Differential Osmotic Behavior of Water Components in Living Skeletal Muscle Resolved by ^{1}H-NMR

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ABSTRACT Using frog sartorius muscle, we observed transverse relaxation processes of ^{1}H-NMR signals from myowater. The process could be well described by four characteristic exponentials: the extremely slow exponential of relaxation time constant $T_2 > 0.4$ s, the slow one of $T_2 \sim 0.15$ s, the intermediate one of 0.03 s $< T_2 <$ 0.06 s, and the rapid one of $T_2 < 0.03$ s. Addition of isotonic extracellular solution affected only the extremely slow exponential, linearly increasing its amplitude and gradually increasing its T_2 toward that of the bulk solution (1.7 s). Therefore, this exponential should represent extracellular surplus solution independently of the other exponentials. At two thirds to three times the isotonicity, the amplitude of the intermediate exponential showed normal osmotic behavior in parallel with the volume change of the myofilament lattice measured with x-ray diffraction. In the same tonicity range, the amplitude of the rapid exponential showed converse osmotic behavior. Lower tonicities increased the amplitude of only the slow exponential. Studied tonicities did not affect the T_2 values. The distinct osmotic behavior indicated that each characteristic exponential could be viewed as a distinct water group. In addition, the converse osmotic behavior suggested that the rapid exponential would not be a static water layer on the macromolecule surface.

INTRODUCTION

Osmotic activity of skeletal myowater has been studied for a long time. So far it is well documented that living skeletal muscle appears to change a part of its volume following the van't Hoff equation leaving the rest osmotically inactive (1). The apparent osmotically inactive fraction was reported to account for 25–34% of the fiber volume (1–3).

Belton et al. (4) inferred that the osmotically inactive volume fraction would coincide with the nonfreezing water component, which could be distinguished from other water even at physiological temperatures by the transverse relaxation of ^{1}H-NMR signals. That is, from the multiexponential decomposition of the relaxation process, they resolved three water components characterized by distinct relaxation time constants (T_2); slow ($T_2 \sim 250$ ms), intermediate (40 ms), and rapid (10 ms) components comprising 15%, 65%, and 20% of total myowater, respectively. With lowering of temperature, they identified the rapid exponential component to be nonfreezing water at −80°C. Considering this water to reside in the vicinity of macromolecules such as protein, they simply inferred that the slow exponential component would represent extracellular water, and the intermediate one would represent water in both myofibrillar and intermyofibrillar space. Since then, water components of distinct T_2 values have been confirmed by various researchers (5,6), but their localization has not been fully established (7,8).

In this study, we intended to correlate apparent osmotic behavior of subcellular divisions of skeletal muscle with that of the myowater components distinguished by T_2. From the distinct osmotic behavior of the components, we reconsidered their local distribution in the muscle.

METHODS

Solutions

Isotonic Ringer solution contained 115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, and 5.0 mM HEPES (*N*-[2-hydroxyethyl] piperazine-*N*′-[2-ethanesulfonic acid]). The solution was adjusted with NaOH to pH 7.4 at room temperature. Ringer solutions of test tonicities were prepared by the reduction or addition of NaCl to the isotonic Ringer solution. A convenient notation of tonicity was adopted that expressed NaCl concentration relative to that of the isotonic Ringer solution. For instance, removal of 57.5 mM NaCl yielded hypotonic Ringer solution of relative tonicity $57.5/115 = 0.5\ T$ or $T^{-1} = 2$. We used this convenient notation for simplicity, keeping in mind that this notation slightly overestimated the tonicity difference from isotonicity.

Specimens

Frogs (*Rana japonica japonica*) were used in accordance with the guiding principles for the care and use of animals approved by the Council of the Physiological Society of Japan. From a pithed frog, sartorius muscle was dissected out under a stereomicroscope with special care not to injure the constituent muscle fibers. When necessary, a dissected specimen was stored in the isotonic Ringer solution at 5°C for at most 4–5 h.

^{1}H-NMR measurement

The specimen was tied at both tendinous ends to a glass capillary (1 mm outer-diameter; Narishige, GD-1, Tokyo, Japan). Sarcomere lengths of the specimens measured with He-Ne laser diffraction were in the range 2.3–2.4 μm. Before the measurement, the specimen was equilibrated with the Ringer

Submitted January 17, 2005, and accepted for publication April 29, 2005.

