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Spin-spin relaxation of 1H-NMR signals from myofibril suspension of rabbit skeletal muscle.

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We observed the spin-spin relaxation process of 1H-NMR signals from myofibril suspension prepared from rabbit psoas muscle. As was the case in tissue skeletal muscle, the decomposition analysis of the relaxation process indicated that water molecules in the suspension could be conveniently grouped into several components based on the transverse relaxation time constants (T₂). The slowest and the second slowest components (components 0 and 1, respectively) dominated over faster ones. Arranging the component 1 around the myofibrils, each myofibril is estimated to affect water molecules as far as 500 nm from its surface. Similar estimation indicated that albumins affect 3 nm water layer, myosin-ghost myofibrils 0 nm, and the dispersed myosin filaments 12 nm. Therefore, at least, the lattice structure of myosin filaments was required for the long ranged (500 nm) interaction between water and myofibrils. Since the component 1 increased with temperature, this interaction may have hydrophobic nature.

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Single molecule measurement of Elasticity of connectin-like 1200K-protein in obliquely striated muscle of a polychaete (Annelida)

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Connectin is a giant elastic protein of vertebrate striated muscle. Because of its elasticity, connectin plays a role in maintaining the thick filaments in the center of the sarcomere. Connectin-like molecules may perform conserved functions in vertebrate and invertebrate striated and oblique muscles, although at present less is known about the structure of invertebrate connectins. We analyzed the partial primary structure of a 1200K connectin-like protein that is expressed in *Neanthes* sp. body wall muscle. We obtained a clone of 1,358bp by immunoscreening from a cDNA library of the body wall muscle. This clone included a PEVK-like repeat sequence flanked by Ig domains. The PEVK rich region is found in vertebrate connectin and is thought to generate elasticity and be responsible for passive tension of the muscle. The force-extension curve of the single recombinant polypeptide containing a GST tag was measured by AFM using a stretch velocity of 2000 nm/sec. Unfolding forces and contour length gain of the Ig domains support that we are stretching single molecules. The force extension curve prior to the first Ig domain unfolding event could be fit well with the WLC equation ($R^2 > 0.95$) and suggests that the curve is likely to be derived from the PEVK-like sequence. Our data indicate that this sequence is largely unfolded and that it provides a high degree of extensibility at force levels that are unlikely to cause Ig domain unfolding. Overall, our results demonstrate that the 1200K-protein is a connectin-like elastic protein.

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An establishment and analysis of recombinant wild-type and mutant human cardiac actins.

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In order to understand a structural change of an actin filament and the relationship between motion and function, it is indispensable to obtain the actin as a recombinant protein. For this purpose, we have been trying to establish an actin expression system using the insect cells and analyze the expressed mutant actins. We selected the human cardiac muscle actin as an isoform of recombinant actin and introduced the L21 sequences in the vector for enhance the expression levels of recombinant proteins. In addition, the tag and the protease site were connected to the N-terminus of the actin to separate the expressed and the intrinsic actin easily. We focused on the Q137. This residue interacts with the gamma-phosphate group of ATP via water molecule which has thought to react as a nucleophile and hydrolysis ATP. Therefore, Q137 may an important role in the actin-ATPase activity. Moreover, the corresponding residue in the deep-sea fish actin is replaced by Lys, suggesting the involvement in the high-pressure adaptation. Hence we made Q137K, A, E and P mutants. In addition, the A108 and the P109 that was structurally adjacent to Q137 were also mutated to make A108G and P109A, respectively. The wild-type and these entire mutant actins were expressed in insect cells and the wild-type, Q137A, A108G and P109A actins were successfully purified in high purity by the same purification conditions. Now we are measuring various kinetics to compare the wild-type and the mutant actins. We are going to report and discuss these results in this meeting.

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Orientations of Resting Myosin Crossbridges in Skeletal Muscles determined by X-ray Fiber Diffraction

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We used the intensities of the myosin-based layer-line reflections of X-ray diffraction patterns from skeletal muscles at full- and non-overlap filament length in the relaxed state to determine the orientation of each head of myosin crossbridges around the fiber axis. It is necessary to remove the sampling effect from the observed layer-line intensity distributions for carrying out the structural modeling of a single thick filament because the myosin-based layer-lines are partially sampled by the hexagonal filament array. Therefore we used a cylindrically averaged difference Patterson function $\Delta Q(r,z)$. We determined the region where the inter-filament vectors contribute in the $\Delta Q(r,z)$ map to remove the most of the sampling effects on the layer-lines (~80%). The recalculated intensity data from the truncated $\Delta Q(r,z)$ of muscles at full- and non-overlap lengths were very similar to each other, indicating that the sampling effect was mostly removed and the intensities of a single thick filament were obtained. The $\Delta Q(r,z)$ showed that the radial position of a crossbridge mass center along a helix was ~12.6 nm and that the two heads of a crossbridge were separated axially. By employing the sampling-free intensity data, we modeled the resting crossbridge structure. In the best-fit model, each myosin head of a crossbridge has also a different orientation around the fiber axis. In top view, the distal ends of two heads appear to orient in the same direction, making a U-shape structure according to the configuration of the myosin molecule.