essential for motility. During the weak binding state, the motor head exhibits Brownian motion along a microtubule (MT) until it finds the next binding site. Thus, the mechanism of the one-dimensional (1D) Brownian motion is important for motility; however, it has not yet understood the mechanism that makes the simultaneous binding and movement possible. In our previous study, 1D Brownian motion along MTs was simulated by using positively charged polyacrylamide particles (diameter: ~ 25 nm), indicating that tubulin-specific binding was not required to maintain the motion. Further quantitative analysis revealed that the particles repeatedly bound to and jumped between the potential holes along the MTs during the 1D Brownian motion.

Then, how is the potential profile along MTs determined? What exactly is the structural entity of the potential holes? To address these questions, in this study, we have generated mutant MTs with variable charge densities or distributions at the glutamine-rich C-terminal region of tubulin and tested the 1D Brownian motion of the charged particles that moved along these mutant MTs. On the basis of this analysis, we expect to elucidate the role of the C-terminal region on the binding and jumping during the 1D Brownian motion. The analysis is underway and we will report the results in a poster.

2P-174 キネシン 2 量体の前後の頭部への ATP 結合の可視化

Observation of ATP binding to the individual motor domains of dimeric kinesin
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Kinesin is a dimeric motor protein that moves along microtubules in a hand-over-hand manner by hydrolyzing ATP. To walk by alternately moving two motor heads, the trailing head should detach from the microtubule prior to the leading head in the two-head-bound state. To explain this mechanism, we have proposed a model that ATP binding to the leading head is prohibited in the two-heads-bound state due to the backward-extending conformation of the neck linker. To investigate whether ATP can bind to the leading head or not, we constructed kinesin heterodimer composed of wild-type and ATP-hydrolysis mutant E236A heads and observed ATP-binding to each heads by measuring fluorescence resonance energy transfer between Cy3 attached to the head and fluorescent ATP (Alexa647-ATP) bound to the head. First, we used monomeric E236A kinesin bound to the microtubule and observed FRET efficiency increase upon addition of Alexa647-ATP using spectrofluorometer. Higher FRET efficiency was observed when Cy3 was attached closer to the ATP-binding pocket, confirming that FRET efficiency increase is caused by ATP-binding. Next, we used heterodimeric kinesin composed of wild-type and E236A heads and observed single molecule FRET between fluorophores attached to two heads. We found that in the presence of ATP this heterodimer stably takes two-head-bound state where the wild-type head is in the front. We are currently trying to observe ATP binding to each heads of this heterodimer.

2P-175 新規イネキネシン K16 の結晶構造解析および他のキネシンの構造との比較

Analysis of Crystal Structure of the Motor Domain of Rice Kinesin K16 and comparison with the structures of other related kinesin

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The novel rice plant specific kinesin K16 has several unique enzymatic characteristics comparing with conventional kinesin. The most interesting property is that the ADP-free K16 motor domain (K16MD) is very stable, contrast to conventional kinesin that is very labile in ADP-free state. Recently, we have determined crystal structure of the novel rice kinesin K16MD in complex with MgADP at 2.5 Å resolution. The overall structure of the K16MD is similar to that of conventional kinesin motor domains, as expected from the high similarity of amino acid sequence (43.2 %). However, the neck-linker of the ADP bound K16MD showed an ordered conformation in a position quite different from that observed in conventional kinesin, which may reflect the unique enzymatic characteristics of rice kinesin K16. In the present study, we analyzed the inner structure of the K16 motor domain in detail and compared the structure with Eg5 and other related kinesins. For crystal structure of MgADP bound KIF5A, Q323 located at the site leading to the neck-linker interacts with K45. And the side chain of I327 in the N-terminal side of neck-linker interacts with the hydrophobic core composed of $\alpha 4$, $\beta 1$, $\beta 3$, $\beta 7$, and $\beta 8$. The interaction may stabilize the docking conformation in the MgADP bound crystal structure. On the other hand, I336 of K16MD does not have interaction with the hydrophobic core. Moreover, K324 in C-terminal of $\alpha 6$ forms salt bridge with D51 in $\beta 2$. The novel conformation of the necklinker of the MgADP bound K16MD may be stabilize by these interactions

2P-176 ニ価反応性フォトクロミック分子による生体分子モーターキネシンの光制御

Photo-control of ATPase activity of the kinesin motor domains intermolecularly cross-linked by bifunctional photochromic compound

