NUCLEAR MAGNETIC RESONANCE TRANSVERSE RELAXATION IN MUSCLE WATER

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ABSTRACT The origin of the nonexponentiality of proton spin echoes of skeletal muscle has been carefully examined. It is shown that the slowly decaying part of the proton spin echoes is not due to extracellular water. First, for muscle from mice with in vivo deuteration, the deuteron spin echoes were also nonexponential, but the slowly decaying part had a larger weighing factor. Second, for glycerinated muscle in which cell membranes were disrupted, the proton spin echoes were similar to those in intact muscle. Third, the nonexponentiality of the proton spin echoes in intact muscle increased when postmortem rigor set in. Finally, when the lifetimes of extracellular water and intracellular water were taken into account in the exchange, it was found that the two types of water would not give two resolvable exponentials with the observed decay constants. It is suggested that the unusually short T_2's and the nonexponential character of the spin echoes of proton and deuteron in muscle water are mainly due to hydrogen exchange between water and functional groups in the protein filaments. These groups have large dipolar or quadrupolar splittings, and undergo hydrogen exchange with water at intermediate rates. The exchange processes and their effects on the spin echoes are pH-dependent. The dependence of transverse relaxation on pH was observed in glycerinated rabbit psoas muscle fibers.

INTRODUCTION

The study of relaxation times of water in different tissues is important not only for the understanding of the state of cell water, but also for the application of the technique of NMR imaging to living systems, which has been rapidly developed recently (1). In the last ten years, ^1H, ^2H, and ^{17}O relaxation of muscle water has been fairly extensively investigated (2, 3). However, interpretations of the experimental data are far from consensus. We have recently offered a new approach to the quantitative interpretation of longitudinal relaxation times (T_1) of ^2H and ^{17}O and the transverse relaxation times (T_2) of ^{17}O in muscle water over wide ranges of frequency and temperature (4). Possible reasons for the unusually short values of proton and deuteron T_2's and their temperature dependences were also briefly discussed (4). Since then, we have made further studies on the transverse relaxation of ^1H and ^2H of water in muscle and in glycerinated muscle. The results have been examined in some detail and the interpretations are given here.

Several authors have shown that the proton spin echoes of muscle water do not decay exponentially with time (5–10). In other words, the logarithmic plot of the amplitudes of the spin echoes versus time is not linear. This is often referred to as nonexponential decay of the spin echoes, and there have been different explanations for this behavior (5–10). We will show that the main factor in determining the short decay time and the nonexponential behavior of the proton and deuteron spin echoes is the hydrogen exchange between water and functional groups in relatively nonmobile proteins.

EXPERIMENTAL

Muscles dissected from the upper hind legs of mature female ARS HA $(ICR)_f$ albino mice (Sprague-Dawley, Madison, Wisc.) were used in most of this work. In vivo deuteron substitution in their body water was accomplished by feeding them D_2O/H_2O mixtures (11). "99.8%" D_2O obtained from Stohler Isotope Chemicals Inc. (Waltham, Mass.) was used for these, and also for preparing modified Krebs solutions (12) for the high resolution NMR experiments. Psoas muscles of albino rabbits obtained from local suppliers were used in some preparations of glycerinated muscle fibers.

Glycerinated muscle was prepared according to the procedure given by Fuchs (13). To prepare glycerol-free samples for NMR measurements, the samples stored in glycerol solutions were soaked in a buffer (pH 7.0) containing 80 mM KCl, 20 mM imidazole, and 2.5 mM dithiothreitol for at least 4 h each time at 4°C, with three changes. Then, the fibers were soaked twice in another buffer with the same compositions but different pH's at room temperature for 2 h each. Finally, the fibers were taken out from the solution and blotted many times with tissue paper to dry all the surface water. The water contents of the glycerinated fibers thus prepared were very consistent, being (82.8 ± 0.2)% for several samples of mouse leg muscle and rabbit psoas muscle soaked at pH 7.0.

The inulin space in the glycerinated muscle fibers was determined in the following way. About 40 mg of washed fibers were soaked for 1 h in a solution having the above composition plus 50 μCi [methoxy-^{3}H]-inulin (New England Nuclear, Boston, Mass.) at pH 7.0. The sample was blotted dry and weighed. It was then solubilized in 0.50 ml FTS-450 tissue solubilizer (Beckman Instruments Inc., Fullerton, Calif.) overnight. 3.5 ml of Ready-Solv NA solution (Beckman) were then added to the solution and mixed. The ^{3}H radioactivity of the solution was counted by using a LS100C liquid scintillation counter (Beckman), and compared with that of the soaking solution.