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0006-3495/05/08/1143/07 $2.00

doi: 10.1529/biophysj.105.059717

solution of test tonicity for 40–50 min. Then the specimen on the capillary was slowly slid up the rim of a beaker four times to drain surplus solution and enclosed in a sample tube (4 mm inner diameter, Shigemi, Tokyo, Japan) to prevent drying. Care was taken to keep the specimen away from the tube wall. When settled in the superconducting magnet of the spectrometer (Varian, Gemini 2000 300BB, Palo Alto, CA), the specimen located within the volume of the sample coil with its fiber axis aligned along the static magnetic field of the magnet.

The specimen was reused several times at various tonicities of ascending or descending order starting from the isotonic Ringer solution. When the contribution of the surplus solution around the specimen was studied, Ringer solution was added to the specimen or the muscle on the capillary was gently blotted on filter paper. The blotted muscle was not reused anymore because its superficial muscle fibers showed fibrillation after the blotting procedure, suggesting damage on the fibers.

Spin-echo signals were elicited by the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence at cycle times of 0.25 ms. Measurements were performed at 5°C. A total of 204 transverse relaxation processes from 53 separate muscles was analyzed.

Multiexponential decomposition

The method adopted to decompose the relaxation process to exponentials was essentially the same as in Hazlewood et al. (5). In brief, we roughly assumed that the process was a summation of several exponentials of discrete T_2 values each representing the signals from non- (or slowly) exchanging water components, i.e.,

$$f(t) = \Sigma f_i(t)$$
$$f_i(t) = f_{0i} \exp(-t/T_{2,i}),$$

where $f(t)$ is the echo amplitude at time t; and f_{0i} and $T_{2,i}$ are the amplitude and apparent T_2 of the ith component, respectively. We started the curve decomposition by finding the slowest exponential $f_1(t)$ that fitted the last part of the recorded relaxation data and subtracted this fraction from the data. From the residuals, the second slowest exponential $f_2(t)$ was found and subtracted. In this manner extraction of the slowest exponential from the residuals was repeated until the root mean square of the final residuals reached the overall noise level of 10^{-3}–10^{-4} of the initial echo amplitude.

Although spectral rather than the discrete distribution of T_2 values may be a more realistic view, the decomposition yielded reproducible results that were suited for the analysis of compiled results from different specimens.

X-ray diffraction

A specimen was tied at the tendinous ends to the hooks of an experimental chamber with a Mylar window. Fresh Ringer solution always perfused the specimen throughout the experiment. Solution tonicity was changed by switching the perfusing solution. Whenever tonicity was changed, 40–50 min were allowed to attain equilibrium. Sarcomere lengths of the specimens measured with He-Ne laser diffraction were in the range 2.3–2.4 μm.

The equatorial x-ray diffraction patterns were recorded on an x-ray film (Kodak, DEF-5, New Haven, CT) with a double-mirror Franks camera (9,10) at room temperature. The x-ray source was a rotating-anode generator (Rigaku, RU-200, Tokyo, Japan) operated at 40 kV and 30 mA with a copper target. The specimen-film distance was 228 mm, and most of the beam path was evacuated. The exposure time was 10–30 min. The spacing of the 1,0 reflections was measured with a comparator (Nikon, V-12, Tokyo, Japan).

Wet and dry weight

The wet weight of the specimen was measured after the gentle draining of surplus solution by slowly pulling it up the rim of a beaker four times as in the case of the NMR experiment. To determine the dry weight, the specimen was dried at room temperature for several days and in vacuum for an additional 6–8 h just before the gravimetry. To calculate the average dry/wet weight ratio, we selected the specimens of wet weight >9 mg because smaller muscles caused large errors. The mean wet weight of the selected specimens was 16.1 mg ($n = 205$), and the dry/wet weight ratio was 0.20 ± 0.04 (mean ± SD; $n = 149$). This ratio agreed well with the value reported in Blinks (1).

