Department of Molecular Physiology

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General Summary

Our efforts have been concentrated on clarifying mechanisms for achieving biological functions from the viewpoint of the cooperative interaction of water and proteins.

Research Activies

Utilization and disorders of water states in the vertebrate locomotive system

The locomotive systems of terrestrial animals should be strong enough to support posture against gravity and compliant enough to enable economical movement. To extract general strategies of vertebrate locomotive systems, we analyzed cartilage and skeletal muscle and evaluated their water state by the use of a nuclear magnetic resonance (NMR)/magnetic resonance imaging (MRI) technique that observes the relaxation process of tissue water protons. Tissue water in both cartilage and skeletal muscle could be grouped into several components on the basis of characteristic relaxation rates. Functional modulation of the tissues accompanied significant changes in the distribution of tissue water among the components without marked changes in the characteristic relaxation properties of each water component. Healthy cartilage of humans and pigs exhibited gradual distribution of the water fraction with slower relaxation rates: smaller in the layers near the bone surface and larger at the synovial surface. Because water with the slower relaxation rates has been shown to be more strongly restricted by the structural proteins, this gradual distribution is consistent with the mechanical robustness of the cartilage near the bone surface and smooth gliding at the articular interfaces. We believe that both cartilage and skeletal muscle utilize the water state to realize their specific functions in locomotive systems.

Effect of pressure caused by subjects' weight on MR images

The transverse relaxation process of muscle tissue as shown with MRI is a powerful indicator of muscular activity in the field of rehabilitation and sports medicine. However, the factors that affect the transverse relaxation process of muscle tissue are poorly understood.

For example, MR images are normally obtained with the subjects lying on their backs, but the effect of the pressure caused by their weights on MR images of the superficial muscles, such as the gluteus maximus and the soleus, has not been investigated in detail. To clarify this point, we acquired transverse relaxation processes of superficial muscles and deep muscles with MRI and analyzed the results on the basis of our multiexponential model for the water state in muscle tissue established by NMR measurements of dissected muscle tissue from animals.

In both types of muscles, the transverse relaxation processes were successfully classified into 2 exponentials with the characteristic time constants. In one of the superficial muscle (soleus) with a longer time constant, the relative amplitude gradually increased after the subjects lay on their backs. Because disturbing venous return with a rubber band did not significantly increase the relative amplitude, an unknown mechanism, other than the reasonable increase in the extracellular water volume caused by the weight of subjects, is likely to be involved.

Water activity in the myofilament lattice evaluated by means of specific gravity measurement

To evaluate the water activity within myofibrils, the specific gravity of myofibril suspensions from rabbit psoas muscle was measured in the presence or absence of polyethylenglycol. If polyethylenglycol does not penetrate the myofilament lattice, the specific gravity of the supernatant after centrifugation of the myofibril suspension should be greater than that of the myofibril suspension before penetration. Polyethylenglycol was found to diffuse into the myofilament lattice at half of the external concentration, indicating that the water activity within the myofilament would be different from that of the bulk water.

Structure of troponin mutant studied by X-ray diffraction

An E244D troponin T (TnT) mutant that causes hypertrophic cardiomyopathy is known to increase maximal tension of cardiac muscle fiber (Ohtsuki et al., 2003). To clarify how this mutation enhances the capacity for tension development, we performed X-ray diffraction experiments with skinned muscle fibers in which endogenous troponin had been replaced with wild-type or E244D TnT at BL15A at the Photon Factory (High Energy Accelerator Research Organization, Tsukuba).

The characteristics of the troponin reflections in both fibers did not differ, suggesting similar structural arrangement of E244D and wild-type TnT on thin filaments. The transition from resting to contracting states accompanied a larger change in the intensity ratio of equatorial reflection (1,1/1,0) in E244D fibers than in wild-type fibers, indicating that a larger fraction of myosin heads are recruited for contractile interaction in E244D fibers.

These results suggest that the rearrangement of local electrostatic bonding triggered by the mutation brings about abnormal interactions between TnT and tropomyosin to cause a larger shift of tropomyosin. This larger shift of tropomyosin would allow increased recruitment of myosin heads to interact with actin and would enhance tension-development capacity in the E244D mutant.