- 1P146 ゲルとしてのスキンド筋の保水性：イオン強度とイオン種の効果
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- 1pC02 Water retaining ability of skinned skeletal muscle as a gel: effects of ionic strength and ion species
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We examined the effects of ionic strength and ion species on the volume of skinned fibers of frog skeletal muscle. Increase in ionic strength in ordinary artificial intracellular solution (mainly composed of K⁺ and organic anions such as methanesulfonate ion CH₃SO₃⁻) was reported to swell skinned fibers with homogeneous widening of myofilament lattice. This is contrary to what is expected from DLVO theory for liquid crystals. Using synchrotron radiation at BL45-XU of SPring8, we found that extraction of thin filaments from skeletal muscle sarcomere with gelsolin treatment affected the volume response of myofilament lattice against the changes in ionic strength to only a small extent. Therefore, we hypothesized that the state of inter-myofilament water exerts significant effects on the spacing of thick filament so as to affect fiber volume. To obtain a support to our hypothesis, we tested various ions which are reported to be structure breaking from their salting-out ability of colloids. Observing optical cross-section of the fibers, we found that increase in ionic strength with the water-structure making ions such as Na⁺ had smaller effects on fiber volume than the water-structure breaking ions such as Cl⁻ and K⁺. Taking the electrolytic dissociation into account, we concluded that the ion species had significant effects on the volume of skinned skeletal muscle. This supports our tentative hypothesis that water structure may affect lattice volume of skeletal muscle sarcomeres.

- 1P148 拍動心筋細胞におけるαB-クリスタリンと Z 帯タンパク質との動的な相互作用の分析
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- 1pC04 Analysis of dynamic interaction of αB-crystallin with Z-disc protein in beating cardiac myocyte
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αB-crystallin, one of small heat shock proteins, is constitutively expressed in heart and skeletal muscle, and functions as molecular chaperone. The expression of αB-crystallin is highly dependent on physiological activity and/or cellular morphology in the skeletal muscles and localizes mainly in Z-bands. Muscles consist of an elaborate lattice of cytoskeletal elements. The peculiar property of the cytoskeleton demands dynamic regulation to maintain the development of tension. It has been reported that αB-crystallin is involved in the stabilization and the regulation of cytoskeleton, such as intermediate filament, actin and tubulin/microtubule. The purpose of the present study is to investigate the dynamic view of αB-crystallin in living myocardial cells by the use of time-lapse imaging system. We used green fluorescent protein (GFP) fused with αB-crystallin and it was expressed in rat neonatal myocardial cells. Fluorescent recovery after photobleaching (FRAP) analysis and fluorescent loss in photobleaching (FLIP) analysis of GFP-αB-crystallin indicated that the mobility of GFP-αB-crystallin in normal state is faster than during heat shock. These results demonstrated that the function of αB-crystallin is to work as molecular chaperone for structural proteins constituting of Z-bands of sarcomere and/or cytoskeleton, which should be also dynamically regulated during normal continuous beating. Beating itself may be associated with folding/unfolding of Z-band proteins including the cytoskeleton.

- 1P147 トレハロースが骨格筋スキンドファイバーの活性張力に及ぼす影響
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- 1pC03 The effects of trehalose on the contractility of mechanically skinned fibers of frog skeletal muscle
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Trehalose is one of the disaccharides that present in various organisms. It not only serves as a source of energy and carbon, but also protects proteins or cellular membranes from inactivation and denaturation caused by a variety of stress conditions such as dehydration, heat and cold. Under these stress conditions, water state drastically changes. On the other hand, trehalose is reported to reduce the dynamics of water molecules through its characteristically stronger hydrogen-bond network compared with those of other disaccharides. With these facts in mind, we hypothesize that trehalose would influence muscle contractility through the change of water state. To investigate our hypothesis, we examined the effect of disaccharides (trehalose and sucrose) on the contractility of skeletal muscle. Mechanically skinned fibers were prepared from sartorius muscle of bullfrogs, and pCa-tension curve was obtained with or without 0.5 M disaccharides. Fibers were treated with 0.5 % tritonX-100 before the experiment to destroy internal membrane systems and their sarcomere length was adjusted to 2.4 μm. Ca sensitivity was decreased by 0.4 units with both disaccharides, while maximum tension was increased by 10 % only with trehalose. The different effects of disaccharides on the maximum tension would not be due to the increase of viscosity which did not differ between two disaccharides. It suggested a possibility that the change of water state induced by trehalose influences muscle contractility stereochemically.

- 1P149 骨格筋収縮におけるクロスブリッジ形成のトロポニン構造変化への影響
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- Influence of crossbridge formation on troponin structure in skeletal muscle
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To examine structural changes of troponin after crossbridge formation, time-resolved x-ray diffraction experiments were made at the BL40XU beamline at SPring-8. Bullfrog muscles were used and stimulated with a single electric pulse of 0.5 ms duration (single-pulse:SP) or two pulses with a 30ms interval (double-pulse:DP) at 16°C. X-ray diffraction patterns were recorded at 1 ms time resolution.

In muscles at rest length, the intensity of the first troponin meridional reflection at 1/38.5 nm⁻¹ increased by ~20% at 5 msec after the stimulus and then decreased both in SP and in DP as the tension developed. In DP, after the second stimulus, the intensity increased to ~115% of that in SP. This value is underestimated because crossbridge formation brings about negative interference to this reflection, so the actual size of structural changes of troponin is considered to be larger.

In overstretched muscles, the intensity of the 38.5 nm reflection increased to ~130% of its resting value both in SP and in DP at 8 msec after the stimulus, and then returned towards resting level in SP. In DP, it began to rise again after the second stimulus and increased to almost the same level as that after the first stimulus. In addition, when muscles were stimulated at 15 msec after the first stimulus, the intensity increased and exceeded the value after the first stimulus slightly.

These results imply that calcium released in SP does not occupy all troponin molecules, and calcium bound to troponin tends to detach quickly in the absence of myosin heads.