All relaxation measurements were made at room temperature (298 ± 1 K) using a home-built pulse NMR spectrometer with a variable field, unshimmed high resolution magnet, and a variable frequency probe (Bruker Instruments, Inc., Billerica, Mass.). Signal accumulations were made with a 1072 signal averager (Nicolet Instrument Corp., Madison, Wisc.). For ^{2}H resonance, a proton external lock unit (Schema Versatae, Berkeley, Calif.) operating at 60.0 MHz was used. T_1 was measured by the 180°-π-90° method, and T_2 by the Carr-Purcell-Meiboom-Gill sequence. After 8–16 scans for proton and 64 scans for deuteron, the noise level was ~0.3% of the intensity of the first echo. The apparent diffusion of coefficients was measured by a pulse field gradient technique (14, 15) with a quadrupole coil (16).

High resolution NMR spectra were recorded with a Varian XL-100 spectrometer (Varian Associates, Palo Alto, Calif.) at 100 MHZ and ambient temperature, using an external ^{19}F lock.

RESULTS

To study the nature of the magnetization decay in the transverse relaxation in muscle water, we measured the spin echoes of proton and deuteron in muscle samples at the same frequency (9.21 MHz) and temperature (298 K). Intact skeletal muscle with in vivo deuteron substitution in the body water of mice was used in the study. Amplitudes of the ^{1}H and ^{2}H spin echoes of a muscle sample are shown in Fig. 1. Each solid curve was calculated from least square fits of the experimental data with two exponential decays. The parameters used in calculating the curves are given in Table I. Samples taken from other partially deuterated mice also yielded a much smaller weighing factor A for deuterium compared with that for proton.

In another set of experiments, the proton spin echoes of intact muscle samples were first recorded at a fixed frequency (16 MHz) and temperature (298 K). Then, the muscle samples were treated with glycerated solutions (7), washed at pH 7.0, and blotted dry of surface water (see Experimental). The proton spin echoes were recorded at the same frequency and temperature. Parameters obtained in two-exponential fits of the spin echo data of an intact

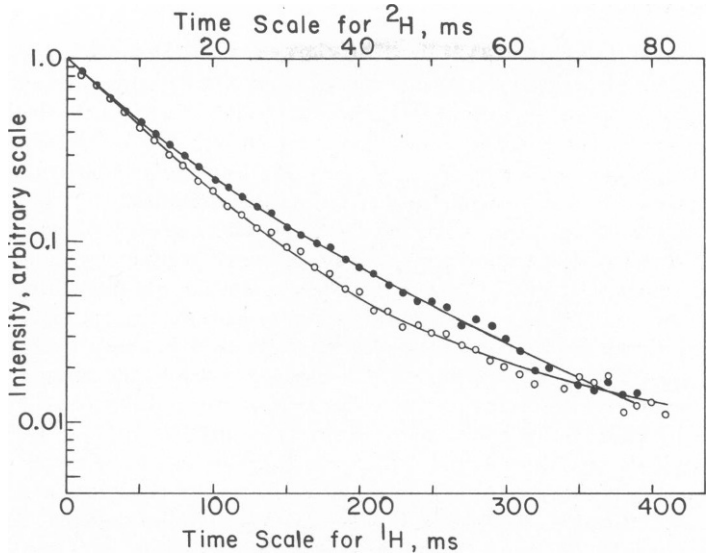

FIGURE 1 Amplitudes of proton (○) and deuteron (●) spin echoes at 9.2 MHz and 298 K for mouse leg muscle with in vivo deuteron substitution. The intensities of the free induction decays are not included in this figure and Fig. 2. The solid lines were calculated according to Eq. 1 for the purpose of comparison only (Table I).

muscle sample and of the same sample after glycerination treatment are given in Table I. The results of other glycerinated muscle samples were very similar.

