxation times of different nuclei in the water molecule. There are several prominent features of the magnetic relaxation of water in muscle and other biological tissues: (*a*) the spin-lattice relaxation times (T_1) of ^1H, ^2H, and ^{17}O are all shorter than those for bulk water, and are strongly frequency-dependent (1–6); (*b*) at a given frequency, the ratio T_1 (^2H)/T_1 (^{17}O) in muscle water is close to that in pure water, while the ratio T_1 (^1H)/T_1 (^{17}O) is 2.1 times greater in pure water than in muscle water (6); (*c*) the temperature dependence of proton T_1's for muscle water becomes smaller when the frequency goes down, which is opposite to that of simple viscous liquids (4, 5); and (*d*) a unique spin-spin relaxation time (T_2) cannot be defined because the spin-echo train is nonexponential and dependent upon the orientation of the muscle sample for striated muscle (7).

In this communication, we report some additional observations on proton and deuteron T_1's of water in muscle. Through the analysis of our experimental results in terms of possible relaxation mechanisms for different nuclei of water, further understanding of the motion of water molecules in muscle can be gained.

Proton T_1's of muscle water as a function of frequency have been reported by several authors (1–6). Deuteron T_1's of muscle water at a few frequencies above 4 MHz have also been reported (5, 6). We have measured T_1 for both protons and deuterons for water in mouse muscle over wide ranges of frequency, and the results are shown in Fig. 1. The proton data cover a frequency range slightly larger than that for frog

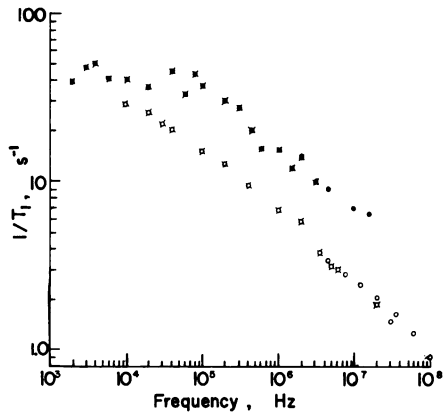

FIGURE 1 Proton (○) and deuteron (●) relaxation rates of muscle water at 25°C as functions of Larmor frequency. The data marked with crosses were taken at the IBM Watson Research Center (see acknowledgment). The deuteron data at 4.5, 9.2, and 15.4 MHz were obtained by using the leg muscle from partially deuterated mice (ca. 30%, ref. 5). Because of intensity problems below 4 MHz, the partially deuterated muscle samples were further soaked in a modified Krebs solution with D_2O as solvent for 1 hr to increase the deuterium content of muscle water. Bathing the muscle in normal Krebs solution increased the total water content slightly; therefore the modified Krebs solution contained 25 g/liter glucose so that the total water content was kept the same as in fresh muscle. Muscle samples treated this way had the same T_1 values as fresh muscle samples.

muscle studied by Held and co-workers (3), and the deuteron data extends to very low frequencies not observed before for muscle water. It is clear from Fig. 1 that the characteristics of the frequency dependence for protons and for deuterons are quite different: the proton relaxation rate increases monotonicly with the decrease of frequency without reaching a plateau down to 10^4Hz, whereas $1/T_1$ for deuterons levels off for $\nu < 10^5$ Hz. Since T_1 for bulk water does not show any frequency dependence at 25°C, it can be readily found that for $\nu \to 0$, T_1 (^{1}H, bulk)/T_1 (^{1}H, muscle) > 100, and T_1 (^{2}H, bulk)/T_1 (^{2}H, muscle) $\approx$ 16.

It is important and interesting to compare the results for muscle water with the results for protein solutions (8, 9). In all protein solutions studied, clear inflection frequencies can be defined on the frequency dispersion curves for both protons and deuterons. For a 210 mg/ml solution of lysozyme (mol wt $\sim 1.47 \times 10^4$) at $\nu \to 0$, T_1 (^{1}H, water)/T_1 (^{1}H, solution) = 11.5, and T_1 (^{2}H, water)/T_1 (^{2}H, solution) = 10.0 at 22°C. For solutions of hemocyanin (mol wt $\sim 9 \times 10^6$) at 25°C and $\nu \to 0$, T_1 (^{1}H, water)/T_1 (^{1}H, solution) $\approx$ 9.5 (normalized to a unit of $M^{-1} \times 10^{-6}$), and T_1 (^{2}H, water)/T_1 (^{2}H, solution) $\approx$ 4.5 (same unit). The results for protein solutions and for muscle water indicate that the difference between the frequency dispersions for protons and for deuterons becomes larger as the motion of the macromolecules slows down (from small proteins, to large proteins, to muscle).