RESULTS

Transverse relaxation process of isotonic muscle

Fig. 1 shows a typical transverse relaxation process (in semilog plot) of the ^{1}H-NMR signals from water protons of sartorius muscle at isotonic condition. Obviously, a single exponential could not fit the whole process, indicating that water in the skeletal muscle could be classified into more than several components.

Usually, at least four exponentials of characteristic T_2 were necessary to obtain acceptable final residuals: an extremely slow exponential of $T_2 > 0.4$ s, a slow one of $T_2 \sim 0.15$ s, an intermediate one of 0.03 s $< T_2 <$ 0.06 s, and a rapid one of $T_2 < 0.03$ s. We hereafter denote the water components represented by the exponentials as the respective exponential components. For instance, the extremely slow exponential component denotes the water component represented by the extremely slow exponential in the relaxation process. The volume of the exponential components would be in proportion to the amplitude of their respective exponentials.

Intentional addition of extracellular solution

To clarify the contribution of extracellular surplus solution around the specimen to the relaxation process, we intentionally

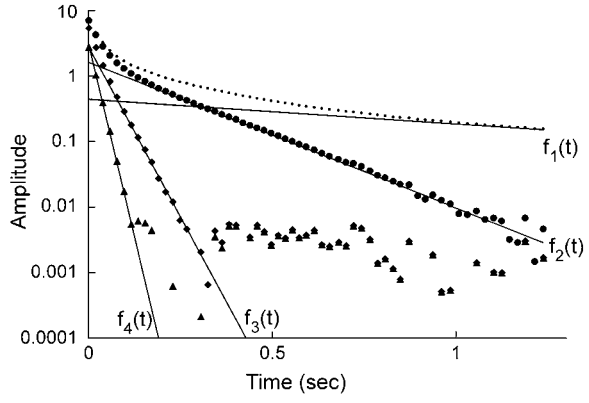

FIGURE 1 Representative CPMG echo decay showing its decomposition into four characterisitc exponentials. Note the logarithmic scale on the ordinate. Small dots show raw data, and the lines represent the fitted exponentials $f_i(t)$. From the raw data, extremely slow exponential $f_1(t)$ was subtracted to yield solid circles, slow exponential $f_2(t)$ was further subtracted to yield solid diamonds, and then intermediate exponential $f_3(t)$ was subtracated to yield rapid exponential $f_4(t)$ (solid triangles). Every 20 data points were shown for clarity. Note the final residuals that little depend on the expansion time representing the measurement noise.

added the isotonic Ringer solution to the specimen. With the volume of the added solution, only the extremely slow exponential linearly increased its amplitude (Fig. 2 *A*) gradually increasing its T_2 toward that of the bulk Ringer solution (1.70 ± 0.02 s, mean ± SE, $n = 9$; Fig. 2 *B*). The amplitudes in Fig. 2, *A* and *B*, are expressed relative to the sum amplitude of the blotted specimens. The oblique line in Fig. 2 *A* shows the expected relationship when the extremely slow exponential component coincides with blottable water. The fair agreement of the results with the oblique line indicates that the extremely slow exponential component is almost identical to the blottable water, and the varied amount of surplus solution around the specimen does not affect the other exponentials. This feature of the extremely slow exponential enabled these analyses of the other exponentials