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Kinesin is a dimeric motor protein carries cellular cargoes along microtubules by hydrolyzing ATP. Azobenzene-dimaleimide (ABDM) is a bifunctional SH reactive

photochromic compounds. We have previously demonstrated that ABDM was incorporated into the functional site of skeletal muscle myosin head (S1), and the global conformational change of S1 was induced by cis-trans isomerization of the cross-linked ABDM upon UV/VIS light irradiation, which may mimic the conformational change accompanied by energy transduction. We have also cross-linked kinesin using ABDM in order to photo-control the ATPase activity of kinesin. We have prepared the mutants of kinesin motor domain T242C, A244C, A247C, I249C, A252C, G272C, and S275C, which have a single cysteine residue in L11 or L12. Only A252C cross-linked by ABDM showed significant alteration of ATPase activity upon UV and VIS light irradiation. In the present study, we prepared novel mutants of kinesin motor domain A21C, R25C, G26C in L1, binding site, Q104C in ATP binding site, T196C in L9, T273C, K274C, H276C in L12 to regulate ATPase activity efficiently. The mutants were cross-linked intermolecularly by ABDM at high efficiency 70-90%. These mutants cross-linked by ABDM showed alteration of ATPase activity between UV and VIS light irradiation. Especially, the kinesin mutant A252C was regulated most effectively.

2P-177 N-グリコシド結合において *Syn* 型のコンホメーションをもつ ATP アナログとキネ シンとの相互作用

Interaction of kinesin with ATP analogue having *syn* conformation with respect to the adenine-ribose bond

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It is known that, in solution, ATP analogues such as 8-Br-ATP with bulky substitution at the eight position of the adenine ring predominantly assume the *syn* conformation with respect to the adenine-ribose bond. Previously we have demonstrated that 8-Br-ATP induces intrinsic trp fluorescence enhancement of smooth muscle myosin that reflect the formation of the $M^{**} \cdot ADP \cdot Pi$ state. Moreover, the phosphorylated smooth muscle myosin supported actin translocation using 8-Br-ATP. Contrary, for skeletal muscle myosin, 8-Br-ATP induced neither trp fluorescence enhancement nor actin translocation. Kinesin is also ATP driven motor protein that has strikingly similar structure of motor domain to myosin. In the present study, interaction of kinesin with 8-Br-ATP has been examined. Interestingly, conventional kinesin supported microtubules translocation using 8-Br-ATP. This suggests conventional kinesin adopts the 8-Br-ATP in the normal conformation. However, the sliding velocities were approximately one-fifth of regular ATP. Moreover single molecular measurement using optical tweezers revealed that for kinesin, 8-Br-ATP induced nearly similar force generation with that of ATP. Interaction of unconventional myosin V with 8-Br-ATP was also analyzed.

2P-178 新規イネキネシン E15 の精製と特徴付け

Preparation and Characterization of Novel Rice Kinesin E15

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Kinesin is a microtubule-based ATP-driven motor protein. Kinesin plays important physiological roles in intracellular transport, mitosis and meiosis, control of microtubule dynamics, and signal transduction. Kinesin species derived from vertebrates have been well characterized. In contrast, plant kinesins have yet to be adequately characterized. E15 is one of the kinesin encoded on rice genome. E15 has the COOH-terminal motor domain and exhibits a high homology with the kinesin-14 family in Arabidopsis thaliana. In contrast, this kinesin is not similar to other kinesin -14 family kinesins derived from animal, e.g., DmNcd, ScKar3, and CeKlp. Consequently, kinesin E15 may belong in the plant-specific kinesins. In this study, we expressed the motor domain of a novel rice plant-specific kinesin, E15, in Escherichia coli. and determined its enzymatic characteristics and compared with other related kinesins. Molecular weight of the E15 motor domain was 37.6 kD. The optimum pH was pH 6.0-6.5. This is similar to rice kinesin K16 we have previously reported. Interaction of E15 with fluorescent ATP analogue, NBD-ATP was also studied prior to the kinetic analysis of E15. The fluorescence of NBD-ATP altered during hydrolysis by E15, suggesting that NBD-ATP is available for kinetic study of E15.

2P-179 光ピンセットを用いた 3 次元力計測の分子モーターへの応用

3D force detection in optical tweezers: application to molecular motors

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An optical system to measure three dimensional (3D) forces was constructed based on both the 3D-tracking technique of single particles and optical tweezers. The force imposed on a trapped microbead along Z-direction was precisely calibrated, by either the distribution of its position or the power spectrum of its thermal motion, to be $0.7 \text{ pN nm}^{-1} \text{ W}^{-1}$ for the size of $0.5 \mu\text{m}$ in diam. First, we applied the system to quantify the protein-protein interaction: the unbinding force between kinesin-1 and a microtubule (MT) was measured. Using optical tweezers kinesin-1 coated bead was positioned to make contact with a MT that was adsorbed onto a glass surface, and pulled upward at a constant speed while monitoring the absolute height of the objective through the bead attaching to the surface as a Z-positional marker. The unbinding force was estimated to be 8.0 pN on average ($n = 9$) from the abrupt displacement of the bead when the protein-protein bond was broken. Next, we tested whether the interaction between

subunits in a surface-immobilized F_1 -ATPase, could be measured. A bead attached to the rotary shaft was pulled upward and the abrupt displacement, corresponding to ~ 20 pN, was observed. Because there were multiple linkages between the bead and the glass surface through single F_1 -ATPase molecule, bond(s) responsible for the displacement was not identified. This point might be revealed by future high-resolution measurement.

