Structure and function of skeletal muscle and locomotive systems: Involvement of water-state transitions

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Abstract Skeletal muscle, fascia, tendon, cartilage, and bone are all of mesodermal origin and constitute a continuous musculoskeletal system. It is necessary that this locomotive system be sufficiently robust to support mechanical impacts and body weight, and to allow friction-free joint movement. As a strategy to fulfill these apparently conflicting requirements, tissues of locomotive systems are proposed here to utilize differential water states and their transitions, on the basis of analyses of structure and function of skeletal muscle and cartilage. Although many studies on physical fitness and sports medicine are now focusing on functional aspects of cytoskeletons and solutes such as proteins and signaling molecules, another breakthrough may be possible from conscious awareness of the role of water molecules supplying a universal field for functioning molecules.

Keywords : Locomotive system, water, MRI, skeletal muscle, cartilage

Introduction

Water is essential for the evolution of life on earth. Except in extremely rare cases, such as tardigrades in a state of cryptobiosis¹⁾, no living organism can survive without water. Any functioning molecules such as proteins and signaling molecules need surrounding water molecules to interact.

Water found in living organisms is distinctly different from bulk water. The well-known differences include physicochemical properties such as freezing point and saturation vapor pressure. Various viable organisms can be found in the freezing cold polar regions because of the low freezing points of their body fluid²⁾. To understand the effect of low saturation vapor pressure, consider the curing process of juicy meat to produce jerky. In this process, some of the water in the meat evaporates easily, while some of the water remaining in the jerky barely evaporates. These facts clearly indicate that living organisms utilize water not only as a convenient solvent but also as an indispensable component.

Differences in water states are generally considered to be a consequence of their interactions with surrounding solutes and structures that form the cytoskeleton. Depression of freezing point and saturation vapor pressure are well-known colligative properties of aqueous solution, which are principally independent of the details of its solutes³⁾. When sweating during summer, our perspiratory glands secrete dilute salt solutions that are more close to ideal solutions with respect to colligative proper-

ties⁴⁾. Even if colligative properties are principally solute independent, they are a consequence of the interactions between water and solutes. With increases in the concentrations and molecular sizes of solutes, solute-specific effects on the properties of solution increase rapidly. Therefore, almost all fractions of our body fluids are far from ideal solutions.

An extreme example of a strong interaction between water molecules and a neighboring molecule is found in hydration water (crystallization water). These water molecules are tightly incorporated into a crystal to form hydrates without forming any covalent bonds with the host molecule. The number of water molecules trapped by the host molecule depends on the temperature of the crystallization process. These water molecules are barely evaporated unless the crystal is heated to a considerable temperature. In case of biomolecules such as proteins, the total volume of hydration water is as much as half the crystal volume.

One may easily imagine that living organisms consist of a continuous spectrum of water states ranging from almost ideal solutions to hydration water. All biochemical reactions in an organism would take place in various water states that are distinctly different from the bulk solutions used in biochemistry laboratories. Thus, it seems natural to assume that evolution and the current functions of life have taken advantage of various water states.

In this review, the ability of living organisms to utilize water molecules in various states and the transition of water molecules between these states is discussed by focusing on the components of vertebrate locomotive systems, i.e., skeletal muscle, fascia, tendon, cartilage, and bone.

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The variety of water states in living organisms

Magnetic resonance (MR) images, which are now widely used in clinical practice, provide convincing evidence of various water states in living organisms. This is because the densities observed in MR images are based on local contents as well as the water states. The MR imaging system identifies water states by detecting nuclear magnetic resonance (NMR) signals from the nuclei of hydrogen atoms (protons). The signals from homogeneous water decay exponentially by the spin-spin relaxation mechanism. The rate of relaxation is generally expressed as the time constant of an exponential decay process, generally referred to as T_2 .

Water in a bulk solution of low viscosity has a high T_2 value of approximately 2 s. The precise T_2 value depends not only on temperature but also on the protocol used. This topic is beyond the scope of this review article. Those interested in this topic should consult specific textbooks such as the one by Cowan⁵.

In contrast, water molecules restricted within ice exhibit extremely low T_2 values. Analysis of a time series of MR images reveals that water proton signals arising from different parts (voxels) decay at varying rates, ranging from those of bulk solution to those of ice (Fig. 1). MR signals of any voxel in soft and solid tissues consist of multiple exponential decay processes. This indicates that water in any minute tissue is heterogeneous and consists of multiple components that can be distinguished by their T_2 values.