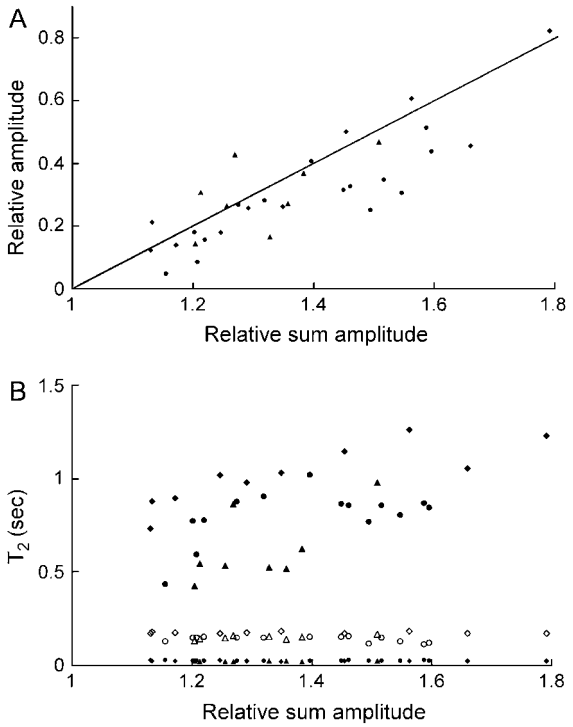

FIGURE 2 (*A*) Amplitude variation of the extremely slow exponential induced by isotonic surplus solution added to muscle specimens. Each symbol shape represents one of three muscle specimens. The ordinate shows the amplitude of the extremely slow exponential, and the abscissa shows the sum amplitude of the four characteristic exponentials. The amplitude on both axes is expressed relative to the sum amplitude of the four characteristic exponentials in the gently blotted specimen. The oblique line shows the expected relationship when whole blottable solution corresponds with the extremely slow exponential component. (*B*) T_2 variation induced by the addition of isotonic surplus solution to the muscle specimens of Fig. 2 *A*. Each symbol shape represents the respective muscle specimen in Fig. 2 *A*. Each set of the vertically aligned three points represents T_2 of the extremely slow (*upper solid*), slow (*middle open*), and intermediate (*lower solid*) exponentials decomposed from a single relaxation measurement. The rapid exponential T_2 is not shown for clarity. As in Fig. 2 *A*, the abscissa shows the sum amplitude of the four characteristic exponentials relative to that of the gently blotted specimen.

without the blotting maneuver, which was observed to damage the constituent fibers (see Methods). We could exclude the procedure dependent blottable water by defining tissue water as the sum of the slow, intermediate, and rapid exponential components. Normalization of volume or amplitude could be accomplished by expressing them relative to those of the tissue water at the isotonic condition ($T^{-1} = 1$). For instance, the slow, intermediate, and rapid exponential components amounted to 23%, 53%, and 24% of the tissue water on average, respectively.

On a likely assumption that the volatile water roughly corresponds with the four exponential components, we can estimate the fraction of the tissue water in the whole tissue volume. Note that the whole tissue volume includes non-water volume and CPMG invisible water volume in addition to the tissue water volume (Fig. 5). In these routinely drained specimens, the extremely slow exponential comprised ~9% of the four exponentials. Because the dry/wet ratio of the whole specimen was 0.2, 9% of the four exponentials amount to $(1 - 0.2) \times 0.09 = 0.07$ of the whole specimen. Subtracting this fraction representing the blottable water from both the water volume and the whole volume of the whole specimen (Fig. 5), the tissue water fraction in the whole tissue volume is estimated to be (tissue water)/(whole tissue volume) = $(0.80 - 0.07)/(1 - 0.07) = 0.78$. We adopted this rough estimate to convert the volume expression relative to the tissue water to that relative to the whole tissue volume in the following analysis.

Effect of osmosis on the water exponentials

In Fig. 3 *A*, typical relaxation processes obtained at hypotonic and hypertonic conditions are shown after excluding the extremely slow exponential from each process. Note that the abscissa of the figure is scaled logarithmically to show the initial part of the relaxation processes. It clearly shows that the tissue water increased and decreased at the hypo- and hypertonicities, respectively. In Fig. 3 *B*, we compiled all the decomposed results by plotting the volume of each exponential component relative to the estimated whole tissue volume at $T^{-1} = 1$ (see above). The highest tonicity examined was $T^{-1} = 0.33$. At $T^{-1} < 1.5$, the volume of the slow exponential component stayed almost constant at 18 ± 0.28% (mean ± SE). The intermediate exponential component increased its volume linearly with T^{-1} and reached a peak at $T^{-1} \sim 1.5$. Its extrapolated volume at $T^{-1} = 0$ was − 4.8 ± 4.3% (95% confidence limit). This means that almost all of the intermediate exponential component behaves as a normal osmometer. At the same tonicity range, the rapid exponential component showed converse osmotic behavior linearly decreasing its volume with T^{-1} until its substantial disappearance at $T^{-1} \sim 2$. The extrapolated volume of this component was 35 ± 2.8% (95% confidence limit) at $T^{-1} = 0$. At the lower tonicity range of $1.5 < T^{-1} < 2$, the slow exponential component increased. So the whole