Fig. 2 shows the temperature dependence of proton and deuteron relaxation rates at several frequencies. At high frequencies both proton and deuteron relaxation rate

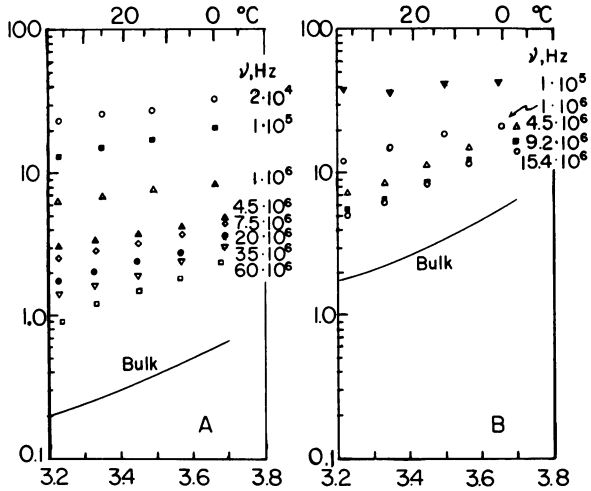

FIGURE 2 Proton and deuteron relaxation rates of muscle water at several frequencies as functions of temperature. A: proton; B: deuteron. The data at 1 MHz and below were taken at the IBM Watson Research Center (see acknowledgment).

for muscle water exhibit a temperature dependence similar to that of bulk water, but the temperature dependence of muscle water is much less at low frequencies. It was pointed out (4, 5) that this unique temperature dependence can be explained by a two-state model. In this model, the relaxation rate is a weighted average of two fractions, the frequency-dependent part having a small temperature dependence, and the frequency-independent part having a large temperature dependence. The relative weight of the latter becomes less as the frequency goes down, and the observed temperature dependence approaches that for the frequency-dependent part at low frequencies. However, it should be recognized that the frequency-dependent part for protons and for deuterons may not have the same relaxation mechanism. For example, at 4.5×10^6 Hz, the deuteron $1/T_1$ still has a large temperature dependence similar to that of bulk D_2O, while at the same frequency the proton $1/T_1$ already exhibits a temperature dependence substantially less than that for bulk H_2O (Fig. 2).

It is well known that, in diamagnetic systems, deuteron relaxation is determined by nuclear quadrupole interaction, dependent only upon molecular rotation. On the other hand, the major relaxation mechanism for protons in diamagnetic systems is nuclear dipole-dipole interaction, which depends upon both translational and rotational motions. Since the gyromagnetic ratio of the deuteron is only 1/6.5 that of the proton, the substitution of deuterons for protons in bulk water reduces both the intramolecular and the intermolecular dipolar interaction for protons, and $1/T_1$ rapidly goes down as the ratio of D_2O to H_2O goes up. However, Civan and Shporer (6) found that isotope substitution did not change the proton T_1 of water in frog muscles. In Fig. 3, we show a more extensive set of experimental data for the effect of isotope substitution on the proton relaxation rate of water in the muscle and brain tissues of

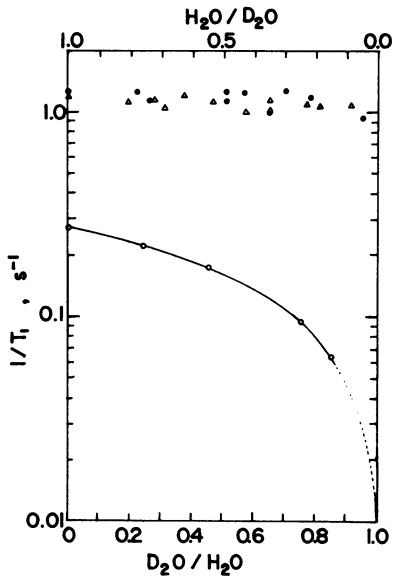

FIGURE 3 Proton relaxation rates of bulk water (○), water in mouse muscle (△), and brain (●) at 25°C and 35 MHz as functions of isotope substitution. The samples were bathed in Krebs solution with different D_2O contents for 1 h. For samples containing more than 70% D_2O, the proton magnetization decay was slightly nonexponential because of the contribution of organic protons (B. M. Fung, to be published); T_1 was taken from the slowly decaying part of the plot. T_1 data for bulk H_2O were obtained from ref. 10.

albino mice. The data clearly show that, in these systems, deuteron substitution has a much smaller effect on the reduction of proton relaxation rate (*ca.* 20% for extrapolation to complete substitution) than that for bulk water (a factor of 24 for complete deuteron substitution) (10).

