Department of Molecular Physiology

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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

To reveal the properties of interaction in the water component of skeletal muscle sarcomeres distinguished by ^{1}H-nuclear magnetic resonance measurement, we observed the phase transition of water in skeletal muscle by means of differential scanning calorimetry, which provides direct information about the interaction energy between molecules. We found significant enthalpy change at around the melting point of water (0˚C) and at lower temperatures (− 25˚C). The enthalpy change at − 25˚C on fresh specimens was smaller than that on denatured specimens. Furthermore, the difference in enthalpy between fresh and denatured specimens almost coincided with the denaturing enthalpy at > 40˚C. The fresh specimens would have a specific heat capacity 0.04 J/˚C/g higher than that of the denatured specimens. This large excess heat capacity would reside in myoproteins and in water molecules, which bear large entropy, and might serve as a heat sink to support muscle contraction.

Viscoelastic properties of water around myosin

We observed the adsorption process of myosin to a gold surface with a quartz crystal microbalance. Viscoelastic properties of myosin adsorbed to the surface of the gold electrode and its surrounding solution as a whole were studied with a quartz crystal microbalance molecular interaction analyzer (AFFINIX QN Pro, Initium, Tokyo). When myosin was adsorbed more sparsely than 0.2 μg/cm^2, the viscoelastic change accompanying the myosin adsorption was almost the same as the viscoelasticity of buffer without myosin. The resonance frequency fell to the level equivalent to weight of the adsorbed myosin. This finding suggests that myosin adsorbed at a low density acts as a solid globular protein. On the other hand, when myosin was adsorbed at a higher density, a large viscoelastic change was observed. Viscoelastic analysis indicated that myosin acts as a protein having viscoelasticity and that the binding of ATP to myosin heads changes the viscoelasticity of the protein. This finding suggests that myosin immobilizes the surrounding solution when adsorbed closely. Half of this immobilized solution was released in the presence of ATP or ADP but not in the presence of ATP-γS.

Structural change of thick filaments in thin-filament-extracted skinned fibers during the ATP hydrolysis cycle

To clarify the mechanism by which myosin heads convert the chemical energy of ATP to mechanical work, we performed an X-ray diffraction study of myosin heads in skeletal muscle fibers without actin filaments to examine structural changes in myosin during the ATP hydrolysis cycle without interaction with actin. When ATP bound to myosin heads, the peak of the myosin layer line shifted outward, indicating the shift of the center of gravity of myosin heads toward their rods accompanying the transition from the state of myosin without nucleotide ("M") to that of myosin with ADP and Pi ("M-ADP-Pi"). The binding of ADP to myosin heads did not shift the peak of the myosin first layer line, indicating that the myosin structure did not differ significantly between the state without nucleotide ("M") and that with ADP ("M-ADP"). Treatment with N-phenylmaleimide, which shifts myosin heads to the ATP-bound state ("M-ATP") from the ADP-Pi-bound state ("M-ADP-Pi"), slightly shifted the peak of the myosin first layer line to resemble that in the "M" state, indicating that the binding of ATP to nucleotide-free myosin itself does not change the structure of the myosin heads but that the succeeding hydrolysis step of ATP does induce structural change of the myosin heads.

Role of polyamines in skeletal muscle cell proliferation and differentiation

The polyamines putrescine, spermidine, and spermine are low molecular weight organic polycations and mediators of cell homeostasis. The polyamines have been proposed to play roles in the functioning of ion channels, nucleic acid packaging, signal transduction, autophagy, DNA and protein synthesis, cell proliferation and differentiation, and the regulation of gene expression. Although the regulation of polyamine levels is associated with skeletal muscle hypertrophy, the underlying mechanisms of polyamines have not been well defined. Here, we studied how polyamines affect the proliferation or differentiation or both of the murine myoblast progenitor C2C12 cell line. To evaluate the role of polyamines in the proliferation process, we counted the number of myoblasts every 24 hours, but polyamines had no effect on myoblast proliferation. On the other hand, during the induction of myogenic differentiation, treatment of C2C12 cells with polyamines significantly increased the number of myotubes. Polyamine-treated C2C12 cells exhibited elongated cell bodies and became multinucleated myotubes. Ultrastructural analysis with transmission electron microscopy revealed that polyamine-treated multinucleated myotubes exhibited abundant myofilaments. Therefore, our study suggests that polyamines play an important role in regulating myogenic differentiation rather than myoblast proliferation.

Publications

Nakano M, Oyamada H, Yamazawa T, Murayama T, Nanba H, Iijima K, Oguchi K. Construction and expression of ryanodine receptor mutants relevant to malignant hyperthermia patients in Japan. *Showa University Journal of Medical Sciences*. 2014; **26:** 27-38.

Reviews and Books

Yamauchi H, Takeda Y, Takeda Y, Tsuruoka S, Takemori S. Effects of aging on unloading-induced skeletal muscle atrophy and subsequent recovery in rats. *Journal of Physical Fitness and Sports Medicine*. 2013; **2:** 417-22.