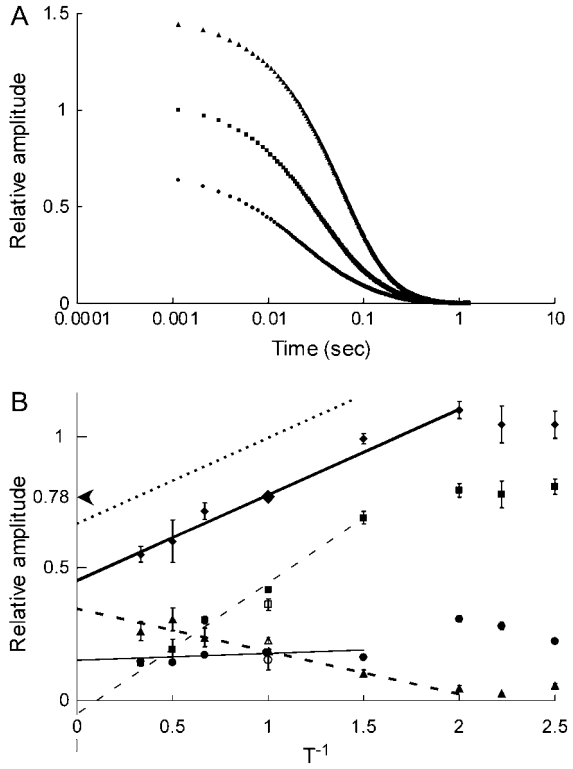

FIGURE 3 (*A*) Typical transverse relaxation processes of tissue water protons at $T^{-1} = 1$(■), 2(▲), and 0.5(●). The ordinate shows the echo amplitude relative to the tissue water amplitude (the sum amplitude of the slow, intermediate, and rapid exponentials) at $T^{-1} = 1$. The extremely slow exponential was subtracted from each data point. Expansion time on the abscissa is expressed on a logarithmic scale. (*B*) The volume of the exponential components as a function of T^{-1} ((●) slow; (■) intermediate; (▲) rapid; and (♦) their sum representing tissue water). The volume is expressed relative to the estimated whole tissue volume at $T^{-1} = 1$ assuming the tissue water fraction to be 0.78 (*arrowhead*; see text). The attached bars, some of which are within the symbol size, show mean ± SE. The thick solid line is the regression line for the tissue water, the thin solid line for the slow exponential component, the thin dashed line for the intermediate one, and the thick dashed line for the rapid one. The dotted line parallel to the thick solid line represents the estimated whole tissue volume. Shown by the open symbols are obtained after the extreme test tonicities ((○) slow after $T^{-1} = 0.33$; (□) intermediate after $T^{-1} = 2.5$; and (Δ) rapid after $T^{-1} = 1.5$). Reversibility after any other test tonicity was better than the shown cases.

volume of the tissue water behaved as a normal osmometer at the tonicity range of $0.33 < T^{-1} < 2$ including apparently osmotically inactive volume fraction of 45 ± 5.4% (95% confidence limit). The whole tissue volume showed a peak at $T^{-1} \sim 2$. At all the tested tonicities, every exponential changed almost reversibly without any marked changes in T_2 (data not shown).

Osmotic changes of the myofilament lattice volume

To observe the osmotic behavior of subcellular divisions in the specimens treated identically with those of the NMR experiment, we performed x-ray diffraction measurements of myofilament lattice. Their mean 1,0 lattice spacing was 35.1 nm (mean of $n = 22$) in isotonic Ringer solution ($T^{-1} = 1$), 30.4 nm ($n = 4$) at $T^{-1} = 0.33$, and 39.4 nm ($n = 3$) at $T^{-1} = 4$. The square of the 1,0 spacing, representing the myofibrillar volume, is plotted against T^{-1} in Fig. 4. It indicates that the myofibrillar volume increased linearly with T^{-1}, began to level off at $T^{-1} \sim 1.5$, and reached a plateau of $\sim 40^2$ nm^2 at $T^{-1} \sim 2$. The regression line at T^{-1} from 0.33 to 1 intersects the ordinate at 60 ± 7.8% (95% confidence limit) of the isotonic value.