2P-180 γ subunit にねじれを加えた F_1 -ATPase 変異体による軸の役割の解明

The role of the axle in F_1 -ATPase revealed by rotation of a mutant containing a twisted γ subunit.

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F_1 -ATPase is an ATP-driven rotary motor in which a central γ subunit rotates inside the $\alpha_3\beta_3$ subunit cylinder. The very recent work using a mutant containing a truncated shaft showed that the presence of the rotor head is sufficient for rotation (Furuie *et al.* 2008), and thus the contribution of the missing hydrophobic bottom region to chemical reaction(s) is now being debated. To investigate the role of the bottom part of the axle, we observed, using high-speed imaging, stepping motions of $\alpha_3\beta_3\gamma(\Delta A275)$ mutant, in which Ala275 in one of the two helices was deleted and thus the shaft was twisted by in the middle part. As Iai *et al.* has previously reported, this is the only mutant known so far that represents altered substep angles; 30° driven by ATP binding and 90° presumably driven by ATP hydrolysis, instead of 80° and 40° respectively in the case of the wild type (Biophysical Society of Japan 41th Annual Meeting, 2003). We confirmed the substep angles to be $\sim 30^\circ$ and $\sim 90^\circ$. We here show that the torque after 30° stepping was estimated to be 38pN nm, which is comparable with the average torque produced by the wild type. Remarkably, short dwelling at $\sim 75^\circ$ was still observed in some 120° steps, which will be further tested in future high-resolution analyses. We will discuss the possibility that the interactions between γ and β in the orifice cause the altered substeps due to the twist whereas those in the lower part are unaffected and drive the same substep as the wild type.

2P-181 X線小角散乱と FRET による新規イネキネシン K16 の構造変化解析

Analysis of Conformational Change of Novel Rice Kinesin K16 Using Small Angle X-ray Solution Scattering and FRET

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Recently, we have successfully dissolved the crystal structure of ADP bound K16 motor domain. The overall structure of the K16MD is similar to that of conventional kinesin motor domains, as expected from the high similarity of amino acid sequence (43.2 %). However, neck-linker region showed an ordered conformation in a position quite different from conventional kinesin. In this study, we designed the K16 motor domain chimera protein fused with GFP at the neck-linker in order to monitor the conformational change of the neck-linker during ATP hydrolysis by small angle X-ray solution scattering and FRET. We determined the Radius gyration (Rg) values of K16-GFP in the presence or absence of nucleotides by X-ray solution scattering. The Rg of nucleotide-free K16-GFP was about 42 Å. In the presence of ADP and ATP, the Rg values were 38 Å and 39 Å, respectively. We also analyzed conformational change of K16 in the solution by FRET. We constructed K16 fused with GFP (acceptor) that have single cysteine at 220 and was labeled with IAEDANS (donor). We could observe change of FRET efficiency during ATP hydrolysis. The FRET efficiency in the absence of nucleotide was lower than that in the presence of nucleotides, reflecting the distance between GFP and DANS in the presence of nucleotides is longer than in the absence of nucleotide. These results may suggest that the neck-linker of nucleotide free K16 is in the docked conformation, on the other hand, the neck-linker of nucleotide bound state is in the novel conformation in crystal structure.

2P-182 クラミドモナスの内腕ダイニン f 調節中間鎖を欠失した新規突然変異株の解析

Phenotypic analyses of novel mutants lacking an intermediate chain of flagellar inner-arm dynein f (I1)

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The flagellar movement in the unicellular alga *Chlamydomonas* is regulated so that the cell can display proper tactic behavior. Previous studies have shown that the phosphorylation of a 138 kD intermediate chain (IC138) of inner arm dynein f (also called I1) plays a critical role in this regulation, and that the central pair/radial spoke system is involved in the phosphorylation. However, the exact function of IC138 in dynein regulation is not clear. In the present study, we isolated novel alleles of *bop5*, a mutant defective in the structural gene of IC138. Unlike the previously isolated mutant (*bop5-1*) that expresses truncated IC138 (Hendrickson *et al.* 2004), one of our new alleles (*ida5-3*) lacks the entire IC138. Yet, it assembles both of the two heavy chains of dynein f and displays only modestly reduced motility. We further found that the axoneme of the *bop5* alleles, as well as various mutants lacking dynein f, lack a novel ankyrin-like protein registered as FAP120 in the flagellar proteome database. Intriguingly, the amounts

of FAP120 in the axonemes closely parallel the contents of IC138. These results suggest a weak but stoichiometric interaction between FAP120 and IC138. We propose that FAP120 functions in the regulatory process as part of a protein complex involving IC138. We are currently studying the activity of dynein f and the cellular behavior in various *bop5* alleles, to correlate the IC138 deficiency with dynein f regulation and cell behavior.