1. Decomposition analysis

To identify various water states in tissue, it is necessary

to decompose the multi-exponential decay processes in the time series of MR signals. For precise decomposition analysis, it is necessary to obtain decaying MR signals as continuously as possible. From this perspective, a tomography protocol is time consuming; thus, acquisition of NMR signals from homogeneous tissue is beneficial (Fig. 2). Acquisition of truly continuous data is, however, technically impossible, even if NMR measurements are performed. It becomes necessary to decompose discrete time series data of decaying NMR signals. With this restriction, and by assuming many elementary exponential decay processes, Whittall and Mackay⁶ attempted to obtain quasi-spectral smooth distributions of water states in various tissue. They found that water states could be grouped into several components with characteristic T_2 values (cf. Fig. 3).

Another method to decompose actual time series data is the data stripping method originally described by Haselwood et al.⁷. Because exponential components with low T_2 values diminish to almost negligible levels in the earlier part of actual time series MR signal intensity data, the exponential component with the highest T_2 value is extracted by applying a single exponential curve-fitting procedure to the latter part of the time series data. After subtracting this extracted component, the procedure of extracting the exponential component with the next highest T_2 value is iterated until all exponential processes that can be detected in the time series data are extracted. This data stripping method appears to yield a set of a minimal number of exponentials that sufficiently reproduce the original time series data. This is confirmed by performing non-linear curve-fitting trials using test time series data

Fig. 1 Time series (20ms time interval; left to right in four successive rows of upper to lower) magnetic resonance (MR) images of a left knee joint. A 1.5T clinical scanner with a Flex-M coil (Philips Medical, Best and Heeren, the Netherlands) was used under the approval of the ethical committee of Tokyo Dental College. A single slice 32-multi-echo sequence was performed with the following acquisition parameters: FOV=180mm, matrix size=256×256, thickness=3mm, TR=5000ms, TE=20ms, total scan time=5m45s for one serial images.

(Fig. 2). The test data are generated by combining several exponential components along with random Gaussian noise. The densities of the time series data and the noise magnitude are set so that they are similar to the actual data found in the study by Takemori et al.⁸⁾. The curve-fitting procedures were iterated assuming an increasing number of exponentials starting from a single exponential (Fig. 4). With an increase in the number of assumed exponentials, the squared sum of deviations decreases dramatically until the number of assumed exponentials reaches the number of original exponentials combined to generate

Fig. 2 Representative time series MR data. The present time series is an artificially generated time series composed of five exponentials and random noise of normal Gaussian distribution. It mimics that of Carr-Purcell-Meiboom Gill pulse sequence obtained from an isolated muscle cell (skinned fiber) in the work of Takemori et al.⁸⁾.

the test data.

Comparing the results of the two extreme decomposition procedures, the quasi-spectral decomposition method and the data stripping method, it has been observed that the latter method is beneficial in grouping water states according to the characteristic T_2 values, even if continuous spectral distributions of water states may be more realistic. Because the spectral distributions of T_2 values are clustered, as reported for various biological tissues⁶⁾, the characteristic T_2 values obtained by the data stripping method represent a cluster of water states with similar T_2 values.

2. Local distributions of water states

Using the data stripping method, Haselwood et al.⁷⁾ identified three water groups in rat skeletal muscle tissues. They assumed that these water groups represent macromolecule-associated water (8%), myoplasmic water (82%), and extracellular water (10%).

Kimura et al.⁹⁾ and Takemori et al.⁸⁾ extended this work using skeletal muscle, with the following advantages, as a sample biological tissue.

(1) Trained muscle physiologists can prepare homogeneous intact skeletal muscle specimens that well retain their physiological properties in vivo. Frog muscle is particularly stable due to its low metabolic rate at rest.