The apparent diffusion coefficient of water (D) in the intact and glycerinated muscle samples was measured by the pulse field gradient method at 16 MHz. For nonoriented samples at 298 K and a diffusion time of 15 ms, the value of D was $(1.24 \pm 0.02) \times 10^{-5}$ cm^2 s^{-1} for intact muscle, and $(1.50 \pm 0.03) \times 10^{-5}$ cm^2 s^{-1} for the sample after glycerination. These are to be compared with the value of $D = 2.20 \times 10^{-5}$ cm^2 s^{-1} for liquid water. In another study with a diffusion time of 40 ms, it was found that $D = (0.74 \pm 0.02) \times 10^{-5}$ cm^2 s^{-1} for intact muscle and $(1.43 \pm 0.06) \times 10^{-5}$ cm^{-2} s^{-1} for glycerinated muscle. The dependence of D on the diffusion time of water in intact muscle has been discussed in detail by Tanner (17).

We also studied the effect of pH on proton relaxation of glycerinated muscle fibers. In this

TABLE I
PARAMETERS USED IN FITTING THE SPIN ECHOES* OF MUSCLE WATER WITH TWO EXPONENTIAL DECAYS, $M/M_0 = A \exp(-t/T_{2A}) + (1 - A) \exp(-t/T_{2B})$

	A	T_{2A}	T_{2B}
		(s)	(s)
Intact muscle, ^{1}H at 9.2 MHz	0.95	0.054	0.27
Same sample, ^{2}H at 9.2 MHz	0.63	0.0090	0.023
Intact muscle, ^{1}H at 16 MHz	0.95	0.048	0.37
The same sample after glycerination treatment, ^{1}H at 16 MHz	0.91	0.042	0.14

*298 K. Data presented in Figs. 1 and 2, respectively.

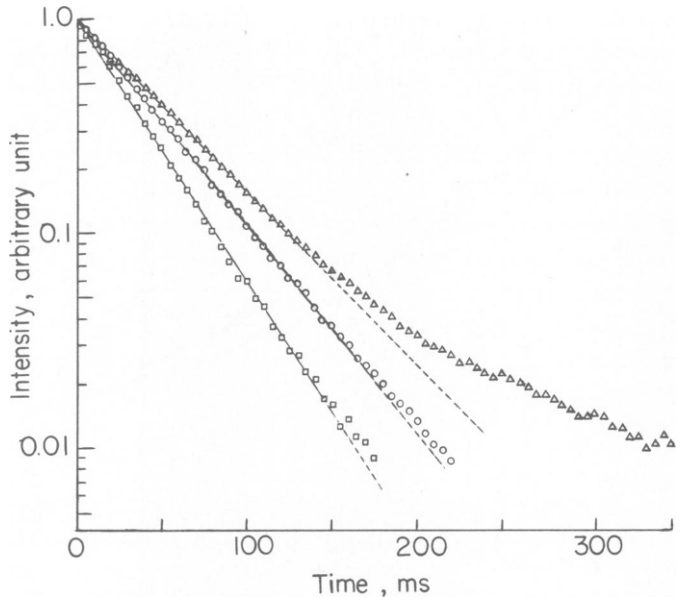

FIGURE 2 Amplitudes of proton spin echoes at 20 MHz and 298 K for glycerinated rabbit psoas muscle fibers. □, pH = 9.0; ○, pH = 7.0, △, pH = 5.0. The straight lines were drawn to show the linear parts of the echoes (Table II).

study, rabbit psoas muscle was used instead of mouse leg muscle, because a larger amount of muscle from the same animal was available for several samples to be treated at different pH values. The results are shown in Fig. 2 and Table II. Glycerinated muscle fibers soaked in solutions with more extreme pH values showed signs of denaturation.

The effectiveness of the glycerination treatment in disrupting the cell membranes was examined by measuring the inulin space of the samples. Inulin $(C_6H_{11}O_6)$ $(C_6H_{10}O_5)_n$ $(C_6H_{11}O_5)$ is a commonly used "marker" for determining extracellular space, mainly because it is water-soluble but cannot go through cell membranes. For three glycerinated mouse muscle samples studied, the inulin space was found to be 76 ± 5% (total water, not total weight). On comparing the inulin space of ~12% (total water) for untreated muscle (18, 19), it appeared that the cell membranes were mostly disrupted.

