

are good substrate for skeletal myosin and induce actin gliding in vitro motility assay. The synthesis of the two ATP analogues have been confirmed by FAB-MS. Excitation and emission maximums in the fluorescence spectrum of the ATP analogues were 474nm and 533nm for NBDTP, and 374nm and 430nm for MANTTP, respectively. NBDTP showed microtubules dependent ATP hydrolysis for conventional kinesin at the almost same level to that of regular ATP. The fluorescence changes of the ATP analogues during ATP hydrolysis have been also studied.

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Photo-activation Of Atpase Activity Of Caged-kinesin

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Photo-responsive caged compounds have high potency in the application concerning functional biological molecules as photo-switching device. Kinesin is a motor protein that moves along microtubule by the energy generated from ATP hydrolysis. The structure of conventional kinesin has been well studied and the key regions related to the function were clarified. In the present study, the photo-regulation of the catalytic activity of mouse brain and *C. elegans* kinesins were investigated by treating with a caging reagent, 4,5-dimethoxy-2-nitrobenzyl bromide (DMNBB). The mouse brain kinesin mutants that have a single reactive cysteine at 96C were prepared and modified by DMNBB in the presence and absence of ATP. For the kinesin modified in the absence of ADP, the ATPase activity was increased by 300% within 10 minutes. In the presence of ADP, the change of the ATPase activity was slower than that in the absence of ATP. Upon UV irradiation, the ATPase activity of the kinesin modified by DMNBB recovered to the level before modification. Wild type of *C. elegans* unc-116 kinesin motor domain derived from *C. elegans*, which has a single reactive cysteine residue. Modification with DMNBB and photo-irradiation on the wild type of *C. elegans* kinesin unc-116 showed also significant reduction and restoration of activity. We have identified the amino acid residue of kinesin unc-116, which affects activity by the modification with DMNBB as Cys16.

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Interaction of Processive Motor Proteins with ATP analogue Having *Syn* Conformation with respect to the Adenine-ribose bond

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It is known that 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**·ADP·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 velocity was 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. Myosin V is also processive motor protein like kinesin. Interaction of unconventional myosin V with 8-Br-ATP was also analyzed. Myosin V supported actin translocation using 8-Br-ATP. Currently, we are examining single molecular measurement of myosin V in the presence of 8-Br-ATP.

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Photo-regulation of Kinesin ATPase Activity using Photochromic Molecule

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Azobenzene is a photochromic molecule that undergoes rapid and reversible isomerization between the *cis*- and *trans*-forms in response to ultraviolet (UV) and visible (VIS) light irradiation, respectively. Previously, we have introduced the sulfhydryl-reactive azobenzene derivative 4-phenylazophenyl maleimide (PAM) into the functional region of kinesin to reversibly regulate the ATPase activity of kinesin by photoirradiation. The five kinesin motor domain mutants, A247C, L249C, A252C, G272C and S275C, which contained a single reactive cysteine residue in loops L11 and L12 were prepared. The PAM-modified S275C and L249C mutants exhibited reversible alterations in ATPase activity accompanied by *cis-trans* isomerization upon UV and VIS light irradiation. In the present study, we prepared the six new mutants (A21C, G26C, S66C, R16C, R25C, M96C) that have single cysteine at near

the ATP binding site in order to regulate kinesin activity more effectively than previous mutants. PAM was stoichiometrically incorporated into the cysteine residues in A21C, resulting in reduction of ATPase activity. However, PAM-modified A21C mutant did not exhibit reversible alterations in ATPase activity on UV-VIS light irradiation. PAM-modified G26C mutant also did not show the change in the ATPase activity upon UV-VIS light irradiation. On the other hand, PAM-modified S66C mutant exhibit reversible alterations in ATPase activity on UV-VIS light irradiation in a preliminary experiment.

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Photo-control Of Atpase Activity Of The Kinesin Motor Domain Intermolecularly Cross-linked By Bifunctional Photochromic Compound

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Kinesin is an ATP driven dimeric motor protein carries cellular cargoes along microtubules. 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, L249C, 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 Q21C, L25C, R26C, in L1, binding site, S66C, Q104C in ATP binding site, T196C in L9, K273C, K274C, Y276C 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. Additionally, We also prepared the mutant of dimmer kinesin A252C or T353C in order to photo-regulate motility of kinesin with ABDM.

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Processive Motility of Heterodimeric Kinesin That Has Defect in the Neck Linker Docking

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Kinesin-1 is a motor protein that moves processively along microtubules in a hand-over-hand manner. The neck linker, a short stretch that connects two motor heads, has been shown to undergo ATP-dependent conformational changes, although its role on the processive motility is still controversial. To address this question, we employed a kinesin mutant at switch I (R203K) that can normally bind ATP but is unable to hydrolyse ATP (Klump et al. JBC 2003). First we observed the neck linker structural state of monomeric R203K bound to the microtubule using single molecule FRET. We found that the neck linker remained undocked conformation even in the presence of saturating ATP, suggesting that ATP-binding is not sufficient to stabilize neck linker docked state. Next we constructed heterodimeric kinesin that is composed of a wild-type head and a mutant R203K head. As recently been shown by Thoresen and Gelles (Biochemistry 2008), this heterodimer showed slow processive movement along microtubules. Then we observed the conformational changes of this heterodimer using a single molecule FRET sensor as previously developed to distinguish one-head-bound and two-head-bound states (Mori et al. Nature 2007), and found that the heterodimer showed hand-over-hand movement. Unexpectedly, they spent most of the time in the two-head-bound state where wild-type head is in the front and the mutant head is in the rear, indicating that the displacement of rear wild-type head to the forward binding site is not the rate-limiting. These results suggest that the neck linker docking in the microtubule-bound head is not essential for the tethered head to translate and bind to the forward tubulin-binding site and rather is required for promoting ATP-hydrolysis and subsequent detachment of the trailing head from microtubule.

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Configuration Of The Kinesin1 Motor Domains In The ATP-waiting State As Revealed By Fluorescence Polarization Microscopy

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The molecular mechanism of coordination between kinesin1 heads during processive walking remains unclear, partly due to the lack of structural information on critical intermediates of the kinesin1 mechano-chemical cycle. To address this issue here we used ensemble and single molecule fluorescence