Higuchi, Science 329, 686 (2010)] clearly showed that the elasticity of myosin is strongly nonlinear. In a previous work (Biophysical Society Meeting in Himeji, 2011) we studied the quick response of tension to the length change of a muscle fiber and the force-velocity relation in isotonic contraction based on the theory proposed by Duke [T.A.J. Duke, Proc. Natl. Acad. Sci. USA 96, 2770 (1999)] with the linear elasticity of myosin replaced by a nonlinear elasticity. It turned out that we have to set the (nonlinear) elastic constant of myosin molecule to about one forth of the value obtained by Kaya and Higuchi to account for the experimental results. A plausible reason for the discrepancy is that actin and myosin filaments are treated as rigid rods in our model as in the Duke model. In the present work, we will carry out numerical simulations taking account of the elasticity of actin and myosin filaments as well as the nonlinear elasticity of myosin molecules to reveal whether we can resolve the inconsistency between the values of elastic constant in the simulation and the experiment.

2H1534 高塩濃度溶液中における熱変性したミオシン分子のミオシンフィラメントへの付着

Adhesion of heat-denatured myosin molecules to myosin filament at high salt concentration

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Myosin filament is formed from myosin molecules mainly by electrostatic interaction. Thus, myosin filament disassociates in salt solutions at above 0.3 M. However, it may be questioned whether the interaction is completely damped out even at the high salt concentration. To explore the question, we investigated the myosin filaments at various salt concentrations by dynamic light scattering (DLS) and transmission electron microscopy (TEM). Myosin from porcine was well dissolved in 1.0 M NaCl solution. Then the myosin solution was diluted to various salts concentration. The myosin filament with hydrodynamic radius of 200 nm was dissolved to myosin molecules with that of 60 nm monitored by DLS, which was not fully disassociated with increasing temperature and salt concentration. The size of myosin filament gradually increased with heating at above 55 °C. Interestingly, the heat-denature myosin molecules adhere onto myosin filaments observed by TEM. These data suggest that the adhesion above the denaturation temperature of myosin decreased with increasing salt concentration through hydrophobic interaction. This experimental system could quantitatively analyze the kinetics of myosin filament-myosin molecule interaction.

2H1546 ラット幼若心筋細胞内サルコメア集団の自動振動(SPOC)特性 Auto-oscillation (SPOC) properties of sarcomeres in rat neonatal cardiomyocytes

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Auto-oscillations of sarcomere length (SL) occur at intermediate activation conditions in cardiac and skeletal muscles. The waveform of sarcomeric auto-oscillation usually consists of slow shortening and quick lengthening. We have termed this phenomenon SPOC. SPOC has properties of auto-oscillation at single sarcomere level and synchronous dynamics of sarcomeres, e.g., propagation of SPOC wave. Therefore, observation of SPOC allows us to understand the dynamic properties of sarcomeres. In order to fully understand the molecular mechanism of SPOC, we examined neonatal rat cardiomyocytes expressing GFP- α -actinin and visualized the motions of sarcomeres. First, we successfully induced SPOC in the neonatal myocytes with the intact inner-membrane system following treatment with ionomycin by controlling the cytoplasmic Ca^{2+} concentrations. And we have termed this phenomenon Cell-SPOC. Our analysis of the Cell-SPOC revealed that the SPOC period of SL oscillations (in the range of 0.2 s to 1 s) increased with the increase in the amplitude of SL oscillations. As the amplitude became smaller, the shortening velocity tended to become larger, especially in the range of 0.2 to 0.5 s of the SPOC period. We also found that the oscillation properties of the Cell-SPOC and those of intact cells activated by electrical stimulation became similar as the oscillation period approaches to 0.2 s from 1 s. Based on these results, we will discuss the similarities and differences between the Cell-SPOC and the oscillations of intact cells.