To examine whether there were protons in organic molecules with fairly long T_2's, we studied the high resolution spectra of several muscle samples. In addition to the main water

TABLE II
CHARACTERISTICS OF PROTON SPIN ECHOES, T_1, AND WATER CONTENT OF GLYCERINATED RABBIT PSOAS MUSCLE AT 20 MHz AND 298 K

pH	Apparent T_2	Linear portion of spin-echo decay	Apparent T_1	Water content
	(ms)		*(ms)*	
9.0	35 ± 2	~4.0 T_2	450 ± 20	85%
7.0	48 ± 2	~3.0 T_2	410 ± 20	83%
5.0	57 ± 2	~2.2 T_2	400 ± 20	81%

peak, there were proton signals at -1.0 to -2.0 ppm from external TMS (tetramethylsilane). The integrated intensity of these protons was ~7% that of the water signal, but varied from one sample to another. In contrast, no proton signal from organic molecules was observed for glycerinated muscle. In another set of experiments, we measured the change in the intensities of the proton signals after deuterium exchange. A modified Krebs solution (12) was prepared from a newly opened bottle of "99.8%" D_2O. The muscle sample was soaked twice in this solution in a beaker tightly covered with parafin film. The amount of H_2O left in the sample was 3.4% (per unit weight). The residual signal from water protons was 2.6%, even after soaking in D_2O Krebs solution four times. The proton signals from organic molecules also diminished in intensity to 13 and 11% of the intensities of the residual water signal after soaking twice and four times, respectively. This indicated that part of these protons might have come from small, water-soluble, organic molecules.

DISCUSSION

The proton transverse relaxation curve (free induction decay, FID, and spin echoes) of muscle is not exponential. It has been customary to analyze the magnetization decay as the sum of several exponential functions, and assign each fraction to a certain type of proton (5–7, 9, 10). The implicit assumption in this kind of analysis is that the lifetime of each fraction is much longer than its decay constant. The validity of this assumption will be discussed later.

It is generally agreed that the fraction with a very short T_2(~24 μs) is due to protons in proteins and other macromolecules. The next fastest fraction (with T_2 ~ 0.4–10 ms) was assigned to water closely associated with macromolecules in the cell in earlier studies (5, 6). Foster et al. (7) suggested that its source was relatively mobile protons in tissue protein and lipid. We compared the ^{13}C and ^{1}H spectra of skeletal muscle (8), and supported their suggestions. Recently, Peemoeller and Pintar (9) and Peemoeller et al. (10) proposed that the fraction with a decay constant of ~5 ms is due to protons in large molecules (~30%) plus protons in water (~70%). This assignment is not contradictory to our estimation that T_2 of the protons in large organic molecules that have a high degree of internal motion is of the order of 0.4–2 ms (8).

In the present study, we are only concerned with the part of the proton signal which decays more slowly. Therefore, only the proton spin echoes of muscle samples recorded from 10 to 400 ms will be discussed. If this part of the signal is decomposed into the sum of two exponentials according to the equation

$$M/M_0 = A \exp(-t/T_{2A}) + (1 - A) \exp(-t/T_{2B}), \quad (1)$$

the decay constants are typically 40–60 ms (fraction A) and 150–400 ms (fraction B), respectively. Several authors have assigned fraction A to intracellular water and fraction B to extracellular water (5–7). Based upon the postmortem change in the proton spin echoes, we questioned this assignment (8). Recently, Peemoeller et al. used a "multiwindow analysis" to study the FID and spin echoes, and assigned fraction A to water, and fraction B to protons in large molecules (9, 10). Here, we will proceed to show that the slowly decaying part of the proton spin echoes of muscle is not due to extracellular water, and suggest that multiexponential fitting of the spin echo data may not be a desirable way to analyze transverse relaxation in

muscle. In the initial discussion, the two-exponential notation in Eq. 1 will be used to analyze the spin-echo data merely for the purpose of examining its validity.

First, let us compare the data of ^1H and ^2H relaxation. The time-dependence of the amplitudes of the deuteron spin echoes was indeed nonexponential, but has characteristics different from the proton spin echoes (Fig. 1 and Table I). If fraction B is due to extracellular water, its weighing factor $(1 - A)$ should be identical for the two nuclei in the same sample. However, the calculated weighing factors of fraction B for ^1H and ^2H differed severalfold for each of several mice studied (for the sample shown in Fig. 1 and Table I, the values were 0.05 ± 0.02 and 0.37 ± 0.02, respectively). Since ~30% deuteron substitution in the muscle water of the mice was gradually accomplished in vivo over a period of 10 d, there is no reason to believe that the distribution ratio of $^1H/^2H$ would be different for extracellular water and intracellular water. Therefore, a comparison of the proton and deuteron spin echoes offered strong evidence that the slow fraction is not due to extracellular water.

