Abstract of Doctoral Dissertation

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Dynamic Control of Microbial Movement by Photoswitchable ATP Antagonists

（光応答性 ATP アンタゴニストによる微生物の運動の可逆的制御）

Adenosine triphosphate (ATP) is essential for various biochemical processes and biomolecular motors in living things. The energy released from ATP hydrolysis is used for various cellular tasks such as muscle contraction, extracellular signaling, cargo transportation, and locomotion of microorganism. The dynamic biomolecular motors are responsible for various cellular movements at molecular level and their combined action leads to the locomotion of organisms at macroscopic level. Reversible photoregulation of these dynamic bimolecular motor proteins has received the center of attention because of their importance in movement and overexpression in various diseases sites, particularly in the tumor environment. Among the reversible photoswitches, azobenzene is predominant because of its ease of synthesis, fast photoisomerization rates, and high fatigue resistance. Our group has previously demonstrated the reversible photocontrol of the motility of kinesin and myosin motor proteins *in vitro* using a photoresponsive ATP analog, AzoTP. How this photoresponsive ATP analog, AzoTP, behaves to the dynein protein systems is unexplored and there are no studies on photocontrol of dynein motors. To address this issue, I have selected dynein as the target motor protein to study the photocontrol of motor activity. Dynein is a large ATP-fueled cytoskeletal motor proteins and exists in two isoforms: cytoplasmic and axonemal dyneins. Axonemal dyneins power the beating of cilia and flagella for the locomotion of eukaryotic organisms and cytoplasmic dyneins are involved in the mitosis and transport of intracellular cargoes. In this dissertation author demonstrate an unprecedented photocontrol of both cytoplasmic and axonemal dyneins using a dynein-microtubule system and a demembranated *Chlamydomonas reinhardtii* model, respectively.

In this thesis, the author reports that, the photoresponsive high-energy molecule, AzoTP cannot drive the gliding motility of microtubule on cytoplasmic dynein (single-motor unit)-coated glass surface. Consequently, the AzoTP act as photoresponsive ATP antagonist for dynein, this is presumably due to the complex structure and powering mechanism of dynein compared to kinesin and myosin. Interestingly the high-energy molecule, AzoTP can undergo hydrolysis (basal ATPase) in a highly efficient manner than ATP. These results of the motility and hydrolysis experiment suggest that AzoTP occupy the recognition site for ATP in dynein in a competitive manner, but the hydrolysis reaction was inefficiently coupled to create the force production for motility. Based on this observation author redesigned the photoresponsive high energy molecule to azobenzene-based non-hydrolysable triphosphate molecules, Azo-Amide-PCP and Azo-propyl-PCP, with "amide" and "propyl" spacer between azobenzene (Azo) and methylene diphosphate (PCP) moieties to work as a photoresponsive ATP antagonist. Hence, the development of optochemical tools that can work as photoresponsive "ATP antagonists" has enormous possibilities in controlling the biochemical processes as well as in designing drugs which potentially leads to the regulation of biological processes in a spatiotemporal manner.

The AzoTP, and Azo-Amide-PCP, served as photoresponsive ATP antagonists by inhibiting the gliding motility of microtubule filaments on cytoplasmic dynein coated glass surface in a dose-response fashion in the presence of ATP. The reversible photo-switching of the gliding velocities of microtubule filaments was observed upon illumination of 365 nm and 430 nm. The velocity increased remarkably after irradiation of 365 nm and significantly decreased with irradiation of 430 nm. Alternate UV and Vis irradiations resulted in the reversible switching of microtubule gliding velocity. To assess the reversible control of dynein motor activity in eukaryotic organism, which is substantially more complex system. *Chlamydomonas reinhardtii*; single-cell green algae uni-1 (single flagella) was selected, which is known to undergo rotational motion through a co-operative action of multiple dynein motors at the flagella. The effect of photoresponsive ATP antagonists was examined using demembranted *C. Reinhardtii* which gives easy access to the motile units. The ATP antagonist, Azo-Amide-PCP, and Azo-Propyl-PCP were applied to the *Chlamydomonas* cells which result in the inhibition of rotational motion. The motion was restarted after irradiation with 365 nm (5s) and again inhibited with irradiation of 430 nm (7s).

To rationally explain the observation of reversible control of motor activity, molecular docking studies was performed AutoDock Vina. The ATP binding site of cytoplasmic dynein (AAA1) chain was superimposed with docked *trans*/*cis*-AzoTP, *trans*/*cis*-Azo-Amide-PCP, and *trans*/*cis*-Azo-Propyl-PCP. Azobenzene motif of the ATP antagonists in its *trans* isomer showed a parallel orientation with the adenine motif of ATP, however, *cis* isomer was in perpendicular orientation showing that *trans* isomer has higher binding affinity than *cis* form. Thus, inhibitory activity was photo-modulated by the conformational changes associated with *cis*-*trans* photoisomerization of azobenzene. This is the first study showing reversible regulation of dynein motor protein at molecular level and organism levels. The author believes that this finding of photo regulation of motor protein from the molecular level to flagellar level indicates that regulating the locomotion of living organisms could be done by utilizing photoresponsive molecules.