Department of Molecular Physiology

Shigeru Takemori, *Professor*
Maki Yamaguchi, *Associate Professor*
Toshiko Yamazawa, *Associate Professor*

General Summary

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

Research Activities

Differential scanning calorimetry measurement of water components in skinned skeletal muscles

Magnetic resonance images reflect not only water content, but also water states in the tissue. By taking advantage of well-organized skeletal muscle, we have recently clarified that magnetic resonance can be used to distinguish localized water clusters of 5 states. However, the nature of each water state has not been clarified in detail. Interaction between water and macromolecules such as myoproteins in skeletal muscle is considered to restrict their mutual motional freedom. From this, it follows that water and macromolecules would gain additional motional freedom absorbing extra heat with temperature similarly to the melting of ice. With differential scanning calorimetry (DSC), we observed the absorbing extra heat with temperature on skinned fibers. We observed 2 significant extra heat absorption at −22˚C, −25˚C and at about the melting point of water. Additionally, we observed more two peaks at 45˚C and 65˚C in a temperature-dependent irreversible manner. These irreversible heat affected on the heat absorption at −22˚C, −25˚C independently. Electron microscopy showed that the peak at 45˚C caused marked deterioration in A-band and the peak at 65˚C caused thorough deterioration in sarcomeres. Functionally, both of the thick and filament denatured specimens developed no tension by Ca^{2+} addition, while once-frozen specimen developed a half tension of native specimen. These results suggest that the melting of water corresponding to heat absorption at −22˚C and −25˚C would entropically drive the contractile processes in muscle and differential scanning calorimetry can be used to effectively explore the water states in sarcomeres.

Spin-spin relaxation process of ^{1}H-NMR signals from thick-filament removed myofibril suspension

We have been observed spin-spin relaxation process of ^{1}H-NMR signals from myofibril suspension in the four major intermediates during the ATP-hydrolysis by myosin. The results implied that the myosin in M and MT states immobilized many water molecules, and that in MDPi and MD state release the water molecules. Although this change of the number of water molecules restricted by myosin heads in the different intermediate states was qualitatively consistent with the results from myosin solution and myosin filament solution, the number of the water molecules restricted by myosin heads was much larger

than those in the myosin filament solution or myosin solution. To confirm the hypothesis that the difference of the number of the water molecules restricted by myosin heads is largely originated from the highly packed lattice structure formed from thick and thin filaments, we performed NMR measurement of myofibril suspension from which thick filaments were removed. The results showed that the number of the restricted waters significantly decreased by the removal of thick filament supporting the hypothesis.

Correlation of molecular dynamics analysis and Ca^{2+} homeostasis in mutant type 1 ryanodine receptors

In excitable cells membrane depolarization is translated into intracellular Ca^{2+} signals. Ryanodine receptors, located in the sarcoplasmic/endoplasmic reticulum (SR/ER) membrane, are required for intracellular Ca^{2+} release. Malignant hyperthermia (MH) is a disorder of Ca^{2+}-induced Ca^{2+} release (CICR) via the type 1 ryanodine receptor (RyR1) in skeletal muscles. More than 200 mutations have been reported in the RyR1 gene of MH patients. The typical symptoms of MH include a rapid increase in body temperature and induction of a hyper metabolic state with skeletal muscle rigidity. Most of those mutations have been found in three "hot spots" regions of RyR1. However, there were only a few experimental results confirming those mutations being responsible for the increment of the CICR sensitivities. We investigated properties of the RyR1 channels carrying disease-associated mutations at the N-terminal region. HEK293 cells expressing the mutant RyR1 channels exhibited alterations in Ca^{2+} homeostasis, i.e., enhanced caffeine sensitivity, decrease of ER Ca^{2+} contents, increases in resting cytoplasmic Ca^{2+} concentration. Molecular dynamics analysis revealed that changes in pattern of electrostatic interaction were correlated with the alteration in Ca^{2+} homeostasis. Increase of electrostatic interaction between domain-A and domain-B was suggested to play key role to enhance sensitivity to CICR, while, decrease of between domain-A and domain-C was suggested to leak of Ca^{2+} from the ER. This result suggests that exploration of the functional mutations of RyR1 is effective in preventive diagnosis of patients associated with MH disease.

Effect of polyamine administration on the structure and function of the heart

Polyamines such as putrescine are poly-cation molecules indispensable for proliferation of the eukaryotic cells. Polyamines are also known as modulators of ion channels regulating physiological excitability of cardiac cells. Therefore, polyamines may play a significant role in the hypertrophy and arrhythmia of athletes' hearts. To examine the effects of oral administration of polyamine, 6-week-old rats were bred for 9 weeks at four combined conditions of presence and absence of 1 mg/ml of putrescine in drinking water and freely accessible wheel for spontaneous running. During the breeding period, electrocardiograph was recorded to monitor the excitability of the heart. Then, the hearts were excised for structural analyses and HPLC measurements of polyamine content. Putrescine concentration in the cardiac cells increased in the putrescine (+) / exercise (−) group, but not in the putrescine (+) / exercise (+) group. Electrocardiograph and structural parameters including heart weight, thickness of ventricle walls, degree of fibrosis, showed no appreciable effect of putrescine administration with and without exercise. Polyamine was suggested to be strictly controlled to regulate exercise induced hypertrophy of the heart.

Publications

Sato C*[1]*, Kinoshita T*[2]*, Memtily N*[1]*, Sato M*[1]*, Nishihara S*[2]*, Yamazawa T, Sugimoto S (*[1]AIST,** [2]***Soka Univ).*** Correlative light–electron microscopy in liquid using an inverted sem (asem). *Methods Cell Biol.* 2017; **140:** 187–213.

Ohno T, Abe T*[1]*, Sugi H*[2] *(*[1]Shibaura Inst Tech,** [2]***Teikyo Univ).*** Effect of antibodies to myosin head on the development of rigor tension and stiffness in skinned muscle fibers. *J Material Sci Eng.* 2018; **7:** 435.