学 位 論 文 内 容 の 要 旨

Abstract of Doctoral Dissertation

博士の専攻分野の名称　博士（生命科学）　氏 名　ｽﾆﾙ ｸﾏｰﾙ ｹｰ ｱｰﾙ

Degree requested　Doctor of Life Science　SUNIL KUMAR K R

学 位 論 文 題 名

Title of Doctoral Dissertation

Synthesis of azobenzene-peptides and their application as a photoresponsive inhibitor for ON/OFF photoswitching of the motility of kinesin-microtubule

（アゾベンゼン- ペプチドの合成とキネシン-微小管の運動機能の ON/OFF 光スイッチのための光応答性阻害剤としての応用）

Protein molecular motors are naturally evolved nanosized machines responsible for mechanical movement, which is essential for many biological functions, including cell division, movement, intracellular transport, and muscle contraction. Kinesin is one among such motor proteins that converts chemical energy, derived from the hydrolysis of adenosine triphosphate (ATP), into mechanical work. The force generated in this process enables kinesin molecules to actively transport designated nano cargo (e.g., vesicles, chromosomes, organelles) to predetermined sites along microtubules, which are cytoskeletal tracks within a cell. The properties of motor proteins—nanometer scale, high fuel efficiency, and force-generating capabilities—make them attractive alternatives to man-made motors, resulting in their use as key components for the construction of highly efficient nanotransport systems. One vision is that the systems based on motor proteins will be used for controlled cargo manipulation on chips, with applications in sorting, separation, purification, or assembly of materials. In the last decade, microtubules have been employed extensively as shuttles to transport attached cargo over surfaces coated with motor proteins. To actualize the utility of such transport systems, it is needed to develop some switching systems allowing the control of their motility. Especially, a complete ON/OFF switching would be necessary in future applications of natural molecular motors for manipulating nano materials as cargos freely in hand from a desired point to the other at any desired timing.

Many researchers have attempted, for the artificial regulation of the motility of the microtubules on kinesin using various methodologies. These include the utilization of caged ATP, caged inhibitor, thermo responsive polymer, electro responsive polymer and photo responsive azobenzene derivatives. Unfortunately all the methods described above suffer from various limitations that disrupt the gliding motility of microtubules in a fully reversible manner. The challenge has remained to develop a system capable of perfect ON/OFF switching of motility with complete control over timing.

In this dissertation, I describe the photoresponsive inhibition properties of azobenzene-tethered peptides, their photoswitchability and structural effect on the complete regulation of kinesin-microtubule motility including complete ON/OFF photoswitching of the motility. I have synthesized and characterized several plain inhibitory peptides and azobenzene tethered-peptides having the peptide sequence derived from the kinesin C-terminus tail domain (known as inhibitor of the motor domain). We tested the photoisomerization behavior of all the synthesized azobenzene-peptides upon UV-visible light irradiation and obtained comparable isomerization behavior.

I performed an *in vitro* kinesin-microtubule motility assay in which all the synthesized peptides were implemented. The motility experiments revealed various factors necessary to achieve complete ON/OFF switching of the motility. Among all the tested compounds, the plain inhibitory peptides showed the inhibitory behavior depending on their structure and peptide sequence order. Whereas in the case of azobenzene tethered peptides, some showed photoresponsive inhibitory behavior and some were not. Among the several tested azobenzene-peptides, we discovered a compound containing a peptide and a terminal azobenzene unit that completely stops and starts the motility of kinesin-microtubule in its *trans* and *cis*-rich states, respectively, obtained after irradiation with visible and UV light, respectively. A gliding motility system utilizing this photoresponsive inhibitor allowed *in situ* control over the motion of microtubules on a kinesin-coated glass substrate. The rapid and repeatable regulation of such motility suggests that this system has great potential for use in the development of nano transport systems.

This is the first demonstration on the complete photoregulation of the "ON" and "OFF" motions upon alternating irradiation with UV and visible light. The advantage of employing photoresponsive unit to the inhibitors to regulate the gliding velocity of microtubules is that the velocity can be completely decreased to zero at sufficiently high concentrations of inhibitor, even in the presence of the less-effective isomer in the PSS. Such a motile property, exhibiting the complete zero velocity in the "OFF state" and the repeated switchability to be able to obtain the complete zero velocity again after attaining the "ON state" with reasonably high velocity, is in general necessary in artificial applications of motor proteins to nano transportation devices. Specifically, it would enable us to make an active spot with photo-generated *cis*-azo-peptide allowing cargo-attached microtubules to move by irradiating with UV light selectively at any desired region. Such an active spot could be moved freely just by moving the position of UV light in an inactivated background irradiated with visible light keeping azo-peptide in *trans*-rich state. In such a manner with a focused UV light we would select one specific microtubule attached with cargos and guide it to a desired point. As a consequence, all kinds of transportation of nano-objects for separation, mixing, concentration would be possible. We expect that the complete photoregulation ability exhibited by an azobenzene-peptide on the motility of kinesin-microtubules will aid in the development of real molecular machines working at will and open up new opportunities to design nano-transportation systems.