Department of Molecular Physiology

Shigeru Takemori, *Professor* Maki Yamaguchi, *Assistant Professor*

General summary

Our efforts have been concentrated on elucidating molecular mechanisms for achieving biological function through the cooperative interaction of water and proteins.

Research Activity

Differential scanning calorimetry of water components in cells

Our previous studies of water in skeletal muscle sarcomeres found at least 5 water components distinguished by characteristic spin-spin relaxation rates. The overall interaction between water molecules and structural macromolecules has been shown to restrict water activity to differentiate these water components. However, details of the interaction remain unknown. To reveal the property of the interaction in these water components, we evaluated the phase-transition of water with differential scanning calorimetry, which gives direct information about interaction energies between molecules. As a first step, we observed the phase-transition process of pure water and confirmed that the pure water was supercool until 253 K and then froze reproducibly.

Dehydrating effects on contraction in skinned skeletal muscle

We recently found that small organic molecules, such as poly-ethylene-glycol (molecular weight, 8,350), which is thought to partially penetrate the sarcomere space, dehydrate skinned skeletal muscle similarly to the macromolecules that dehydrate osmotically. We investigated the contractile properties of skinned muscle dehydrated by macromolecules, such as Dextran T-500 (molecular weight, 500,000), or small organic molecules. Fibers dehydrated with Dextran showed increased maximum force and no change in Ca^{2+}-sensitivity. On the other hand, fibers dehydrated with polyethylene glycol showed significantly decreased maximum force and Ca^{2+}-sensitivity. This result suggests that the water groups removed from the sarcomere by polyethylene glycol differ from those removed by Dextran.

Viscoelastic change of the myosin solution evaluated with a quartz crystal microbalance

The viscoelastic properties of the myosin adsorbed to the surface of gold electrodes and its surrounding solution as a whole were studied with a molecular interaction analyzer (AFFINIX QN Pro, Initium, Inc., Tokyo).

When myosin was adsorbed at a density less than 0.2 μg/cm^2, the viscoelastic change accompanying myosin adsorption was almost the same as the viscoelasticity of buffer without myosin. The resonance frequency falled with the weight of adsorbed myosin. This finding suggested that myosin adsorbed at a low density acts as a solid globular pro-

tein. On the other hand, when myosin was adsorbed at a higher density, a remarkable viscoelastic change was observed. Also, the binding of ATP to the myosin head changed the viscoelasticity of the protein. These results indicated that myosin acts as a protein having characteristic viscoelastic property.

Mutations of ryanodine receptor in malignant hyperthermia

Malignant hyperthermia (MH) is a pharmacogenetical complication of general anesthesia resulting from abnormal Ca^{2+}-induced Ca^{2+} release (CICR) via the type 1 ryanodine receptor (RyR1) in skeletal muscles. Although more than 200 mutations of the RyR1 gene have been reported in patients with MH, only a few of these mutations have been confirmed with experiments as being responsible for increases in CICR sensitivities, because complicated procedures are required to make the desired mutations in the long complementary (c) DNA of RyR1 and because of the low transfection efficiency of the mutant DNAs. We characterized the functional mutations of RyR1 in nonmuscle cells, specifically HEK293 cells with tetracycline-regulated RyR1 expression, prepared by improved method for making MH mutants in the cDNA of RyR1. Some mutations of the RyR1 were found to enhance CICR sensitivity; therefore, these mutations would be responsible for the incidence of MH. These results suggest that exploration of the functional mutations of RyR1 is effective for the preventive diagnosis of patients with MH disease.

Structural analysis of cardiac muscle fibers causing hypertrophic cardiomyopathy

The E244D- and K247R-troponin-T (TnT) mutants, which cause familial hypertrophic cardiomyopathy, have been shown to enhance calcium-dependent contraction on cardiac muscle fibers. To clarify the mechanism of this enhancement, we performed X-ray diffraction experiments with skinned muscle fibers to which wild-type/E244D/K247R mutant troponin T had been introduced. When E244D/K247R-TnT was introduced into the cardiac muscle fibers, intensity of the second actin layer line, which reflects structural change of tropomyosin on thin filaments, increased on contraction compared with that of fibers to which wild-type TnT had been introduced. This result indicates that in the E244D/K247R mutant, a larger shift of the tropomyosin would induce enhanced tension development and trigger the hypertrophy of cardiac muscle.

Publications

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