Considering the above experimental results, we suggest the following description for the relaxation mechanism of different nuclei of water in muscle. For ^1H, the major relaxation mechanism is the *inter*molecular dipole-dipole interaction between each proton in the water molecule in the first hydration layer and the protons in the relatively immobile proteins and other macromolecules. About 10% of the total amount of water molecules are in the first hydration layer of the macromolecules, and they are probably all involved in this type of relaxation. The motion(s) responsible for this must have a relatively long correlation time ($>10^{-5}$ s), and the most likely one is the diffusion of water molecules to and from the first hydration layer of the macromolecules. The importance of this type of diffusion has been suggested by Pintar and co-workers (11, 12), but they did not explicitly consider the dipolar interaction between the water protons and the protons in the macromolecules. Since the hydrated water molecules are in fast exchange with the rest of the water molecules on the NMR time scale, the observed relaxation rate is a weighted average. For ^2H and ^{17}O, the fast-relaxing part in the exchanging fractions seems to involve only a small

portion of the water molecules in the first hydration layer. These water molecules may be more tightly bound to the polar groups of the macromolecules through one or more hydrogen bonds. Their rotational motions are considerably slowed down, and the correlation time is of the order of 10^{-6} s instead of *ca.* 2×10^{-12} s for the rest of the water molecules (the motion of the bound water molecules is most likely anisotropic and has to be described by more than one correlation time. The estimation of 10^{-6} s is for the longest one; internal rotations probably have shorter correlation times).

The above description of the state of water in muscle seems to be consistent with all the available experimental data to date. More experimental work is being performed and a quantitative calculation based upon the above model will be presented separately.

The low frequency data in Figs. 1 and 2 were taken in the laboratory of Drs. R. D. Brown and S. H. Koenig at the IBM Watson Research Center using a field-cycling technique (13). The author gratefully acknowledges them for their great help and useful discussions.

The author is a recipient of a National Institutes of Health Research Career Development Award.

Received for publication 24 January 1977.

REFERENCES

1. BRATTON, C. B., A. L. HOPKINS, and J. W. WEINBERG. 1965. Nuclear magnetic resonance study of living muscle. *Science (Wash. D.C.).* **147**:738.
2. OUTHRED, R. K., and E. P. GEORGE. 1973. Water and ions in muscles and model systems. *Biophys. J.* **13**:97.
3. HELD, G., F. NOACK, V. POLLAK, and B. MELTON. 1973. Protonenspin-relaxation und Wasserbeweglichkeit in Muskelgewebe. *Z. Naturforsch. Sect. C. Biosci.* **28C**:59.
4. FUNG, B. M., and T. W. MCGAUGHY. 1974. The state of water in muscle as studied by pulsed NMR. *Biochim. Biophys. Acta.* **343**:663.
5. FUNG, B. M., D. A. DURHAM, and D. A. WASSIL. 1975. The state of water in biological systems as studied by proton and deuteron relaxation. *Biochim. Biophys. Acta.* **399**:191.
6. CIVAN, M. M., and M. SHPORER. 1975. Pulsed nuclear magnetic resonance study of ^{17}O, ^{2}D, and ^{1}H of water in frog striated muscle. *Biophys. J.* **15**:299.
7. FUNG, B. M. 1975. Orientation of water in striated frog muscle. *Science (Wash. D.C.).* **140**:800.
8. KOENIG, S. H., K. HALLENGA, and M. SHPORER. 1975. Protein-water interaction studied by solvent ^{1}H, ^{2}H, and ^{17}O magnetic relaxation. *Proc. Natl. Acad. Sci. U.S.A.* **72**:2667.
9. HALLENGA, K., and S. H. KOENIG. 1976. Protein rotational relaxation as studied by solvent ^{1}H and ^{2}H magnetic relaxation. *Biochemistry.* **15**:4255.
10. SMITH, D. W. G., and J. G. POWLES. 1966. Proton spin-lattice relaxation in liquid water and liquid ammonia. *Mol. Phys.* **10**:451.
11. KNISPEL, R. R., R. T. THOMPSON, and M. M. PINTAR. 1974. Disperson of proton spin-lattice relaxation in tissues. *J. Magn. Res.* **14**:44.
12. DIEGEL, J. G., and M. M. PINTAR. 1975. Origin of the nonexponentiality of the water proton spin relaxation in tissues. *Biophys. J.* **15**:855.
13. KOENIG, S. H., and W. E. SCHILLINGER. 1968. Nuclear magnetic relaxation dispersions. I. Apotransferrin. *J. Biol. Chem.* **244**:3283.