2H1558 横紋筋サルコメアのフィラメント格子の安定性

Stability of Myofilament Lattice in Striated Muscle Sarcomere

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Removal of cell membrane from striated muscle cells to prepare skinned muscle specimens not only increases inter-myofilament spacing but also destabilize lattice structure of myofilaments. Takemori et al (2007) revealed that this destabilization is accompanied by diffusion of macromolecular solutes out of inter-myofibrillar space. Therefore, we expected that exogenous macromolecules that penetrate into inter-myofibrillar space may recover the stability. Adopting a set of myosin layer lines on the two dimensional X-ray diffraction patterns (obtained at BL-6A of KEK and at BL-45XU of SPring8) as one of the indicators of lattice stability, we found that 3% polyethylene glycol molecules (M_w less than 3,360) that penetrate into myofilament lattice restore myosin layer lines of skinned specimens of striated (rabbit skeletal and rat cardiac) muscles. Polyethylene glycol also dehydrated the skinned specimens to restore physiological inter-filament spacing. Since the dehydrating effect mainly depends on the concentration of hydrophobic $-CH_2-$ unit in the solution (Kimura and Takemori, 2008), we consider this stabilizing effect of polyethylene glycol to support our hypothesis that construction of myofilament lattice involves structured water that serves as free-energy sink for contractile interaction. From this view point, cause of differential radial stiffness of relaxed and rigor muscle specimens are reconsidered.

2H1610 羽ばたくマルハナバチ中で拮抗する2種の飛翔筋の超高速X線回折像同時記録

Simultaneous ultrafast X-ray recordings of actions of two antagonistic flight muscles during wingbeat of live bumblebee

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In bees and other insects, their flight muscles drive their wings indirectly by deforming the thoracic exoskeleton (indirect flight muscle). The deformation is caused by alternating force generation by two antagonistic flight muscles (dorsal longitudinal muscle, DLM, and dorsoventral muscle, DVM). Here we studied how the actions of these two antagonistic muscles are coordinated by simultaneously recording the X-ray diffraction patterns from these muscles during wingbeat. Intense X-ray beams from the BL40XU high-flux beamline of SPring-8 were irradiated from the side of the thorax of a live bumblebee, where the two antagonistic muscles cross. The diffraction patterns were recorded at 5,000 frames/s by using a fast CMOS video camera. A second fast CMOS video camera recorded its wingbeat. The frames of the two video cameras were synchronized in a master-slave connection. Besides the strong equatorial reflections, the 1st myosin meridional reflection (1MM) and the 2nd troponin off-meridional reflection (2TN, reporting myosin binding to actin) were clearly recognized for both DLM and DVM after summing the frames for ~5 wingbeat cycles. The intensities of these reflections, as well as the lattice spacing (d10), changed basically in an anti-phase fashion for DLM and DVM but not always in a sinusoidal manner as generally assumed. Further analyses are underway and they will give more detailed information about the timing of action of the two antagonistic flight muscles during wingbeat.

2H1400 海洋性ビブリオ菌の周毛性べん毛形成の抑制に関与するDnaJモチーフを持った新規遺伝子の解析

A novel gene of dnaJ family plays a role in the suppression of flagellation in *Vibrio alginolyticus*

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Bacterial flagella are formed in various numbers and locations in different kinds of bacteria. A *Vibrio alginolyticus* strain VIO5 that lacks lateral flagella and possesses wild-type flagella, has a single polar flagellum by expressing FlhF and FlhG. FlhF positively regulates the number and location of flagella, while FlhG does so negatively. The Δ flhFG strain, derived from VIO5, has mostly no flagellum, but a small population of the Δ flhFG cells have some peritrichous flagella and only slightly form the motility ring on the soft-agar plate. In our laboratory, the suppressor mutant recovering motility on the soft-agar plate was isolated from the Δ flhFG strain (named Δ flhFG-sup). The Δ flhFG-sup cells were peritrichously flagellated. We identified the sup mutation by whole genome sequence, on the unknown gene which has a DnaJ motif and was specific in *Vibrio*. We named this gene sflA (suppressor of Δ flhFG). We showed that the Δ flhFG strain expressing mutant SflA from plasmid recovered motility and biosynthesis of flagellin, and the Δ flhFG-sup strain expressing