(2) Because of the extraordinarily large dimensions of skeletal muscle cells and the intense MR signals from water protons, NMR measurements can be performed for a

Fig. 3 Results of our quasi-spectral decomposition analysis of an actual MR time series obtained from an isolated cell (skinned fiber) of frog skeletal muscle. Non-linear non-negative curve-fitting algorithm implemented in MATLAB software (Mathworks, Natick, MA, USA) is used. With an increase in the number of assumed exponentials (n_{exp}), yielded quasi-spectral results converge to discrete spikes of a limited number. The amplitudes and T_2 values of characteristic exponentials are reasonably close to the decomposition results obtained with the data stripping method.

Fig. 4 The squared sums of deviations plotted in a logarithmic scale against the numbers of exponentials assumed in obtaining optimized fits. No-linear non-negative curve-fitting algorithm of MATLAB was used to obtain optimized fit to the time series shown in Fig. 2. Note that the time series is artificially composed of five exponentials and normal Gaussian noise.

single cell.

(3) The cell membrane of a skeletal muscle cell can be mechanically removed under a stereomicroscope to prepare a specimen of the cytoplasm (myoplasm) called a skinned fiber¹⁰⁾. In the absence of the diffusion barrier of the cell membrane, the solution used to bath the myoplasm of skinned fibers can be directly controlled.

(4) The cytoplasm (myoplasm) of a skeletal muscle cell is a simple repeat of half-sarcomere units (Fig. 5). The volume of this unitary structure¹¹⁾ is $10\text{nm} \times 35\text{nm} \times 1,000\text{nm} = 350,000\text{nm}^3$. This structure contains at most 10 million water molecules, which is a tractable number.

(5) The spacing of longitudinal and cross-sectional repeats of the half-sarcomere units can be monitored using light diffraction techniques under physiological conditions without the need for fixation. The longitudinal sarcomere spacing can be easily monitored using a laser diffraction technique, and the cross-sectional myofilament repeats can be monitored using an x-ray diffraction technique.

Kimura et al.⁹⁾ identified more than five water groups in intact living frog muscle and determined their locations referring to the sarcomeric construction of the myoplasm (Fig. 5). After intentionally adding an extracellular solution to the muscle specimens, they first ascribed extracellular water as the main component of the water groups with the highest T_2 values of approximately 1 s. To determine the intracellular locations of other water groups, they equilibrated intact muscle specimens in Ringer solutions of varying tonicities and decomposed their data using the data stripping decomposition method. They searched for corresponding spatial expansion or shrinkage in myoplasmic spaces using light diffraction techniques.

When soaked in Ringer solution of low tonicity, the potential gradient of water molecules drove the flow of

water from the solution to the muscle tissue. Note that the potential μ of water is generally given as follows:

$$\mu = \mu_0 + RT \ln \gamma [\text{H}_2\text{O}] + \pi \bar{V},$$

where μ_0 is the standard potential of a mole of water molecules; R is the universal gas constant ($8.31\text{ JK}^{-1}\text{mol}^{-1}$); T is the absolute temperature (K); γ is the activity coefficient of water; $[\text{H}_2\text{O}]$ is the concentration of water molecules; π is the hydrostatic pressure; and $\bar{V}$ is the molar volume of water³⁾. Among these variables, μ_0 and γ can vary largely depending on the water states. When a lower T_2 value is considered to represent lower mobility caused by stronger restrictions from the surrounding solutes, water groups with lower T_2 values would have larger γ and smaller μ_0 values.

The flow of water to the muscle tissue expands the water group with the third highest characteristic T_2 value of approximately 0.05 s. On the basis of sub-cellular structural considerations of the intra-sarcomere space, this water group is assigned to be the water fraction residing in the sarcomere space. The expansion of this water group shows no marked changes in its characteristic T_2 value. This suggested that volume expansion is not due to an ordinary osmosis process in which the water molecule potential increases with the dilution of the solute; i.e., an increase in water concentration. Marked dilution of solutes would cause significant increases in the characteristic T_2 value toward that of bulk solution. Rather, an almost stable T_2 value with volume expansion suggests that the expansion would proceed with a shift of solutes from another water group, i.e., with an increase in water potential through mechanical work ($\pi \bar{V}$) exerted on the cross-sectional lattice structure of a sarcomere.

The water group that supplies the shifted solutes would be the group with the fourth highest T_2 value of approximately 10 ms. This group shrinks with a decrease in the tonicity of the bathing solution without a significant change in its characteristic T_2 value. It is considered to reside close to the surfaces of structural proteins, such as the thick and thin filaments in the intra-sarcomere space. The surfaces of structural proteins would act to stabilize water molecules of this water group by decreasing their standard potential (μ_0). The increase in the equilibrating water potential μ in the bathing solution limits this water group to a smaller volume, where the interaction from the protein surfaces can increase at a sufficient intensity.