Second, let us examine the results of glycerinated muscle. The glycerination procedure disrupts cell membranes and removes most of the sacroplasmic proteins and low molecular weight compounds, but keeps the contractile mechanism of the muscle fibers intact (20). Tanner (17) suggested that the decrease in the apparent diffusion coefficient (D) of water in muscle compared with that of liquid water, and the strong dependence of D on diffusion time, are mainly due to the barrier effect created by the cell membranes. For water in glycerinated muscle fibers, we observed a smaller decrease in D as well as a much weaker dependence of D on diffusion time compared with water in intact muscle. These data and the result of inulin space showed that the cell membranes are mostly disrupted in the glycerinated muscle, and it can be safely assumed that there is little distinction between extracellular water and intracellular water in these samples. However, the amplitudes of their proton spin echoes were still nonexponential with respect to time, with a relatively small weighing factor (~9%) for the slowly decaying part (Table I). Obviously, this part of the spin echoes is not due to extracellular water in the case of glycerinated muscle.

A third argument against the assignment of the slow part in the proton spin echoes to extracellular water was presented previously (8). In each of the many muscle samples we have studied in the past few years, the slow fraction was not very obvious when the spin echoes were measured right after the mouse was killed. It gradually increased in 15–60 min (depending on the temperature) when postmortem rigor of the muscle set in (8). The corresponding change in the spin echoes of porcine muscle appeared much more slowly, and a postmortem increase in the amount of extracellular water was given as the explanation by Pearson et al. (21). However, we know of no other evidence that there is a postmortem redistribution of extracellular water in muscle, and do not think that their interpretation is convincing.

Let us now examine this problem from a theoretical viewpoint. If there are two fractions of water in slow exchange, their respective transverse (spin-spin) relaxation times T_{2A} and T_{2B} can be obtained from fitting the spin echo data to Eq. 1. This would require (22)

$$\tau_A \gg T_{2A} \text{ and } \tau_B \gg T_{2B}, \tag{2}$$

where τ_A and τ_B are the lifetimes of the two fractions, respectively. In a recent detailed study on the diffusion of water in muscle (17), Tanner estimated that the barrier spacing for cell water is 43 μm, roughly equal to the average diameter of muscle cells, and the permeability is

0.01 cm/s. This means $\tau_A \sim 0.43$ s. If the B fraction is extracellular water, $\tau_B = \tau_A(1 - A)/A$, and has a value of 2.3–4.8 $\times 10^{-2}$ s (for $A = 0.95$–0.90). This is much smaller than the value of T_{2B} (0.15–0.40 s) obtained from a two-exponential analysis (Fig. 1) of the proton data. Therefore, the slow exchange condition (Eq. 2) is not satisfied. One may still argue that the nonexponential character of the spin echoes is a result of intermediate exchange between intracellular water and extracellular water, and their respective relaxation times T_{2in} and T_{2ex} are related to T_{2A} and T_{2B}. Then, one can analyze the spin echo data by using the values of $\tau_{in} = \tau_A$ and $\tau_{ex} = \tau_B$ as estimated above and the values of T_{2A} and T_{2B} listed in Table I, or the corresponding values obtained by other authors (5–7). For an intermediate exchange rate with the condition $T_{2in} < T_{2ex}$, the expressions are (22):

$$\frac{1}{T_{2in}} + \frac{1}{T_{2ex}} = \frac{1}{T_{2A}} + \frac{1}{T_{2B}} - \frac{1}{\tau_{in}} - \frac{1}{\tau_{ex}}, \tag{3a}$$

$$\frac{1}{T_{2in}} - \frac{1}{T_{2ex}} = \left[\left(\frac{1}{T_{2A}} - \frac{1}{T_{2B}}\right)^2 - \frac{4}{\tau_{in} \cdot \tau_{ex}}\right]^{1/2} + \frac{1}{\tau_{ex}} - \frac{1}{\tau_{in}}. \tag{3b}$$

However, in each case the calculated value of T_{2ex} was either imaginary or negative, clearly an unacceptable result. Therefore, we conclude that the nonexponential behavior of proton spin echoes in muscle is not due to slow or intermediate exchange between intracellular and extracellular water.

