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 within muscle cells.

Research Activities

Functional water in skeletal muscle evaluated with differential scanning calorimetry
Magnetic resonance images reflect not only water content but also water states in physiological tissue. By taking advantage of well-organized skeletal muscle, we have previously clarified that magnetic resonance can be used to distinguish localized water clusters of 5 states. Although water state in the tissue are not clarified yet in detail, interaction between water and macromolecules is generally considered to restrict motional freedom of the water molecules similarly to the freezing of water. As is the case with melting of ice, differential scanning calorimetry can represent the restriction as extra heat absorption at a certain temperature.
Differential scanning calorimetry measurement with skinned fibers of the sartorius muscle from bull frogs at a rigor condition showed extra heat absorption at −24, −21, 0, 46, and 65 degrees centigrade. The peaks at 46˚C and 65˚C would represent denaturation of myosin and actin filaments, respectively, because selective removal of myosin or actin filaments diminished the corresponding peaks and because the temperature values are close to those reported for the denaturation temperature of corresponding proteins from rabbit psoas muscle. The heat denaturation and selective removal of myosin and actin filaments differentially affected the peaks at −24˚C and −21˚C. The peak at −24˚C was affected mainly by the manipulation of actin filaments, and the peak at −21˚C was affected by both myosin and actin filaments. Integrated heat capacity in the range from −80˚C to +20˚C was decreased by denaturation of actin. The integrated heat capacity per protein mass, after the solution mass was subtracted, was 150% of the control muscle with myosin removal and 25% of the control muscle with actin removal. These results suggest that actin and myosin independently and cooperatively restrict surrounding water and that extra integrated heat capacity mainly depends on actin filaments.

Insights into channel modulation mechanism of skeletal muscle type ryanodine receptor mutants using Ca^{2+} imaging and molecular dynamics
Type 1 ryanodine receptor (RyR1) is a Ca^{2+} release channel in the sarcoplasmic reticulum in skeletal muscle and plays an important role in excitation-contraction coupling. Mutations of the ryanodine receptor 1 gene (*RyR1*) cause severe muscle diseases, such as malignant hyperthermia, which is a disorder of Ca^{2+}-induced Ca^{2+} release via RyR1. So

far, more than 300 mutations in *RyR1* have been reported in patients with malignant hyperthermia. However, due to a lack of comprehensive analysis of the structure–function relationship of mutant RyR1, the mechanism remains largely unknown. Thus, we combined functional studies and molecular dynamics simulations of RyR1 bearing disease-associated mutations at the N-terminal region. When expressed in HEK293 cells, the mutant RyR1 caused abnormalities in Ca^{2+} homeostasis. Molecular dynamics simulations of wildtype and mutant RyR1s were performed using crystal structure of the N-terminal domain (NTD) monomer, consisting of A, B, and C domains. We found that the mutations located around interdomain regions differentially affected hydrogen bonds/salt bridges. Particularly, mutations at R402, which increase the open probability of the channel, cause clockwise rotation of the B and C domains with respect to the A domain by altering the interdomain interactions. Similar results were obtained with artificial mutations that mimic alteration of the interactions. Our results reveal the importance of interdomain interactions within the NTD in the regulation of the RyR1 channel and gain insights into the mechanism of malignant hyperthermia caused by mutations at the NTD.

Effect of homogenate extract of adult skeletal muscle on expression pattern of myosin heavy chain

In adult skeletal muscle, satellite cells are maintained in a state of quiescence until tissue damage or other stimulus causes these cells to become activated, to proliferate, and, subsequently, to differentiate into mature myofibers. Meanwhile, myosin heavy chains (MyHCs) play a critical role in contractility and have various fiber types, such as slow (I), fast (IIa, IIb, and IIx), and extraocular specific types. Previous studies are unclear as to whether activated satellite cells from adult muscle are predisposed organogenetically to express the phenotype of their founder muscle or they differentiate to a standardized post-mitotic stage dependent on extrinsic signals from the host muscle for determination of mature phenotype. To address this issue, satellite cells from mouse extraocular muscles, the diaphragm, and various hindlimb muscles were isolated, expanded, and differentiated under laminin i211-coated culture dishes. We harvested cells during 3 distinct time courses, which were: without differentiation, 3 days after differentiation, and 8 days from differentiation. Proliferating myoblasts and differentiated myofiber cultures were analyzed via sodium dodecylsulfate-polyacrylamide gel electrophoresis and mass spectrographs for expression of MyHCs. The MyHC profile of differentiated primary satellite cells was equivalent across all cultures with embryonic type MyHC and MyHC cardiac beta (MYH7B). Interestingly, slow type MyHC was expressed in satellite cells from the diaphragm but was in satellite cells from extraocular muscle or hindlimb muscles. Mass spectrographs showed expression of nonmuscle MyHC 2A (NM2A) across all cultures. These results suggest that differentiation of myofibers from satellite cells is similar to that of myogenesis and that the determination of phenotypes is due to intrinsic factors or extrinsic factors or both. The expression of NM2A indicates that NM2A plays a major role in myotube fusion.

Effect of homogenate extract of adult skeletal muscle on proliferation and differentiation of myoblasts

Residing next to mature skeletal muscle fibers, satellite cells are known to serve as the progenitor of myoblasts when triggered by various stimuli, including damage to the muscle fibers *in vivo*. The homogenate extract of various types of adult chicken skeletal muscles was reported to induce differentiation of primary culture of myoblasts prepared from chicken embryo to express MyHC isoforms of the source skeletal muscle of the extract. Inspired by this report, we examined the effect of homogenate extract from adult mouse skeletal muscle on primary cultures of satellite cells prepared from young mice. Unexpectedly, the extract seemed to enhance proliferation of the satellite cells without inducing obvious differentiation in the expression pattern of MyHCs. However, the extract's effect on satellite cell proliferation was unclear because of cellular contaminants, such as fibroblasts and adipogenetic cells. Therefore, we examined the effect of muscle extract on C2C12 myoblasts. The proliferation of C2C12 myoblasts was evaluated with the cytotoxic assay method. The extract was found to dose dependently enhance C2C12 myoblast proliferation confirming the stimulating effect of the extract on the growth of the myogenic precursor cells.

Publications

Yamazawa T, Ogawa H, Murayama T, Yamaguchi M, Oyamada H, Suzuki J, Kurebayashi N, Kanemaru K, Oguchi K, Sakurai T, Iino M. Insights into channel modulation mechanism of RYR1 mutants using Ca^{2+} imaging and molecular dynamics. *J Gen Physiol.* 2020 Jan 6; **152**(1): e201812235. doi: 10.1085/jgp.201812235. PMID: 31841587; PMCID: PMC7034096.

Sugi H, Yamaguchi M, Ohno T, Okuyama H, Yagi N. X-ray Diffraction Studies on the Structural Origin of Dynamic Tension Recovery Following Ramp-Shaped Releases in High-Ca Rigor Muscle Fibers. *Int J Mol Sci.* 2020 Feb 13; **21**(4): 1244. doi: 10.3390/ijms21041244. PMID: 32069889; PMCID: PMC7072990.