2P217**Cross-linking of kinesin using bifunctional photochromic compounds**

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As a photochromic compound changes its property and shape reversibly by UV/VIS light irradiation, it is expected that activity of the dimer kinesin cross-linked with bifunctional photochromic compounds could be photo-controlled by light irradiation due to alteration of the steric coordination between the two kinesin motor domains. In the present study, we have tried to crosslink the kinesin using bifunctional thiol reactive photochromic compound, 4,4'-azobenzene-dimaleimide (ABDM) resulting in formation of kinesin dimer. To incorporate the photochromic compound site specifically, E340C kinesin motor domain mutants, which have one cysteine in the C-terminal, were prepared. Reaction time and concentration dependency of cross-linking was examined. The maximum crosslinking efficiency reached to 20 %. The crosslinked kinesin was isolated from uncrosslinked kinesin using gel permeation chromatography. And the microtubules dependent ATPase activity of the isolated kinesin crosslinked by ABDM under UV or VIS light irradiation was measured. In addition, we have also tried to crosslink with a new bifunctional photochromic compound that has longer crosslinking span than ABDM.

2P219**Interaction of a novel rice kinesin O12 with fluorescent ATP analogue**

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Kinesin is a motor protein that plays important physiological roles in intracellular transport, mitosis and meiosis, control of microtubule dynamics and signal transduction. Kinesin converts chemical energy from ATP into mechanical force. Kinesin family is classified into some subfamilies. Some species of kinesin derived from vertebrate have been well studied on their characterization. However, not so many studies for kinesins of plants have been done yet. In the present study, we have succeeded to express the rice plant specific novel kinesin O12 motor domain with *E. coli* BL21 (DE3) LysE. The biochemical characterizations of the kinesin were studied and compared with conventional kinesin and another rice kinesin K16 we have previously characterized. ATPase activities and interactions with microtubules of the kinesin were apparently different from conventional kinesin. Furthermore, interaction of the kinesin with the fluorescent ATP analogues were studied and compared with that of conventional kinesin from mouse brain. It has been revealed that rice kinesin O12 may have different conformation of ATP binding site from that of conventional kinesin.

2P218**Characterization of a Novel Rice Kinesin D04 and Its Interaction with Fluorescent ATP analogue**

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Kinesin is an ATP-driven motor protein that plays important physiological roles in intracellular transport, mitosis and meiosis, control of microtubule dynamics, and signal transduction. The kinesin family is classified into subfamilies. Kinesin species derived from vertebrates have been well characterized. On the other hand, plant kinesins have been less well known. We have previously expressed a novel rice plant specific kinesin K16 and N14 motor domains in *E. coli* BL21 and have shown the kinesin has different enzymatic properties from conventional kinesin. In this study, we expressed additional novel rice plant kinesin D04 motor domain (N-terminal motor domain), in *E. coli* BL21 (DE3) and studied its enzymatic characteristics. Furthermore we examined the formation of D04-ADP-fluorometals complexes using phosphate analogues of aluminum fluoride (AlF₄) and beryllium fluoride (BeFn), which may mimic transient state intermediates in the kinesin ATPase cycle, using fluorescent ADP analogues. The microtubule dependent ATPase activity and affinity for microtubule of the D04 were apparently lower than that of conventional kinesin. When fluorescent ATP analogue, NBD-ATP bound to the ATPase site of D04, its fluorescent intensity was increased by 150%. After complete hydrolysis to NBD-ADP, the fluorescent intensity slightly decreased. Subsequent addition of phosphate analogues induced further different fluorescence intensity level, which may reflect the formation of D04-ADP-fluorometal ternary complex.

2P220**Insigh into The molecular rotation of c-subunit ring in Thermophilic Bacillus PS3 ATP synthase**

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In this decade, the driving force for the rotation of *c*-subunit ring in membrane-integrated ATP synthase was proposed to be generated by the conformational changes in trans-membrane helix (TMH)-2 of one *c*-subunit relative to the stationary TMH-4 of *a*-subunit due to its swivel motion, revealed with the cross-linking studies and NMR structural analysis of *Escherichia coli* *c*-subunit. Recently, molecular structures of *c*-subunit ring have been resolved by X-ray analysis of V-Type Na⁺-ATPase crystal from *Enterococcus hirae* and Na⁺-ATPase crystal from *Ilyobacter tartaricus*. Since the solution structures are tightly packed with surrounding residues, the fact implies that the conformational changes may not occur during *c*-subunit ring rotation. In this study, the coupling of proton translocation and rotation in the F_o of ATP synthase was investigated using a stable thermophilic *Bacillus* PS3 enzyme functionally expressed within *E. coli* cells. Some mutants with single or double cysteine replacements on TMHs of *c*-subunit ring were constructed based on the substantive structural information. Double cysteine residues, which introduced into TMH-1 and TMH-2, respectively, were efficiently cross-linked via disulfide bond without the addition of any oxidants. However no cross-linked product was obtained whenever a single cysteine residue was introduced into TMH-1. Remarkably, the cross-linked products were active in both proton-pumping and ATPase. These results suggested that the swivel motion in TMH-2 is not necessarily required for the F_o function.