Recently, Peemoeller and Pintar (9) and Peemoeller et al. (10) offered the interesting suggestion that the slow component of the proton signal in muscle (with $T_2 \sim 0.14$ s) is due to protons in large molecules. The assignment was based upon the study on "fully deuterated muscle" obtained by soaking intact muscle twice in a buffered D_2O solution. However, we found that it was impossible to completely replace H_2O by D_2O in muscle. First of all, hydrogen in phosphate salts used in the buffer system and very slowly exchanging amide groups in the proteins would contribute to the proton content. In addition, there is always an appreciable amount of H_2O in commercial D_2O, which is claimed to be 99.8% or higher in deuteration. This is mainly due to proton exchange with moisture in air upon storage. It was pointed out in the previous section that a residual H_2O signal several times more intense than the organic signal was always detected in the high-resolution spectrum of the deuterated muscle. In their proton relaxation study of deuterated muscle, Peemoeller et al. (10) reported that they did not detect any proton signal in the distillate under the same spectrometer settings as were used for the muscle experiments. This is not surprising, because proton T_1 of H_2O in the distallate would be very long (~60 s) due to the small magnetic moment of deuterium (23); unless the pulse period is several minutes or longer, the proton signal may be easily saturated and escape detection. Thus, we conclude that the contribution of residual water to the proton signal in deuterated muscle is usually more important than that of protons in small organic molecules or molecular segments. Furthermore, the protons in organic molecules were not observed in the high resolution spectra of glycerinated muscles, but the slow component was still present in their proton spin echoes (Table I and Fig. 2). Therefore, it is evident that the slow fraction in the proton spin echoes in muscle cannot be exclusively assigned to organic protons.

In two recent papers (4, 24), it was pointed out that in muscle water the ratio T_1/T_2 is about the same for ^1H and ^2H, but much smaller for ^{17}O. We suggested that the unusually small T_2

values of ^1H and ^2H are most likely caused by hydrogen exchange between water and macromolecules (4). Here we will argue that the nonexponential behavior of the spin echoes is also a result of hydrogen exchange.

In relatively nonmobile proteins such as the actin and myosin filaments, the $-NH_2$, $-OH$, and $-SH$ groups would have dipolar splittings $\Delta\omega$ (or quadrupolar splittings for deuterium) because of extremely slow motions. Even though these splittings may not be resolvable because of severe overlapping, their effect on the transverse relaxation of proton or deuteron in water would be very significant. The effect of exchange between a major fraction a and an extremely small minor fraction b on relaxation times has been well-documented (25, 26). If fraction b has a paramagnetic shift or a "static" splitting $\Delta\omega$ due to spin-spin, dipolar, or quadrupolar interaction, the line shape in the frequency domain would not be Lorentzian under the condition

$$\frac{\Delta\omega}{10} \lesssim \frac{1}{T_{2b}} + \frac{1}{\tau_b} \lesssim 10\,\Delta\omega, \tag{4}$$

where T_{2b} is the spin-spin relaxation time of the minor fraction and τ_b is its lifetime. In the time domain, the magnetization decay would be nonexponential if the conditions in Eq. 4 are satisfied.

The dipolar splitting between two protons is given by (27)

$$\Delta\omega = |3\gamma^2 \hbar\,(1 - 3\cos^2\theta)/2r^3|, \tag{5}$$

where γ is the gyromagnetic ratio of proton, r is the proton-proton distance, and θ is the angle between the magnetic field and the r vector. Let us now consider the case of a terminal $-NH_2$ group in a protein filament. For $r = 1.57 \times 10^{-10}$ m, the maximum dipolar splitting $\Delta\omega$ is $2.93 \times 10^5\ s^{-1}$. The rotation of the $-NH_2$ group about the C—N axis, which is perpendicular to the proton-proton vector, would reduce the splitting by a factor of 2. Imperfect alignment of the C—N axis ($\theta \neq 0°$) and segmental motions of the side chains in the proteins would further reduce the value of $\Delta\omega$. Therefore, a reasonable estimate of $\Delta\omega$ for various $-NH_2$ groups would be 10^4–$10^5\ s^{-1}$. T_2 for the protein protons is $\sim 2.4 \times 10^{-5}$ s (9, 10). The internal rotation of $-NH_2$ would cause T_2 to increase. Therefore, we estimate that $T_{2b} \sim 10^{-4}$ s. The lifetime of hydrogen on an amine group depends on the hydrogen ion concentration (in moles decimeter^{-3}):

