rate (0.5nm x 0.5nm/pixel). Consequently, the image tailing at steep ramp-off area was reduced. Topographs of dynein-c were aligned, classified and class-averaged based on comparing head shapes. In class-averaged topographs, a ring of ~4nm diameter was observed at the center of the head showing the left-view. The ring swelled up from the head surface. We observed the small ring in the independent observations using different probes. These suggest that the observed ring wasn't an artifact resulted from probe shapes. In observations of destruction processes of dynein-c, we often observed that the head broke into 4-7 fragments. In ~60% of head destruction, the fragments were distributed in radial pattern. At the focus of the radial pattern, we observed that a ring, which was similar to the ring observed at the center of head, tethered the fragments. By analogy with other AAA proteins, we suppose that the observed ring was a complex of the parts of respective AAA domains.

3P171 Bead-size dependent dwell time of step rotation exhibited by GT mutant of F1-ATPase

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The $\alpha\beta\gamma$ subcomplex of F1-ATPase is a rotary molecular motor. It hydrolyzes ATP into ADP and inorganic phosphate; the hydrolysis of one ATP drives discrete 120° rotation of the γ subunit relative to the other subunits. At a high ATP concentration, ATP binding is so fast that we cannot resolve the step rotation at video rate. But previously, we found that a mutant containing G181A, T165S, and Y341W mutations in the β -subunit (GT mutant) frequently exhibited steps even at 2 mM ATP. The angular position of the dwell coincided with that of ATP waiting state. In the present study, we investigated the influence of the size of the bead attached to the γ subunit on the stepping rotation. In the dwell time histogram, the first bin contained many more events than expected from a single exponential decay that were fitted well with the data after the second bin. The dwell time derived from the data after the second bin got longer as the size of the bead increased. The plot of the dwell time against the drag coefficient of the bead was not linear but tended to saturate. The tendency means that the dwell exhibited by the GT mutant is not simply explained by a Kramers' mechanism. We also found a tendency that when the variance of the bead position in the dwell was larger, the dwell time was shorter. We will discuss possible mechanism for the anomalous dwell exhibited by the mutant.

3P172 ミオシン S1 およびアクチン S1 による ATP 加水分解反応に及ぼすハライドイオンの影響 Effects of halide ions on the ATP hydrolysis activity of myosin S1 and actin-S1

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Myosin binds with F-actin and produces mechanical force in the presence of ATP. The ATPase activity depends on the affinity between myosin and F-actin. As shown by Katoh et al (1996) the affinity in the presence of ATP reduces as increasing ionic strength by NaCl. The aim of this study is to examine the effects of halide ions on the ATPase activities of myosin S1 and actin-S1 and the relations between the activity and the hydration state of myosin S1. Actin and myosin were taken from chicken pectoral muscle, and myosin S1 was obtained by Papain treatment. From the ATPase activity measurement by malachite green method the K_m of myosin S1 and F-actin in the presence of ATP increased by 3 times when changing from Cl^- 20 mM to I^- 20 mM. On the other hand V_{max} did not change apparently. It indicates that the ATPase activity is governed by the affinity between myosin and F-actin in this condition. When changing Cl^- to F^- the V_{max} became about half and K_m increased about 70%. The presence of F^- decreased the affinity between myosin and F-actin and the cycle rate about a half. From the hydration measurement using microwave dielectric spectroscopy the constrained water and hyper-mobile water increased by changing from Cl^- to I^- , corresponding to K_{cat} decrease. Thus, halogen ions affect both the ATPase activity of myosin S1 and actin-S1 and hydration properties of myosin S1.

