Department of Molecular Physiology

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

X-ray diffraction study of extraocular muscle

To explore the structural bases of the unique function of extraocular muscle at the molecular level, an X-ray diffraction experiment was performed. First, we measured 1,0 lattice spacing of extraocular muscle fibers in the function of sarcomere length, which is the most representative variable of myofilament structure. The 1,0 lattice spacing of extraocular muscle fibers in distal and end plate regions was found to exhibit the same relationship with psoas fibers. The relationships did not differ from each other for the subgroup consisting of orbital and global layers and indicate that extraocular muscle fibers have the same sarcomere structure from the end plate to the distal region in both layers.

Next, we observed profiles of equatorial reflections of extraocular muscle fibers which give information about the mass distribution of muscle proteins arising from the 1,0 and 1,1 lattice planes. Equatorial reflections of extraocular muscle fibers showed patterns essentially similar to those of psoas fibers, as suggested with electron microscopic observation. However, equatorial reflections, especially 1,0 reflection, appeared to be wider in extraocular fibers than in psoas fibers. Also, within the results from extraocular fibers, 1,1 reflection in samples from the end plate region tended to be lower than that from the distal region. Averaged (1,1/1,0) intensity ratios of equatorial reflections of each species of muscle fiber specimens which have statistically the same sarcomere length distribution showed that the (1,1/1,0) intensity ratio was lower in extraocular muscle fibers than in psoas muscle fibers, suggesting that different kinetic properties of myosin may be a cause of the unique function of extraocular muscle.

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 by absorbing extra heat with tempera-

ture similarly to the melting of ice. With differential scanning calorimetry, we observed the absorption of extra heat with the temperature on skinned fibers. We observed 2 significant extra heat absorptions at −22˚C, −25˚C, and at about the melting point of water. Additionally, we observed 2 more peaks at 45˚C and 65˚C in a temperature-dependent irreversible manner. The 2 peaks at less than −10˚C were affected by the denaturation and the presence of thick/thin filaments. Furthermore, the effect of the presence of thick filaments was different from that of the presence of thin filaments. We found that specimens with intact thin filaments had a larger specific heat capacity (accumulated heat from −80˚C to +20˚C). These results suggest that 1) differential scanning calorimetry can be used to effectively explore the interaction between myoproteins and water and that 2) thin filaments serve a special heat capacity.

Property of water around myoprotein studied with quartz crystal microbalance and nuclear magnetic resonance

We observed the adsorption process of myosin to the gold surface by quartz crystal microbalance to study viscoelastic property of the myosin and its surrounding solution as a whole by means of a quartz crystal microbalance molecular interaction analyzer (AFFINIXQN Pro, Initium, Inc., Tokyo).

When myosin adsorption was sparser than 0.2 μg/cm^2, viscoelastic change accompanied by myosin adsorption was almost the same as the viscoelasticity of buffer without myosin. The resonance frequency falls as does the weight of adsorbed myosin. This implies that myosin adsorbed at low density plays as a solid globular protein. On the other hand, when myosin adsorbed at a higher density, large viscoelastic change has been observed. Viscoelastic analysis indicates that myosin plays as a protein having viscoelasticity and that ATP binding to the head domain of myosin changes the viscoelasticity of the protein. This change suggests that myosins immobilize a surrounding solution when it is closely adsorbed. Half of this immobilized solution was released in the presence of ATP or ADP but not in the presence of ATP-γS.

We also observed the spin-spin relaxation process of ^{1}H nuclear magnetic resonance signals with the suspension of myosin filaments of rabbit psoas muscle in the 4 major intermediates during the ATP-hydrolysis by myosin. The results imply that myosins in the M and M.T states immobilize many water molecules and that myosins in the M.D.Pi and M.D states release water molecules.

Exocrine glands observed in aqueous solution with atmospheric scanning electron microscopy

Exocrine glands, e.g., salivary and pancreatic glands, play an important role in digestive enzyme secretion, while endocrine glands, e.g., pancreatic islets, secrete hormones that regulate blood glucose levels. The dysfunction of these secretory organs immediately leads to various diseases, such as diabetes and Sjögren's syndrome, by poorly understood mechanisms. Glands and gland-related diseases have been studied by optical microscopy, and at higher resolution by transmission electron microscopy of Epon-embedded samples, which necessitates hydrophobic sample pretreatment. We challenged the direct observation of tissue in aqueous solution by atmospheric scanning electron microscopy (SEM).

Salivary sublingual glands, lacrimal glands, and pancreas were fixed, sectioned into slabs, stained with phosphotungstic acid, and inspected in radical scavenger D-glucose solution from below with inverted SEM, guided by optical microscopy from above to help target the tissue substructures. A specimen thickness of 2 to 3 μm was visualized with SEM. In secretory cells, cytoplasmic vesicles and other organelles were clearly observed at high resolution, and the cytoplasmic vesicles could be classified according to the degree of phosphotungstic acid staining. In the islets of Langerhans, the microvascular system used as an outlet by the secretory cells was also clearly observed. The microvascular system is also critically involved in the onset of diabetic complications and was clearly visible in subcutaneous tissue observed with atmospheric SEM. The results suggest that in-solution atmospheric SEM can be used for histology and to study vesicle secretion systems. Furthermore, the high-throughput of atmospheric SEM makes it a potential tool for diagnosing exocrine-related diseases, such as Sjögren's syndrome.

Publications

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Reviews and Books

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