A non-nucleoside triphosphate for powering kinesin-microtubule motility with photo-tunable velocity

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We synthesized three types of non-nucleoside triphosphates among which azobenzene-tethered one shows high motile activity of a kinesin-microtubule system with an observed maximum velocity of almost half of that with ATP. The cis-trans photoisomerization of azobenzene moiety controls the motile properties of kinesin reversibly and repeatedly.

Kinesin\(^1\) is a microtubule based motor protein, which plays substantial roles in many cellular processes in eukaryotic cells. Kinesin motor transports various cargos along the microtubule filaments by utilizing adenosine tri-phosphate (ATP) as energy source.\(^2\)\(^3\) Kinesin-microtubule motility system is an exquisite model for the biologically derived nano-scale motion. Several ATP analogues have been reported to study the motile activities of kinesin motor protein.\(^5\)\(^6\) Among the various substrate analogues, multifunctional substrate analogue gives more importance on in vitro kinetic studies of biological motor proteins.\(^6\)\(^7\)

Azobenzene, one of the most studied photochromic compounds,\(^8\) which undergoes cis-trans photoisomerization. The cis and trans isomers can be switched with particular wavelengths of light. Various applications of the photo-induced isomerization of azobenzene moiety have been reported for switching functions of biomolecules.\(^9\) It remains a challenge to control reversibly the motile activities of kinesin motor protein by the photoisomerization of azobenzene.\(^10\)\(^11\) Recently, our group has synthesized ATP analogues substituted with azobenzene at ribose group and demonstrated driving and reversible photo-controlling of kinesin-microtubule motility.\(^12\) However, the observed change with the ATP analogue in the motility upon photo-irradiation was far from satisfactory.

In the present study, we introduce a novel photo-responsive non-nucleoside triphosphate used as a substrate to drive microtubule gliding on a kinesin coated glass surface and to regulate its motility reversibly by the alternative irradiation with two different wavelengths.\(^11\)

NPhAETP (Fig. 1) has been reported as a substrate for actomyosin motor protein system.\(^13\) NPhAdMTP (Fig. 1) is a newly synthesized amide version of NPhAETP to investigate the effect of the linkage between aromatic ring and triphosphate, whereas AzoTP is synthesized by exchanging the nitro group of NPhAdMTP with a photo-responsive phenyl azo group (Fig. 1).

Fig. 1 Structures of non-nucleoside triphosphates, AzoTP, NPhAETP and NPhAdMTP in comparison with adenosine triphosphate (ATP).

![UV-vis absorption spectra of AzoTP (5.85 x 10^{-5} M) in water. (Black line) Before irradiation. (Red line) Photostationary state (PSS) at 366 nm. (Blue line) PSS at 436 nm. Inset: Absorbance changes at 327 nm after the alternative irradiation at 366 nm and 436 nm up to 10 cycles.](image-url)
The compounds were characterized by $^1$H-NMR and Electrospray (ESI) mass spectrometry. The in vitro microtubule gliding assay\textsuperscript{14} was carried out using flow cell method. In this assay, the gliding motility of NPhAdMTP capable of driving microtubule motility. NPhAETP and microtubules in 40 seconds.

The optical surface was visualized by fluorescence flow cell method. (3 mM)

| 1) Before irradiation. 2) After UV (366nm) irradiation for 10 s. 3) After subsequent irradiation at 436 nm for 30 s. 4) After subsequent UV irradiation for 10 s. 5) After subsequent irradiation at 436 nm for 30 s. | 0 s. 5) After subsequent irradiation at 436 nm for 30 s. | 436 nm leads to its trans-rich photostationary state by recovery conversion of cis to trans. We examined the effect of photo-induced isomerization of AzoTP on microtubule gliding velocity. Fig. 3 shows the fluorescence images of gliding microtubules at 0 sec and 20 sec for non-irradiated and 366 nm photostationary states, respectively, in the same flow cell (videos are provided in ESI). By the direct UV light irradiation of the flow cell, trans AzoTP is converted to cis form, which results in reducing the gliding velocity of microtubule from 0.33 to 0.07 μm/s. Subsequent visible light irradiation induces the recovery of velocity of microtubule to 0.28 μm/s due to reverse cis to trans isomerization of AzoTP. Fig. 4 indicates the changes in the motility in two cycles by the alternative irradiations with UV and visible lights. The maximum change in the velocity upon photorradiation was 79 % of the initial velocity in the trans form at 3 mM of AzoTP. The repeatable photo-controlled velocity changes between cis-rich and trans states were investigated up to 10 cycles of UV and visible light irradiations (Fig. S7 in ESI). To check the effect of the irradiation light on the original microtubule motility, we carried out a control experiment with 1mM ATP and confirmed that there were no significant changes in the motility by the alternative irradiations with UV and visible lights (Fig. S6 in ESI).

The red and blue circles indicate the gliding velocities corresponding to non-irradiated (100 % trans) and irradiated (cis-rich) states, respectively. It was estimated with $^1$H-NMR that cis-rich photostationary state contains 92 % of cis and 8 % of trans isomers (Fig. S4 in ESI). The black line indicates the microtubule gliding velocity evaluated for 8 % of trans isomer (Fig. 5). Here, the gliding velocity observed for cis-rich photostationary state is slightly higher than that evaluated for 8 % of trans isomer of AzoTP. The contribution from 92% of cis isomer of AzoTP is estimated by the difference in the values of the velocities between the experimental data at cis-rich photostationary state and the theoretical black line for 8% of trans isomer of AzoTP. According to the estimation, the efficiency of cis isomer for microtubule motility is only 1/29 of that of trans isomer at 3 mM concentration of AzoTP.

It is well known that kinesin-microtubule system is driven by some ATP analogues modified at its base or ribose unit.\textsuperscript{5,6}
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AzoTP to such sandwiched by the stacking and hydrophobic interactions. The azobenzene is feasible for the mismatched methylene group of arginine from below and by hydrophobic interaction with proline with any amino acid residues of kinesin in the binding site. In the present study, it is demonstrated for the first time that non-nucleosides are good enough to replace the base and ribose units in a substrate of kinesin.

In sharp contrast to the photo-responsive ATP derivatives substituted at the ribose with a 4-tert-butylazobenzene, the present AzoTP showed a quite large change in the activity upon photosomerization to kinesin-microtubule system. It is known that the ribose group in ATP does not make a special interaction with any amino acid residues of kinesin in the binding site, while adenine is sandwiched by a stacking interaction with histidine from below and by hydrophobic interaction with proline and the methylene group of arginine from above. The azobenzene part of AzoTP should have the role of the aromatic group in place of adenine in ATP. Azobenzene in trans state has a flat shape which is feasible for the stacking interaction. On the other hand, cis azobenzene has a round shape that would prevent it from being sandwiched by the stacking and hydrophobic interactions. The large change in the activity of AzoTP in trans and cis states can be explained by the difference in shapes inducing the change in such interactions.

In summary, we achieved the substantial control of kinesin-microtubule motility with 79% of change in velocity in photo-induced and reversible manner by a newly synthesized non-nucleoside triphosphate, AzoTP. We expect the application of AzoTP to bio-motor based transportation system and to the artificial operation of natural motor proteins in living cells.

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† Electronic Supplementary Information (ESI) available: [Synthesis and characterization of compounds and experimental conditions of kinesin-microtubule motility system]. See DOI: 10.1039/b000000x.
16 The concentrations of AzoTP solutions were calculated based on the molecular weight of tetra sodium triphosphate. The error of the values of concentrations within 10% can be included due to the contamination of less sodium species (mono-, di- and tri-).