DISCUSSION

Curve decomposition

In this study, four characteristic exponentials were extracted from the transverse relaxation process of ^{1}H-NMR signals from skeletal muscle (Fig. 1). We assumed that each exponential represented a noninterexchanging group of water molecules. Of course, each group does not necessarily represent a single uniform water compartment. A more realistic view might be a group of water molecules with spectral T_2 distribution and slow interexchange with other groups. However unique spectral distribution of T_2 values could not be obtained because of the experimental noise (11). The reproducibility of the discrete decomposition method, on the other hand, facilitated the analyses on the compiled results from different specimens. Therefore, we conveniently adopted this method of decomposition.

In our preliminary study, using the semispectral nonnegative least square method with and without a smoothing condition for the spectrum (11), we confirmed the clear-cut distinction between the water groups in the muscle. Further justification for our temporal grouping of water comes from this observation of the distinct behavior of each water group against the solution addition (Fig. 2) and osmosis (Fig. 3).

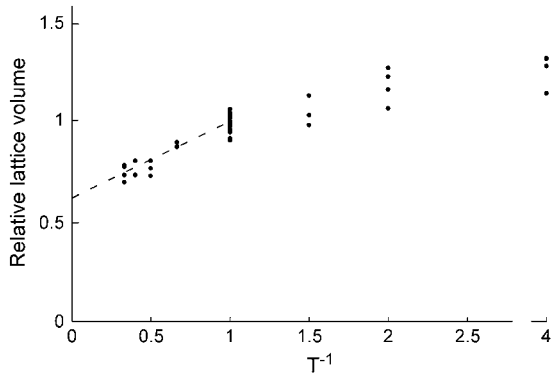

FIGURE 4 Volume of the myofilament lattice as a function of T^{-1}. The volume is expressed relative to that at $T^{-1} = 1$ where the square of the mean 1,0 spacing was 1.23×10^3 nm^2. The dashed line represents the regression line in the range of $0.33 < T^{-1} < 1$, which intersects the ordinate at 60% of the isotonic value.

For instance, T_2 values of the exponentials were little affected by osmosis despite the marked changes in their amplitudes. This indicates that the proton exchange between the exponential components would not be, at least, critically affecting the relaxation process.

Extremely slow exponential

The extremely slow exponential had not been reported by previous researchers (4,5). This supports our identification of the extremely slow component as the extracellular blottable water because they had blotted the surplus solution around their specimens. Massive expansion of the extracellular space induced by the addition of surplus solution increased T_2 of the extremely slow exponential without developing an additional exponential component. This indicates that the protons in the massively expanded space could keep sufficiently rapid diffusional exchange with the protons of shorter intrinsic T_2 within the component. Since the effective diffusion of bulk water is expected to extend no more than 0.1 mm in a second from the diffusion constant of the bulk water (e.g., 1.3×10^{-9} m^2s^{-1} at 5°C) (12), the added surplus solution would be held in the swollen network of the interstitial matrices around the specimen without forming a bulk separate phase of the solution.

Particularly notable about the extremely slow exponential component is the finding that it behaved independently of the other three exponential components. This indicates that the protons of the extremely slow exponential component do not appreciably exchange with the protons of the other exponential component at a timescale of T_2. This is a surprising feature because the extracellular nonblottable water would contain the slow exponential component as discussed below and because, according to Fung and Puon (13) and Cole et al. (7), cell membrane does not set an effective boundary for the exponential components. In any event, this particular feature enabled this study without the blotting maneuver, which was observed in this study to damage the constituent fibers of the specimen (see Methods).

These characteristic exponentials other than the extremely slow exponential agreed reasonably well with those of previous researchers despite the difference in specimen size, source, and handling procedures.

Slow exponential

The slow exponential component stayed almost constant at $0.33 < T^{-1} < 1.5$, consistent with its assignment by previous researchers (4,5) as nonblottable extracellular water. If the slow exponential component is identical with the nonblottable extracellular water, however, an unlikely situation arises where tonicity affects extracellular water compartments without affecting the fiber volume. This is because at the tonicity range of $1.5 < T^{-1} < 2$, only the slow exponential component increased its volume without affecting the intermediate and rapid exponential components which are considered intracellular. Therefore, at least at the extreme hypotonicity, a part of the slow exponential component represents intracellular water. Since myofilament lattice volume was observed to stay at a plateau in this tonicity range (Fig. 4), the increased intracellular slow exponential component would reside in the intermyofibrillar space. A likely explanation is that the expansion of the intermyofibrillar space makes some water molecules there relatively free from the influence from the macromolecules so as to convert them to the slow exponential component from a faster (probably intermediate) exponential component.

