Department of Physiology (I)

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General Summary

Our efforts have been concentrated on clarifying the mechanism of skeletal muscle contraction.

Research Activities

Dynamics of the sarcomere lattice structure of striated muscles

Regulated contractile interaction of striated muscle is performed within the liquid crystalline structure consists of thin actin filaments and thick myosin filaments. To understand the physiological contraction mechanism of structurally organized striated muscles, it is essential to understand the dynamics of its lattice spacing upon sarcomere-length perturbation. Using gelsolin to selectively remove thin actin filaments from skinned sarcomeres, we prepared the specimen which consists of thick myosin filament lattice without thin actin filaments. On the 45XU beam line at the Large-scale Synchrotron Radiation Facility (Spring 8, Hyogo Prefecture), we performed an x-ray diffraction study of the gelsolin-treated skinned striated muscles. The gelsolin-treated muscle was found to preserve physiological fine structures except for those related to thin filaments. Monitoring sarcomere spacing with the laser diffraction technique and passive tension with a force transducer, we observed the dynamic response of the gelsolin-treated lattice against sarcomere-length perturbation. The obtained results indicate that the lattice spacing was not determined by any of the mechanisms so far proposed, including the Y-shaped elastic connectin filament model and the Derjaguin, Landau, Verwey, and Overbeek model. We are now attempting to develop a novel model that accounts for the dynamics of lattice spacing and our previous results obtained with nuclear magnetic resonance experiments on the dynamics of myowater. This work was performed in collaboration with Drs. Okuyama and Toyota of Kawasaki Medical University and Dr. Yagi of the Large-scale Synchrotron Radiation Facility.

Structural change of mutant troponin related to hypertrophic cardiomyopathy

Many troponin mutants causing familial cardiomyopathy have been reported. To clarify the molecular mechanism of cardiomyopathy related to troponin, we performed a molecular dynamics study of the structure of troponin mutants related to familial hypertrophic cardiomyopathy (HCM). Two different troponin-T mutants, Glu244Asp and Lys247Arg, which are related to HCM were studied.

Dynamics was calculated with the Amber software package (version 9). Iteration was done in a TIP3P water sphere with 0.5- or 1-femtosecond time-step in periodic boundary condition at constant temperature (310 K) and pressure. Model structures of troponin mutants were constructed by introducing the mutation to the crystal structure of human

cardiac troponin (core region of the TIC complex) obtained from Protein Data Bank (ID number 1J1E). More than 8 trajectories of 1 nanosecond were obtained for wild and mutant structures.
We found that that electrostatic interaction between troponin I and troponin T which linked alpha helices of troponin T and troponin I in the wild type was lost in the mutant. Furthermore, when a terminal residue of troponin I was pulled toward an actin molecule mimicking the intramolecular force on relaxation, the structural change observed differed between the mutant and the wild type. This difference is likely involved in the enhanced tension development in mutant troponin.

The effect of trehalose on muscle contractility
Trehalose is a disaccharide present in various organisms which protects proteins or cellular membranes from inactivation and denaturation due to dehydration, heat, and cold, which can cause drastic changes in the water state. On the other hand, trehalose is reported to reduce the dynamics of water molecules through its hydrogen-bond network which is stronger than those of other disaccharides. Under the hypothesis that trehalose influences muscle contractility through changes in the water state, we examined the effect of the disaccharides trehalose and sucrose on the contractility of skeletal muscle.
Mechanically skinned fibers were prepared from sartorius muscles of bullfrogs, and pCa-tension curves were obtained with or without 0.5 M disaccharides. Before the experiment, the internal membrane systems of the fibers were destroyed and the sarcomere length was adjusted to 2.4 μm. The Ca sensitivity was decreased by 0.4 units with both disaccharides, whereas maximum tension was increased by 10% with trehalose alone. The differing effects of the disaccharides on the maximum tension would not be due to the increase in viscosity, which did not differ between the disaccharides. These results suggest that the change in water state induced by trehalose affects muscle contractility stereochemically.

Specific volume of myofibril suspension with polyethyleneglycol
Polyethylenglycol narrows the lattice spacing of skinned skeletal muscle sarcomeres. Because the polyethylenglycol molecule (molecular weight, 3,350) is several nanometers in size, a lattice spacing of 40 nm seems to be large enough to allow polyethylenglycol to penetrate. To determine whether polyethylenglycol penetrates the sarcomere, 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 sarcomere, the specific gravity of the supernatant after centrifugation of the myofibril suspension would be larger than the specific gravity of the myofibril suspension. Our results suggest that polyethyleneglycol does not penetrate the sarcomere.