3P173 F1-ATPase の β サブユニット (Y341W) 単体へのヌクレオチド結合の温度依存性 Temperature dependence of nucleotide binding to β subunit of F1-ATPase revealed by fluorescence measurement

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In the $\alpha\beta\gamma$ subcomplex of F1-ATPase, the central γ subunit rotates 120 degree against $\alpha\beta$ ring while hydrolyzing one ATP molecule; thus F1-ATPase is a rotary molecular motor. Its rotary mechanism is now well understood. However, thermodynamic aspect of the process is still unclear. We examined the thermodynamics of nucleotide binding to the isolated β subunit by use of

the genetically engineered Trp at the nucleotide binding site (β Y341W). The fluorescent intensity of the Trp decreases upon nucleotide binding. By measuring the fluorescent intensity of this mutant under various concentrations of nucleotides at a temperature range from 277 to 323 K, we measured the temperature dependency of the nucleotide binding for ATP, ADP, GTP, GDP, ITP and IDP. The affinity to the isolated β subunit decreased in the order of ANP, GNP, and INP. But there was little difference in the affinity between diphosphate and triphosphate. The van't Hoff plot gave similar slope for ANP, GNP, and INP, suggesting that the difference in the affinity arises from the difference in the entropic effect.

3P174 2mM ATP の DMSO 存在下における F1-ATPase のステップ運動 Stepwise rotation of F1-ATPase at 2 mM ATP in the presence of DMSO.

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F1-ATPase is the catalytic moiety of FoF1-ATP synthase which catalyzes ATP synthesis from ADP and inorganic phosphate using the electrochemical potential difference of proton across the mitochondrial inner membranes, chloroplast thylakoid membranes and bacterial plasma membranes. Previously, De Meis examined the free energy of hydrolysis of PPi in the presence of various inorganic solvents. He found that under these conditions, the free energy approached to zero Ref (De Meis et al, JBC 7869-7873,1989). In the presence of DMSO, Yoshida reported the formation of bound ATP from bound ADP and medium Pi Ref (Yoshida et al, BBA 850(1986)429-435). With these observations, we expected that DMSO also modulates the free energy of ATP hydrolysis and examined the effect of DMSO on the ATP-driven rotation of $\alpha\beta\gamma$ subcomplex of thermophilic F1-ATPase. We found that the ATPase activity was severely attenuated and clear stepwise rotation was observed at 2mM ATP in the presence of 30% DMSO. In addition, the stepping velocity decreased significantly. We also examined the effect of DMSO on the viscosity of the medium by the fluctuation of a bead particle trapped by a optical tweezers. In the presence of 30 % DMSO, the viscosity coefficient increased two times than the pure water. However, the decrease in the stepping velocity was not solely explained by the change of viscosity and it was strongly suggested that the torque generated during the stepping motion decreased in the presence of DMSO.

3P175 ミオシン V の運動性におけるトロポミオシンとトロポニンの影響 Effect of tropomyosin and troponin on the single-molecule motility of myosin V

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In muscle, tropomyosin (Tm) and troponin (Tn) play essential roles in regulating the interaction between the thin (actin) filament and myosin II molecules. *In vitro* motility assays revealed that in the presence of Ca^{2+} the decoration of actin filaments with Tm and Tn results in the generation of higher forces and faster sliding velocity, whereas in the absence of Ca^{2+} no force generation and sliding was observed. The single-molecule motility of processive motors, such as myosin V, might also be modulated by Tm (and Tn), however, nothing is known about the effect of the decoration of actin filaments with Tm and Tn on the motility of myosin V molecules. At the last year meeting we reported that in the absence of Ca^{2+} the single-molecule motility of myosin V on the filaments decorated with only Tm remains almost unchanged, but when actin was decorated with both Tm and Tn, the velocity and run length decreased. However, we were unable to examine the effect of Ca^{2+} due to the dissociation of calmodulin (CaM) from myosin V, which resulted in the loss of motility. Here we examined the effect of Ca^{2+} on the motility of myosin V along the filaments decorated with both Tm and Tn by using myosin V construct (Δ all-MV) in which native CaM was replaced with a non- Ca^{2+} -binding mutant. We examined the motility of Δ all-MV, both in the presence and the absence of Ca^{2+} , on the filaments decorated with both Tm and Tn by the direct measurements of the single-molecule velocity, run length, and stall force using TIRF and optical tweezers.

3P176 Highly purified inner-arm dynein e from *Chlamydomonas reinhardtii* studied with *in vitro* motility assay

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Green alga *Chlamydomonas reinhardtii* has two flagella at the front of the cell and beats them to swim. A flagellum is a biomolecular machine that consists of various proteins in a highly regulated manner. It is considered that an align-