Osmotic behavior of fiber volume

From the regression line for the volume at $T^{-1} < 2$, 67% of the whole tissue volume appears to be osmotically inactive (Fig. 3 *B*). This is incompatible with the reported values of the osmotically inactive volume fraction in isolated fibers (33%, 1; 34%, 3) if the osmotic behavior of the constituent fibers of muscle tissue is assumed to be the same as that of single isolated fibers. This is because, in this case, nonblottable extracellular water would occupy the unlikely large fraction of 50% of the whole tissue volume to account for the osmotically inactive volume fraction as $0.5 + 0.5 \times 0.34 = 0.67$. On a more probable assumption that the steady slow exponential component at $0.33 < T^{-1} < 1.5$ (18% of the whole tissue volume; Fig. 3 *B*) represents the extracellular nonblottable water, the osmotically inactive volume fraction of fibers in tissue is estimated to be $(0.67 - 0.18)/(1 - 0.18) = 0.60$. Therefore, the osmotic behavior of the fibers in tissue would be different from that of the isolated fibers. Fair reversibility of these results indicates that the difference is not due to the degeneration of the constituent muscle fibers. We consider that the difference is due to the rich connective matrices that are removed from the isolated fibers. They would limit the fiber volume through the elevation of intracellular hydrostatic pressure as inferred in (14), and as a result, the osmotically inactive fraction of the fiber volume in tissue would appear larger than that of isolated fibers.

Osmotic behavior of lattice volume

To complement information on the osmotic behavior of subcellular divisions in these specimens, we performed the myofilament lattice volume measurements. The lattice volume also showed an osmometer-like behavior at $0.33 < T^{-1} < 1$ (Fig. 4). Various previous researchers have observed similar behavior of the lattice with osmotically inactive volume fraction of 38% (15), 53% (16), and 28–31% (17). All these values are smaller than the value here of 60%. The cause of the difference is not clear, but it is not due to the blunting of the correlation at extreme tonicities (2,17) because elimination of the data points at $T^{-1} < 0.5$ did not appreciably affect the regression line in Fig. 4.

Since lattice volume naturally represents myofibrillar space, the osmotically inactive volume fraction of the myofibrillar space would roughly coincide with that of the fiber volume (60%; see above). This suggests that not only the myofibrillar space but also the intermyofibrillar space behaves as a normal osmometer.

Intermediate exponential

Since the intermediate exponential component showed the normal osmotic behavior (Fig. 3 *B*), this component is likely to reside in both the myofibrillar and intermyofibrillar space. From the linear relationship with T^{-1}, the volume of this component at $T^{-1} = 0$ is extrapolated to be negative ($-4.8 \pm 4.3\%$). Reminding us that our convenient notation of tonicity overestimates the difference from isotonicity, the intermediate component expanded to a larger extent than the ideal osmometer with T^{-1}. Probably this exponential component accepted some solutes from another component, for instance, the dissolving rapid exponential component with T^{-1} (see the possibilities 2 and 3 in the following subsection).

Rapid exponential

The localization of the rapid exponential component is not evident from its osmotic behavior. However, it is natural to consider the rapid exponential component to be in the vicinity of the macromolecule surface as proposed by previous researchers (4,5). In this case, the rapid exponential component would occupy a considerable volume fraction in the myofilament lattice where myoproteins are packed densely.

