学 位 論 文 内 容 の 要 旨
Summary of Doctoral Dissertation

博士の専攻分野の名称 Degree requested　博士（生命科学） Doctor of Life Science　氏 名 NISHAD PERUR

学 位 論 文 題 名
Title of Doctoral Dissertation

Driving and reversible controlling of motor proteins by photochromic non-nucleoside triphosphates
（フォトクロミック非ヌクレオシド三リン酸によるモータータンパク質の駆動および可逆的制御）

A huge amount of biological research in recent decades has led to the realization that the living cell can be viewed as a miniature factory that contains a large collection of dedicated motor proteins. These cytoskeletal motor proteins use the energy of adenosine triphosphate (ATP) hydrolysis to perform mechanical tasks such as cell division, cell motility (migration), intracellular transport, muscle contraction, and organization of the organelles within the cell. There are two classes of biomolecular motors: linear and rotary. Linear motors include the kinesin, dynein and myosin motors while the rotary motors include the F_0-F_1 ATP synthase. The latest advances in the field of biomolecular motors in nanotechnology have made it clear that these motor proteins possess a number of intrinsic characteristics that make them exquisite candidates for powering or manipulating nanoscale engineered devices.

An important demand in the nanotechnology is artificial controlling of the activities of motor proteins. In an effort to control the activity of biomolecular motors, various methodologies have been developed so far. These include utilization of magnetic field, electric field, ionic gradient, temperature, light, artificial tracks and so on. Although each method has shown promise to control motor protein activity, these approaches have not yet clearly demonstrated the ability to reversibly control motor activity over several cycles of activation. When considering the biodevices, light-control would be most favorable because light has several advantages over other external stimuli such as high temporal and spatial resolution. Light can be applied to the targeted site without any physical contact and act without producing chemical waste.

In this dissertation, a set of non-nucleoside triphosphates to drive and control the activities of motor proteins have been described. One of the synthesized non-nucleoside triphosphates, NPhAETP, has previously been reported as a substrate for actomyosin motor protein system. In addition, we synthesized another three novel non-nucleoside triphosphates. Among these, NPhAdMTP is a new amide version of NPhAETP to investigate the effect of the linkage between the aromatic ring and the triphosphate; we also synthesized azobenzene triphosphate, AzoTP, by exchanging the nitro group of NPhAdMTP with a photoresponsive phenylazo group. The second azobenzene triphosphate, *p*-AzoTP synthesized in such a way that amide-triphosphate part incorporated to the *para* position of azobenzene.

I performed an *in vitro* kinesin-microtubule motility assay in which all of the four compounds were used as substrate. The motility experiments revealed that all of the tested compounds are capable of driving kinesin-microtubule motility. NPhAETP and NPhAdMTP showed low efficiency in driving the microtubule gliding and results suggested that the changing the linkage between the aromatic ring and triphosphate unit from an amino to an amido group did not affect the activity significantly. In contrast, AzoTP provided high efficiency in driving the microtubule gliding with a velocity of half of ATP-driven motility, whereas *p*-AzoTP showed low efficiency same as NPhAETP and NPhAdMTP. These results indicate that both the structure of the aromatic group and position of the triphosphate moiety on the aromatic group have significant effect on the activity of kinesin. To check the generality on the motor proteins, AzoTP was employed as a substrate on actomyosin motility assay and F_1-ATPase rotation assay systems. The obtained results suggested that AzoTP is generally active to motor proteins.

Furthermore, the effect of photoisomerization of azobenzene moiety of AzoTP on the activities of motor proteins upon alternating irradiation with UV and visible lights is investigated. The observed

results suggested that there are considerable reversible changes in the activity of motor proteins by *cis*-to-*trans* photoisomerization of AzoTP upon alternating irradiation with UV and visible lights.

This is the first demonstration of the substantial control of the activity of a motor protein in a reversible manner. It is expected that AzoTP could be applied in biomotor-based transportation systems and in the artificial operation of natural motor proteins in living cells.