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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

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 absorption of extra heat with temperature on skinned fibers. We observed two significant extra heat absorption peaks at $-22^{\circ}C$, $-25^{\circ}C$ and at about the melting point of water. Additionally, we observed two more absorptions peaks at 45˚C and 65˚C in a temperature-dependent irreversible manner. These irreversible heat absorption peaks affected on amplitude of the heat absorptions at $-22^{\circ}C$, $-25^{\circ}C$ independently. Electron microscopy showed that the development of the absorption peak at 45˚C markedly deteriorated A-band structure and that at 65˚C extensively deteriorated sarcomere structure. Therefore, it was found that the peak at 45˚C reflects mainly denaturation of the thick filaments and the peak at 65˚C reflects that of the thin filament. These results suggest that differential scanning calorimetry can be used to effectively explore the water states in sarcomeres.

Property of water around myoprotein studied by quarts crystal microbalance and nuclear magnetic resonance

We observed spin-spin relaxation process of ^{1}H-NMR signals from suspension of myofibrils prepared from rabbit psoas muscle. In the absence of ATP myofibril affects water molecules within 500 nm from its surface differently from water molecules in the bulk solution, and release many water molecules in the presence of ATP.

We also observed the adsorption process of myosin to the gold surface by QCM (quartz crystal microbalance). Viscoelastic property of the myosin adsorbed to the surface of the gold electrode and its surrounding solution as a whole was studied using the AFFINIXQN Pro (Initium, Tokyo).

When myosin adsorption was sparser than 0.2 μg/cm^2, viscoelastic change accompanied by myosin adsorption was almost the same as to 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 myosin head changes the viscoelasticity of the protein. This suggests that myosins immobilize surrounding solution when it is closely adsorbed. The half of this immobilized solution was released in the presence of ATP or ADP but not in the presence of ATP-γS.
Finally, we 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 implies that the myosin in the M and M.T state immobilized many water molecules, and that myosins in M.D.Pi and M.D states release the water molecules.

Role of polyamines in skeletal muscle hypertrophy
The polyamines putrescine, spermidine and spermine are considered to be essential growth factors in virtually all cells. The proposed roles of polyamines are the functioning of ion channels, nucleic acid packaging, signal transduction, cell proliferation, and differentiation, as well as regulation of gene expression. In skeletal muscle, regulation of polyamine levels is associated with muscle hypertrophy and atrophy, yet the underlying mechanisms of polyamine actions are not well defined. Here, we studied how polyamines may affect the proliferation and/or differentiation of murine myoblast progenitor C2C12 cell line. Upon polyamine treatment of C2C12 cells during induction of myogenic differentiation, the number of myotubes significantly increased. Morphologically, polyamine-treated C2C12 cells exhibited elongated cell body and became multi-nucleated myotubes. On the other hand, the polyamine did not have influence on myoblasts proliferation. Furthermore, compensatory muscle hypertrophy of C57BL6 mice underwent sciatic nerve transection of the left hindlimb was enhanced by administration of polyamines. These results demonstrate that polyamines may play an important role in regulating myogenic differentiation rather than myoblasts proliferation to enhance muscle hypertrophy.

Effect of polyamine on calcium dynamics and electrophysiological property of cardiac cells
Polyamines are poly-cation molecules which are indispensable for proliferation of the eukaryotic cells. On the other hand, polyamines modulate biological functions of ionic channels to modify excitability of the cardiac cells in the physiological condition. Considering these facts, increased polyamine concentration within the cardiac cells may possibly interfere with the function of the ionic channels to induce arrhythmia in athletes who have hypertrophic hearts. To address this issue, intracellular calcium dynamics and electrophysiological property of the cardiac cells were measured *in vitro* and *in vivo* system. Calcium dynamics and electrophysiological property of the isolated cardiac cells were evaluated by fluorescent dyes. Excitability of cardiac cells in the whole body was evaluated by electrocardiograph of the rats under anesthesia. Polyamines increased the duration

of a spontaneous discharge of cardiac cells both *in vitro* and *in vivo*. Polyamine increased intracellular basal calcium concentration in isolated ordinary cardiac cells without corresponding membrane potential change. Amplitude of T-wave of electrocardiograph was increased by the addition of polyamine. Increased intracellular polyamine concentration in cardiac cells may affect hypertrophic hearts of athletes to modify electrophysiological property.

Publications

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