Now, what is the mechanism for the converse osmotic behavior of the rapid exponential component? Is there any inevitable reason for the coincidental leveling off of hypotonic fiber expansion with the disappearance of the rapid exponential component at $T^{-1} \sim 2$ (Fig. 3 *B*)? We considered the following three possibilities for the dissolving mechanism of the rapid exponential with T^{-1}:

1. With the increase of T^{-1}, macromolecular solutes responsible for the rapid exponential component gradually leaked out from the muscle fibers constituting the specimen to show its apparently converse osmotic behavior. Although this possibility could set the expansion limit of the fiber volume at the disappearance of the rapid exponential component, it can be ruled out because of the fair reproducibility of the tonicity effects (Fig. 3 *B*).
2. With the increase of T^{-1}, the rapid exponential component gradually dissolved into other exponential components such as an intermediate one in response to the increase in the hydrostatic pressure that we considered to limit the fiber volume in tissue as described above. This possibility may link the expansion limit with the disappearance of the rapid exponential component. Our preliminary experiment, however, indicated that the application of air pressure of up to 1.5×10^5 Pa to the specimen caused no appreciable effect on the exponentials.
3. The rapid exponential component represents not a static water layer on the macromolecule surface but the water molecules that are simultaneously affected by multiple surfaces of the macromolecules. Releasing the overlapping influence from the multiple surfaces, fiber expansion with the increase of T^{-1} would decrease the multiply affected water molecules to dissolve the rapid exponential component to the other components. In this possibility, the coincidence of the swelling limit and the disappearance of the rapid exponential component would be accidental. This is because isolated fibers were reported to expand beyond the expansion limit of this tissue although fiber isolation would not affect the overlap of influences from macromolecule surfaces on the intracellular water molecules. To account for the considerable amount of the rapid exponential component, the influence should be a long-range one such as electrostatic force and hydrophobic interaction. Further study is awaited to verify this possibility and to clarify the nature of the influence.

CONCLUSION

The osmotic behavior of the T_2 exponentials of ^{1}H-NMR was examined in frog sartorius muscle. Comparing with the osmotic behavior of subcellular divisions in the muscle, we reconsidered the localization of the water components represented by the exponentials as summarized in Fig. 5:

1. We found an extremely slowly relaxing exponential of $T_2 > 0.4$ s. This exponential was identified to represent blottable solution around the specimen and was found to behave independently of other exponentials.

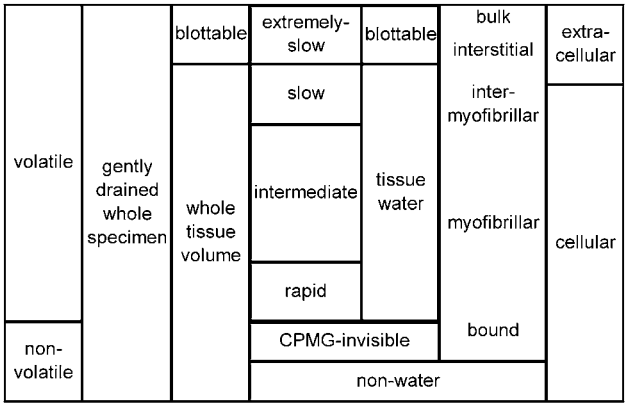

FIGURE 5 Simplified diagram summarizing the proposed interrelationship between various volume divisions in the whole muscle specimen. The nonwater fraction in the extracellular space was neglected for simplicity.

2. The amplitude of the slow exponential stayed almost constant with moderate tonicity changes, whereas it increased at $1.5 < T^{-1} < 2$ where fibers expanded without the expansion of the myofilament lattice. The slow exponential is, therefore, considered to represent extracellular nonblottable water as well as some water molecules in the intermyofibrillar space.
3. The intermediate exponential of $0.03\ \text{s} < T_2 < 0.06\ \text{s}$ appeared to expand linearly at $0.33 < T^{-1} < 1.5$. This exponential is likely to represent water in the major part of myofibrillar and intermyofibrillar space.
4. The rapid exponential of $T_2 < 0.03$ s exhibited converse osmotic behavior. This exponential would mainly represent water in the vicinity of macromolecules. From its interesting behavior, we consider that the rapid exponential may not represent a static water layer on the macromolecule surface but the water molecules that are influenced by multiple macromolecule surfaces.
5. The osmotically inactive volume fraction of whole sartorius muscle tissue was 67%. It is much larger than that of isolated fibers, suggesting that the osmotic behavior of fibers in whole muscle tissue is different from that of isolated fibers.

A part of this work was supported by grants-in-aids for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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