elements that influence nucleotide exchange rate of actin. In this work, we characterized nucleotide binding of carp (*Cyprinus carpio*) muscle actin, which contains five amino acid substitutions compared to chicken muscle actin. In carp actin, both the association and the dissociation rate of ϵ -ATP were significantly large. At high Ca^{2+} concentration, the dissociation rate constant of ϵ -ATP was $8.3 \times 10^{-5} \text{ s}^{-1}$ which was about 10-fold larger than that in chicken actin. Also in the absence of Ca^{2+} , the dissociation rate of ϵ -ATP from carp actin was about 5-fold larger than that from chicken actin. On the other hand, the dissociation rates of Ca^{2+} were similar in two actins. These results suggest that carp actin more frequently undergoes conformation change. An amino acid substitution of Ala/Ser-155 is located on one of the nucleotide binding β -hairpins (P1:Asp11-Lys18, P2:Asp154-Met161, P3:Met299-Tyr306) of actin. The amino acid of this position will regulate the conformational change to exchange bound nucleotide.

1P-118 Cy3 蛍光標識されたアクチン繊維の運動の光感受性阻害
Photosensitive inhibition of motility of actin filaments covalently conjugated with Cy3 dyes

Kuniyuki Hatori (1), Syunsuke Matsushita (1), Hirotugu Kimijima (1) ((1) Department of Bio-System Engineering, Yamagata University)

In order to examine the functional modulation of actin molecules, both chemical modification and labeling of actin molecules were simultaneously carried out by the conjugation with amino-reactive dyes. Cy3-NHS was employed as the chemicals in this study. We found that the motility of actin filaments on myosin molecules was inhibited by the conjugation of Cy3-NHS to them over a 3-fold molar ratio. In the case of conjugation at 2-fold excess, Cy3-conjugated actin filament (Cy3-F-actin) moved, but after exposure to excitation light stalled in a short duration. The duration time of movement was decreased with an increase of filament length. The stalling (or the decrease of velocity) occurred suddenly, indicating a cooperative manner of motility. We also measured the dissociation rate of Cy3-F-actin from a single HMM molecule in the absence of ATP. Dissociation rate constants for Cy3-F-actin and for intact one were 0.06 s^{-1} and 0.03 s^{-1} , respectively.

Inhibition of motility of Cy3-F-actin was extensively suppressed by adding dithiothreitol (DTT) in the solution during the observation, which is likely to act as an antioxidant. In the absence of DTT, the duration time was below 1 sec. On the other hand, in the presence of 100 mM DTT, the duration time was increased above 10 sec. This result indicates that local oxidation of actin molecules may affect the motile function of actomyosin complex. We are attempting to identify the Cy3-conjugated sites in actin molecules by a mass spectrometry.

1P-119 デスミンはアクチンモーターの滑り運動に影響を与える
Desmin affects the sliding movement of actomyosins

Asami Matsuda (1), Kuniyuki Hatori (1) ((1) Department of Bio-System engineering, Yamagata University)

Desmin, which is one of the constituents of intermediate filaments, is widely found in various muscular tissues. Although desmin is thought to strengthen the cell structure, its functional roles in muscular contraction are unknown.

We examined effects of desmin molecules on the actomyosin function both in the superprecipitation of natural actomyosin suspension and in the motility assay. Skeletal muscular actin and myosin were employed in this study. Monomeric desmin was prepared from a chicken gizzard and was added to an assay solution just before experiments. In the superprecipitation experiments, desmin enhanced the contractile condensation of actomyosins in the presence of Ca^{2+} ions. On the other hand, in the absence of Ca^{2+} ions, desmin was more effective in suppressing superprecipitation. This results show that desmin may vary the binding strength between actin and myosin which were regulated by concentration of Ca^{2+} ions. In the motility assay, sliding velocity of actin filaments on myosin molecules was decreased by 20 % with an increase of concentration of desmin in the range of 0 - 0.1 mg/ml. Also, the length of actin filaments during the movement was decreased with the increase of concentration of desmin.

Desmin was found to affect directly both the contraction of actomyosins and the sliding movement of actin filament on myosin molecules.

1P-120 格子間隔に依存した 2 状態モデルにおける筋肉の状態相図
Lattice spacing-dependent two state model of striated muscle contraction: Phase diagram and dynamic behavior

Katsuhiko Sato (1), **Masako Ohtaki** (2), Yuta Shimamoto (3), Shin'ichi Ishiwata (4) ((1) Dept of Physics, Tohoku Univ.; (2) Dept of Physics, Faculty of Science & Engineering, Waseda Univ.; (3) Laboratory of Chemistry and Cell Biology, Rockefeller Univ.; (4) Dept of Physics, Faculty of Science & Engineering, Waseda Univ.)

see 1TA2-06

1P-121 分子動力学による理論的研究：分子の揺らぎからわかる F₁-ATPase のサブユニット協調性

Structural fluctuation and cooperativity in F₁-ATPase: a molecular dynamics study

Yuko Ito (1), Mitsunori Ikeguchi (1) ((1) Graduate School of Nanobioscience, Yokohama City University)