Water groups represented by the remaining exponential components were little affected by changes in tonicity. These water groups would be tightly confined in the vicinity of protein surfaces by adjusting their μ_0 in accordance with the equilibrating bathing value of μ .

These propositions were confirmed by the study of Takemori et al.⁸⁾ in which the decomposition results of proton NMR signals from intact and skinned muscle cells were compared. As expected, diffusion of macromolecular solutes from a cell after removal of its membrane in-

Fig. 5 A schematic representation of an elementary unit of skeletal muscle. The right panel shows the longitudinal section of sarcomere and the left panel shows its cross section. A represents a thin filament, M represents a thick filament, and S1 represents a head of a myosin molecule protruding out from the thick filament.

creased the third highest T_2 component at the expense of the fourth highest T_2 component. The indiffusible solutes supporting the third highest T_2 component would be the protruding mobile parts of sarcomere proteins such as myosin heads.

Use of water states in locomotive systems

1. Physiological roles of water states in muscle contraction

During the processes involved in the evolution of life, there is another good reason to consider that water in various states plays an indispensable role. It is now documented that many proteins require proper interactions with surrounding water molecules to realize their physiologically folded states that are necessary for performing their respective functions. Thus, it is interesting to consider the work of Suzuki's group. By performing dielectric dispersion measurements, they found that clusters of water molecules in the vicinity of thin filaments had higher mobility than those in bulk solution¹²⁾. They coined the phrase 'hyper-mobile water' to characterize such water clusters. They also observed larger clusters of hyper-mobile water in the vicinity of thin filaments in the presence of myosin¹³⁾. They presented the idea that hyper-mobile water clusters afford asymmetric fields of mobility or viscosity along a thin filament to realize unidirectional migrations of myosin heads at the expense of free-energy derived from MgATP hydrolysis (chemomechanical coupling).

According to the theoretical work of Kinoshita and Suzuki¹⁴⁾, thin layers of hyper-mobile and restricted water molecules are distributed periodically around a negative charge-rich domain of an actin molecule. According to them, the thicknesses of these layers should be less than the diameter of a water molecule. Therefore, on the time scale of NMR measurements, the mobility of water molecules wandering around these layers would have been averaged and prevented the detection of hyper-mobile water clusters.

In any event, the presence of water in states of widely ranging mobility strongly suggests that skeletal muscles

use these states as scaffolds for mechanical propulsion and as temporary reservoirs of free energy.

2. Use of water states in skeletal muscle as a locomotive tissue

Skeletal muscle is one of the major tissues that provides support and realizes locomotive activity in animals. Other component tissues include fascia, tendon, cartilage, and bone. These locomotive tissues are all of mesodermal origin and constitute a continuous musculoskeletal system. They are required to provide mechanical strength of considerable magnitude and, at the same time, friction-free joint movement.

For example, the extensor muscles of terrestrial animals must shorten rapidly and generate forces of considerable magnitude for the animals to be able to jump to a particular height against gravity. Mechanical robustness sufficient to transmit force and smooth muscle shortening with minimal friction are requirements that are difficult to fulfill simultaneously. In addition, counteracting flexor muscles should elongate passively without any apparent resistance. The sarcomere structure tends beneficial characteristics to skeletal muscle for the simultaneous fulfillment of these requirements.

Iwamoto et al.¹⁵⁾ showed that the perfectly crystalline structures of sarcomeres enabled self-organized oscillatory contraction-relaxation cycles in the flight muscles of insects that beat their wings at high frequencies without oscillating intracellular Ca^{2+} concentrations. This type of self-organized contraction-relaxation cycle of sarcomeres has also been observed in vertebrate skeletal muscle as spontaneous oscillatory contractions. In Natori's laboratory, repetitive propagating contraction waves were observed in intact muscle cells in the presence of quinine¹⁶⁾. Later, during the course of experiments on relaxing factors of skeletal muscle, Goodall and Szent-Györgi¹⁷⁾ noticed spontaneous oscillations in glycerin-extracted skeletal muscle fibers under sub-maximally activated conditions. Natori¹⁸⁾ observed that repetitive contractions in membrane-devoid muscle cells (skinned fibers) propa-