2P-183 F_1 -ATPase の β サブユニット協調性に関する理論モデル

Theoretical model for the concerted operation of the three beta subunits in F_1 -ATPase

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A rotary molecular motor, F_1 -ATPase has three catalytic sites on three β subunits and makes a stepwise rotation of 120° every time an ATP molecule is consumed; the 120° step consists of two substeps of 80° and 40° . Recent experiments (e.g., K. Adachi *et al.*, Cell, 2007; T. Ariga *et al.*, Nat. Struct. Mol. Biol., 2007) clarified the mechano-chemical coupling and the cooperated operation of β subunits in some detail. On each β subunit chemical events proceed in the following sequence: the binding of ATP, the cleavage of ATP, the release of ADP, and then the Pi release. The ATP binding and the Pi release are associated with the 80° and 40° substeps, respectively. One cycle of the chemical events on a single β subunit completes in a full revolution of the motor instead of a 120° rotation, and all the three β subunits participate to drive a single 120° step of rotation in a concerted manner.

We are interested in constructing a theoretical model of F_1 -ATPase that accounts for the features explained above in order to help understanding the mechanism of this marvelous molecular machine and to make predictions to be tested by experiments. For this purpose, a ratchet-type model for describing a single 'molecular chemical engine' (KS *et al.*, J. Phys. Soc. Jpn., 2007) is extended in such a way that three engines work in tandem. We are investigating whether this model works properly and hoping that it will provide useful information on a recently suggested (K. Shimabukuro *et al.*, Biophys. J., 2006) alternative reaction pathway.

2P-184 微小管上粒子拡散の温度依存性

Temperature dependence of particle diffusion along microtubules

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Motor proteins move along their "track" filaments by a cyclic repeat of weak and strong binding. Since strong binding is a specific interaction between local sites on both sides of the motor protein and the filament, acting more as an anchor, the actual motility depends more on the motion during the weak binding state. Thus investigation of the weak binding state is crucial to understand the physics behind the motility of motor proteins and ultimately their control. We have previously shown that positively charged polyacrylamide particles interact with negatively charged microtubules and can diffuse along their long axis. Since the diffusion constant of the particle changes as a function of its charge density, it is evident that an electrostatic interaction is involved. However, the free energy potential is the most important to consider and it is not at all clear if mere electrostatic interaction and thermal fluctuations of the particle can explain the above observation. Considering the fact that a microtubule has a diameter comparable with the size of the particle and is also situated in a thermal environment, entropic effects from the microtubule may also contribute to the diffusion of the bead. In the first step to explore such possibilities, here we analyze the diffusion of charged particles as a function of solution temperature. The results will provide more information on the free energy potential for the particle and thus more physical insight underlying the weak binding state.

2P-185 クラミドモナス軸系外腕ダイニンの in vitro 運動解析: 3つの重鎖の機能の違いについて

Motility of *Chlamydomonas* flagellar outer-arm dynein studied in vitro: distinct properties of the three heavy chains

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Outer arm dynein of cilia and flagella is a complex containing 2-3 heavy chains (HCs) that generate force for beating. To help understand how different HCs coordinate force production in beating axonemes, we systematically compared the motile properties between *Chlamydomonas* native outer arm dynein ($\alpha\beta\gamma$) containing α , β and γ HCs and mutant complexes ($\alpha\beta$, $\alpha\gamma$, and $\beta\gamma$) devoid of a particular HC. To facilitate comparison under controlled conditions, we constructed biotinylated outer arm dyneins using specific labeling. The velocity of microtubule gliding on the glass surface, to which the biotinylated dynein complex was fixed through avidin-biotin interaction, increased with the ATP concentration and the dynein density. It also increased when the dynein was preincubated with ADP. The duty ratio of the $\alpha\beta\gamma$ dynein was calculated to be as low as 0.08. Comparison of the motile properties of $\alpha\beta\gamma$, $\alpha\beta$, $\alpha\gamma$, and $\beta\gamma$ suggested that the β HC is the main engine while the γ HC functions as a brake. Remarkably, the α HC accelerates microtubule gliding without increasing the ATPase rate in the $\alpha\beta\gamma$ complex, indicating that the α HC functions to enhance the mechano-chemical coupling of the entire outer arm. These results indicate that the three HCs contribute differently to the entire motile activity of outer arm dynein complex.