F₁-ATPase is an ATP-driven rotary motor enzyme. We investigated the structural fluctuations and cooperative motions of subunits in F₁-ATPase using molecular dynamics (MD) simulations. Firstly, MD simulations were carried out for the isolated β_E subunits of bovine mitochondrial F₁-ATPase (MF₁) and thermophilic Bacillus PS3 F₁-ATPase (TF₁) over the time scale of 100 ns. Consistent with NMR experiments, for both species, the average conformations remained open, and large hinge-bending motions around the open conformations were observed. Secondly, a MD simulation for the $\alpha_3\beta_3\gamma$ complex was carried out for a 30 ns time period. While large fluctuations of the N-terminal domain observed in the isolated β_E simulations are suppressed in the complex simulations, fluctuations of the C-terminal domain are clearly different among the three β subunits (β_E , β_{TP} and β_{DP}). Despite fairly similar conformations of β_{TP} and β_{DP} , β_{DP} exhibits smaller fluctuations of the C-terminal domain than β_{TP} due to their dissimilar interface configurations. In addition to well-known $\alpha_{DP}\beta_{DP}$ tight packing, the β_{DP} subunit is also tightly packed by the adjacent α_E subunit. This sandwiched configuration leads to suppression of the fluctuations in the β_{DP} subunit. The relationship between this suppression of the fluctuation and ATP hydrolysis/synthesis reaction is discussed. Also, the correlation matrix revealed that the β_{DP} subunit undergoes concerted motions with the γ subunit, suggesting that the structural change occurring in the catalytically active β_{DP} subunit can be transmitted to movements of the γ subunit.

1P-122 ゼルニケ位相差法によるビブリオ菌べん毛フック基体の低温電子線トモグラフィー

Zernike phase contrast cryo-electron tomography of *Vibrio* flagellar hook-basal body

Naoki Hosogi (1), Hideki Shigematsu (1), Hiroyuki Terashima (2), Michio Homma (2), Kuniaki Nagayama (1) ((1) Laboratory of Nano-Structure Physiology, Okazaki Institute for Integrative Bioscience; (2) Graduate School of Science, Nagoya University)

Vibrio alginolyticus can swim with flagella, which function as a rotary machine like a corkscrew. The flagellar motor uses the electrochemical potential of sodium ions across the cytoplasmic membrane as the energy source. One single flagellum consists of the filament, the hook and the basal body. The basal body, which takes part in the rotor and the bushing, is constituted of the rod and several ring structures. This study investigates the structure of the T ring which is a unique component of the sodium ion-driven flagellar motor of *V. alginolyticus*. Using Zernike phase contrast (ZPC) cryo-electron tomography, 3D structures of the purified hook-basal body (HBB) from wild type and deletion mutant of MotX and MotY (ΔmotX ΔmotY), which are thought to be responsible for the T ring, have been compared. Due to the in-focus high-contrast feature of ZPC electron microscopy, ZPC images of HBB were highly improved in the signal-to-noise ratio, compared to the conventional phase contrast images. We could observe the overview of HBB, and find the T ring only in the HBB from wild type. This structure existed beneath the LP ring and took a ring shape (about 31 nm in diameter). It was lost in the basal body from a ΔmotX ΔmotY strain.

1P-123 回転分子モーター F₁-ATPase の触媒部位へ結合したヌクレオチドの高精度位置検出

High-Accuracy Localization of Single Nucleotides Bound to the Catalytic Sites in Rotary Motor F₁-ATPase

Kengo Adachi (1), Tomoko Masaie (1), Kazuhiro Oiwa (2), Takayuki Nishizaka (1) ((1) Gakushuin Univ.; (2) Kobe Adv ITC Res Ctr)

F₁-ATPase is an ATP-driven rotary molecular motor in which a central γ subunit rotates against hexagonally arranged subunits $\alpha_3\beta_3$. Three catalytic sites have a place in α - β interfaces, mainly hosted by a β subunit, and they hydrolyze ATP sequentially to generate the rotation of γ . To see how the chemical reactions in three catalytic sites are coupled to mechanical rotation, we had observed the binding and release of a fluorescent ATP analog (Cy3-ATP) to and from F₁ (Nishizaka *et al.*, *Nar. Struct. Mol. Biol.*, 2004; Adachi *et al.*, *Cell*, 2007). We also succeeded in detecting the orientation of fluorophores by polarization (Nishizaka *et al.*, 2004). To determine the occupied catalytic site directly, we here localize the precise position of single fluorescent nucleotides bound to the catalytic sites. To achieve this, we first improved a mechanical stability of a microscope. An optical bench was compartmented with thermal insulating materials, and a temperature around the bench was calmly controlled within the change ≤ 0.1 °C/hour without forced air flow. The drifting displacement of the sample stage was reduced < 10 nm/hour for x and y-axis, and < 50 nm/hour for z-axis. With this setup, Cy3-ATPs fixed on a glass surface were localized with the spatial accuracy of ± 3 nm (SD), by fitting its fluorescent spot, which was captured at 35 frames/s and then averaged over 15 frames, with Gaussian distribution. We now observe simultaneously the stepwise rotation of magnetic beads attached to γ with 60 nM Cy3-ATP and 60 nM unlabeled ATP.