gate along the myofibrils. Similar propagating contraction waves have been reported under various conditions in skeletal muscle¹⁹⁻²¹⁾ and cardiac muscle²²⁾. Okamura and Ishiwata²³⁾ reproduced these propagating contraction waves in isolated myofibrils and named them spontaneous oscillatory contractions (SPOCs) of sarcomeres. Sasaki et al.²⁴⁾ found a linear relationship between physiological heart rates and the characteristic frequencies of SPOCs in dissected myofibrils of cardiac muscle. This evidence seems to confirm that the crystalline order of the sarcomere structure is required to realize instantaneous switching between the states of contraction and relaxation, even with control of the Ca^{2+} concentration.

Another aspect of the sarcomere structure that should be noted is the effective osmotic coefficient of intracellular diffusible solutes. Perforation of the membrane of a skeletal muscle cell results in the diffusion of various molecules from the cell²⁵⁾. However, Maughan and Godt²⁶⁾ measured the osmotic coefficient of parvalbumin in intact sarcomeres and determined that it was comparable to the osmotic coefficient in a simple salt solution in a 4% agarose gel. This suggests that parvalbumin dissolves in the water compartment having a state not drastically different from the state of bulk solution. This intracellular water compartment would be in the water group characterized

by the third highest T_2 values in NMR and MRI measurements. The main difference between water molecules in a homogeneous solution and those in a sarcomere structure is considered to be due to the crystalline alignment of structural proteins. Kimura et al.⁹⁾ proposed that the crystalline order integrates the elementary effects from constituents to generate domains with distinct water states.

Taken together, skeletal muscle is considered to use water states and their transitions for transducing the free energy derived from MgATP hydrolysis to mechanical contractile force (chemomechanical coupling) and realizing mechanical robustness and low compliance simultaneously.

3. Use of water states in cartilage

In case of cartilage, the requirements are mechanical robustness and friction-free sliding at the interfaces of joints. To fulfill these requirements simultaneously, the cartilage microstructure is designed to realize an ice-like water state.

As well known in ice skating, from a vertical aspect, ice is sufficiently mechanically robust to endure the impact of landing by skater, while horizontally, ice provides sufficient lubrication to allow friction-free sliding along the tangential path. These features of ice originate from the strong hydrogen bonding network of water molecules²⁷⁾. This network spreads the vertical impact of landing both deeply and widely through the ice; melting of the hydrogen bonds allows horizontal friction-free skating along a particular path.

To realize an ice-like robustness at body temperature, cartilage near bone should be comprised of collagen fibrils aligned at right angles to the bone surface. The collagen fibrils are accompanied by abundant amounts of chondroitin sulfate. Negative charges on chondroitin sulfate attract cations and its counterions into a brush of collagen fibrils that restrain water molecules to achieve an osmotic equilibrium²⁸⁾ (Fig. 6). These strongly restricted water molecules provide perpendicular robustness to cartilage in order to endure perpendicular impacts and support the body weight against gravity.

In contrast, collagenous fibrils near lubricating interfaces are aligned tangentially along the surface, thus exposing their outer halves to the interface covered by synovial fluid. The tangential element of impacts imposed on the interface liberates water molecules on the cartilage surface from restrictions, thereby enabling friction-free sliding at the interface.

4. Use of water state transitions in other locomotive systems

When viewed in the context of the strategies employed by skeletal muscle and cartilage, the histological constitution of other locomotive tissues would have evolved to utilize water states and their transitions to fulfill the mechanical and kinetic requirements imposed on them. To elucidate the details of the strategies employed by these

Fig. 6 A schematic representation of water states in cartilage structure. The upper panel shows the general microscopic structure of cartilage, in which cartilage cells are embedded in abundant matrices. The lower panel shows osmotic water restriction exerted by negative charges on chondroitin sulfate.

locomotive tissues, further experimental work from the view point of water states and their transitions is necessary. Any progress in our understanding of the roles of water molecules to supply a universal field for functioning molecules would also assist in the advancement of studies on physical fitness and sports medicine, not only in the field of locomotive musculoskeletal systems but also in other fields.

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