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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 both water content and water states in tissue. Recently, taking advantage of well-organized skeletal muscle, we clarified that magnetic resonance distinguished 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, is believed to restrict their mutual motional freedom to result in one of the water states. Therefore, it follows that water and macromolecules would gain additional motional freedom by absorbing extra heat with temperature similarly to the melting of ice. With differential scanning calorimetry, we observed the changes of skinned fibers with or without thick filament to find the extra heat absorption with temperature. There were endothermic peaks at 50˚C and 65˚C in a temperature-dependent irreversible manner. The denaturing peaks at approximately 50˚C were affected by the presence of thick filament, but those at approximately 65˚C were not. Additionally, we found 2 significant endothermic peaks at < -10˚C and at about the melting point of water. The peaks at about -21˚C were affected by the presence of thick filament, whereas the peaks at about -23˚C were not. Accumulated enthalpy as an index of overall heat capacity was affected by the presence of thick filament. These results suggest that peaks at -21˚C and 50˚C originate from mainly thick filament and that peaks at -23˚C and 65˚C originate from mainly thin filament.

Viscoelastic property evaluated with quartz crystal microbalance

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). Samples were measured in both solution and air. The adsorbed protein volume was calculated from the data in the air.

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. As the weight of the adsorbed myosin decreased, the resonance fre-

quency decreased. 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, large viscoelastic change was observed. Viscoelastic analysis indicates that myosin plays as a protein having viscoelasticity and that binding ATP to the head of myosin changes the viscoelasticity of the protein. This suggests that when adsorbed closely, myosin immobilizes the surrounding solution. Half of this immobilized solution was released in the presence of ATP or ADP but not in the presence of ATP-γS.

Chlorogenic acid, a polyphenol in coffee, protects neurons against glutamate neurotoxicity

The study was designed to explore the molecular mechanism of chlorogenic acid (CGA) in protecting against glutamate-induced neuronal cell death. We investigated the protective effects of CGA on glutamate-induced neuronal cell death using primary cultures of mouse cerebral cortex because the release of glutamate during brain ischemia triggers the deaths of neurons. Cortical neurons in primary culture were exposed to 300 μM L-glutamic acid or vehicle, with or without 10 μM CGA or 10 μM MK-801. After 16 hours, primary cultures were stained with propidium iodide/Hoechst or calcein. Double-staining with propidium iodide and Hoechst was performed to confirm whether the cell death induced by glutamate was apoptotic. Intracellular concentrations of Ca^{2+} were observed with the Ca^{2+}-indicator fura-2. Glutamate-induced neuronal cell death was inhibited by treatment with CGA. In addition, CGA prevented the increase in the intracellular concentration of Ca^{2+} caused by the addition of glutamate to cultured neurons. On the other hand, CGA had little effect on cell death induced by nitric oxide, which is downstream of the ischemic neuronal cell death. Our results suggest that the polyphenol CGA in coffee protects neurons from glutamate neurotoxicity by regulating the entry of Ca^{2+} into neurons. Therefore, CGA in coffee may have clinical benefits for neurodegenerative diseases, such as ischemic stroke.

Intrinsic structural change of helically arranged myosin heads in skeletal muscle sarcomere in the absence of thin filaments

Myosin converts the chemical energy of ATP to mechanical work in combination with actin. The molecular mechanism of this chemomechanical transduction is still unknown, mainly because mechanical work significantly deforms the molecules. Therefore, it is of interest to follow the intrinsic structural changes of myosin in the absence of actin.

We followed intrinsic structural changes of myosin heads in sarcomeres, where conformational freedom of myosin would be highly restricted in a range optimized for the physiological path, unlike in the purified solution system.

Actin was removed from sarcomeres of skinned fibers with gelsolin treatment, and helically arranged myosin heads were observed with X-ray diffraction (at BL6A of PF) following the ATP hydrolysis steps of M, M-ATP, M-ADP-Pi, M-ADP, and M, in which "M" represents myosin. Compared with M and M-ATP (trapped with N-phenylmaleimide) states, myosin heads in the M-ADP-Pi state was retracted close to the backbone of the thick filament. Retrograde binding of ADP to M to yield M-ADP did not cause this marked transition of myosin heads. Because the orthograde conformational change in the

M–ADP state following Pi release is generally considered to be coupled with the mechanical work of myosin, radial retraction and the following protrusion of myosin heads would likely be the prime mover, as in the case of crawling bristle grass in your gripping hand.