Department of Molecular Physiology

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General Summary

Our efforts have been concentrated on clarifying molecular mechanisms of the dynamic function of living cells from the aspect of protein-water interaction.

Research Activities

Model of intracellular water

Magnetic resonance (MR) images depict biological tissues on the basis of the differential transverse relaxation of water proton nuclear MR signals. Using skeletal muscle sarcomeres as a biological model of general biological tissues, we have shown that the relaxation process represents the restriction of water molecules from the surrounding tissue structure. To find nonbiological materials that restrict water molecules similarly to biological tissues, we tested nanoporous silica and reverse micelles. Water states in the nanoporous silica were similar to those in skeletal muscle sarcomeres, whereas those in reverse micelles were far less restricted by the interface of the micelles. These results confirmed that the restriction of water molecules is highly dependent on the nature of the interface between the water phase and the surrounding structure. Because the coexistence of myosin and actin in sarcomeres has been shown to strongly restrict sarcomeric water molecules, it is reasonable to consider that the contractile interaction between myosin and actin involves the transition of water molecules as a reservoir of free energy. We further hypothesized that biomolecular systems generally adopt similar strategies involving the restriction of neighboring water molecules to realize their physiological functions.

Evaluation of water state in living muscle from MR images for effective training

The obturator internus and externus muscles are important for stabilizing the hip joint. Patients with locomotor disabilities often need to train these muscles as part of their rehabilitation. To develop effective training methods, the activity of these muscles must be evaluated with MR because they are difficult to access with electromyography or ultrasonography. In this study, we evaluated the activity after exercise of the obturator internus and externus muscles with MR, which is generally considered effective from the view point of anatomical observation.

Before and immediately after 3 minutes of external rotation of the right hip joint at a frequency of 0.25 Hz, transverse relaxation processes were obtained with the multiecho Carr-Purcell-Meiboom-Gill sequence with 32 equidistant echos. The processes obtained from the obturator internus and externus muscles were assigned to 2 water groups with moderate time constants of transverse relaxation (T_2) out of 4 water groups

classified on the basis of data from dissected frog skeletal muscle. The water group of longer T_2 increased its relative content after exercise compared with the resting state in the obturator internus and externus muscles on both sides. The increase was especially prominent in the obturator externus muscle on the exercised side. These results are consistent with data from dissected frog muscle in the contracting state.

X-ray diffraction study of molecular mechanisms of cardiomyopathy due to troponin mutation

The E244D troponin T (TnT) mutant that causes hypertrophic cardiomyopathy increases the maximal tension of cardiac muscle fibers. To clarify how this mutation enhances the capacity to develop tension, we have been performing structural analysis by means of simulations of molecular dynamics and X-ray diffraction experiments.

Molecular dynamics at a steady state showed that the formation of electrostatic bonds between troponin I (TnI) and TnT involving the mutated amino acid was suppressed in E244D troponin, although its main-chain structure was essentially identical to that of the wild type. Molecular dynamics studies in a stressed condition showed that the mutation did not significantly affect the connectivity of TnI to TnT and suggested that the mutation has no significant effect on signal transduction from TnI to TnT.

To verify this hypothesis, we performed X-ray diffraction experiments on skinned muscle fibers in which endogenous troponin was replaced with wild-type or E244D TnT. The intensity of troponin reflection in both fibers suggested that the structural arrangement of E244D and wild-type TnT on thin filaments was similar. In fibers in which E244D TnT was introduced, the transition from the resting state to the contracting state was accompanied by a greater change in intensity of the myosin layer lines than in wild-type fibers; this finding indicates that a larger fraction of myosin heads are recruited to contractile interaction in E244D TnT fibers.

These results suggest that the rearrangement of local electrostatic bonding triggered by the mutation causes an abnormal interaction between TnT and tropomyosin and leads to a larger shift of tropomyosin. This larger shift of tropomyosin would allow increased recruitment of myosin heads to the actomyosin interaction so as to enhance the tension-development capacity in the E244D mutant.

Evaluation of the water state by viscosity measurement

Polyethylene glycol (PEG) narrows the lattice spacing of skinned skeletal muscle sarcomeres. Because the estimated size of PEG (molecular weight, 3,350) is several nanometers and the myofilament lattice spacing of 40 nm would allow PEG to penetrate, water within the sarcomere might have a different property to inhibit penetration by PEG. To test this hypothesis, the concentration of PEG within the sarcomere was estimated by measuring the specific gravity of myofibril suspensions from rabbit psoas muscle in the presence or absence of PEG. If PEG does not freely penetrate the sarcomere, the specific gravity of the supernatant after centrifugation of the myofibril suspension is expected to be larger than that before centrifugation.

The concentration of PEG within the sarcomere was half that outside the sarcomere, indicating that water within the sarcomere has a different property from the bulk water

surrounding the myofibrils. This finding supports our hypothesis.

Publications

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