$$\tau_b \sim 2.7 \times 10^3 \times [H^+]\ s \tag{6}$$

(28). Therefore, Eq. 4 is satisfied for the $-NH_2$ protons for pH <10. As pH is lowered, the exchange rate decreases and the effect of the amine groups on T_2 of water would reduce (25). Then, the apparent T_2 would increase and the contribution of the slowly decaying part would become more prominent. This was indeed observed for glycerinated muscle (Fig. 2 and Table II). In these samples, the water content decreased slightly when pH was lowered. There was a corresponding decrease in T_1, consistent with previous observations on intact muscle (29). However, both T_2 and the contribution of the slowly decaying part increased with the lowering of pH (Table II), indicating the importance of hydrogen exchange on transverse relaxation. The pH in intact muscle cannot be adjusted as freely as in glycerinated muscle fibers, but it does decrease to ~5.7 during postmortem rigor (31). Accompanying this decrease in pH, an

obvious increase in the apparent T_2 and the nonexponentiality of the proton spin echoes was observed in the postmortem rigor of intact muscle (4), giving another indication that transverse relaxation in muscle water is affected by hydrogen exchange. This pH-dependent change of proton T_2 of water in muscle and in glycerinated muscle should be distinguished from that in liquid water. Proton T_2 in liquid water is slightly smaller than T_1 due to effects of $^1H—^{17}O$ spin-spin splitting and proton exchange (natural abundance of $^{17}O \approx 0.037\%$, $\Delta\omega \approx 580\ s^{-1}$). T_2 shows a minimum at pH 7.0 and increases at higher and lower pH, but its total change with pH is quite small ($\Delta\ [1/T_2] = 0.1\ s^{-1}$; reference 30).

For other exchangeable groups such as amide, $-OH$, and $-SH$, the proton dipolar splitting $\Delta\omega$ would be smaller than that of $-NH_2$ because of longer distances from the nearest protons (Eq. 5). Except for phenolic hydrogens, the lifetimes of hydrogen on these groups are longer (32, 33) than that on $-NH_2$. Therefore, the condition in Eq. 4 may also be satisfied by some of these groups. The quadrupolar splittings of deuterium are one order of magnitude larger than the dipolar splittings and depend on the type of functional group (34). The rate of hydrogen exchange would be only slightly affected by isotope substitution, but T_{2b} would be one order of magnitude smaller. Consequently, Eq. 4 would also be applicable to deuterium for some functional groups, and nonexponential decay which has characteristics different from that of hydrogen would be observed (Fig. 1 and Table I). The dependence of proton and deuteron spin echoes on the direction of the muscle axes with respect to the magnetic field (35, 36) can be explained by the angular dependence of $\Delta\omega$ of the exchangeable hydrogens in the proteins. The temperature dependences of proton and deuteron T_2's in muscle also indicated the importance of hydrogen exchange in the transverse relaxation of muscle water (4).

The arguments given above are only order-of-magnitude estimations, but do offer reasonable explanations for several observations in the study of spin-spin relaxation of water in muscle. In a complete analysis of the problem, cross-relaxation between nonexchangeable protein protons and water protons in muscle (37, 38) must also be considered. However, due to the complexity of the problem, quantitative solutions seem to be intractable. It has also been suggested (39, 40) that the nonexponential decay of the spin echoes may be due to effects of the diffusion of water in the cells.

In summary, we have shown that the slowly decaying part of the proton spin echoes in muscle is not due to extracellular water, and not likely due to organic protons. The most likely cause of this and the unusually short T_2's for ^1H and ^2H is the hydrogen exchange of intermediate rates between water and functional groups in the protein filaments. Since the characteristics of the magnetization decay depend on $\Delta\omega$'s and exchange rates in a complicated way (25, 26), a multiexponential fit of the spin echo data does not appear to be a desirable way of analyzing the results of spin-spin relaxation of water in muscle.

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