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

Nishad Perur, Masao Yahara, Takashi Kamei, and Nobuyuki Tamaoki*

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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.[2-4] 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).

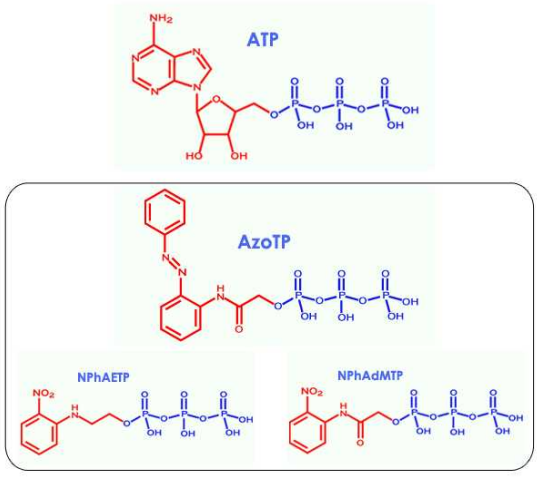

Fig.1 Structures of non-nucleoside triphosphates, AzoTP, NPhAETP and NPhAdMTP in comparison with adenosine triphosphate (ATP).

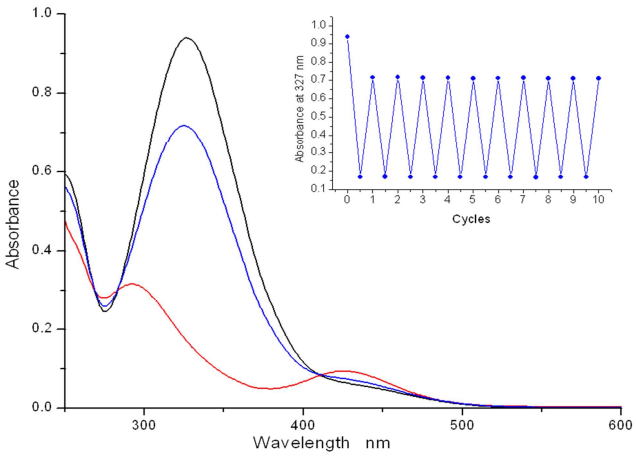

Fig.2 UV-vis absorption spectra of AzoTP (5.85 x 10^{-5} M) in water. (Black line) Before irradiation. (Red line) Photostationary state (PSS) at 366 nm. (Blue line) PSS at 436 nm. Inset: Absorbance changes at 327 nm after the alternative irradiation at 366 nm and 436 nm upto 10 cycles.

The compounds were characterized by ^{1}H-NMR and Electrospray (ESI) mass spectrometry.

The *in vitro* microtubule gliding assay[14] was carried out using flow cell method. In this assay, the gliding motility of fluorescently labeled microtubules[15] on kinesin coated glass surface was visualized by fluorescence optical microscopy. The gliding velocity was calculated by tracing the leading point of microtubules in 40 seconds.

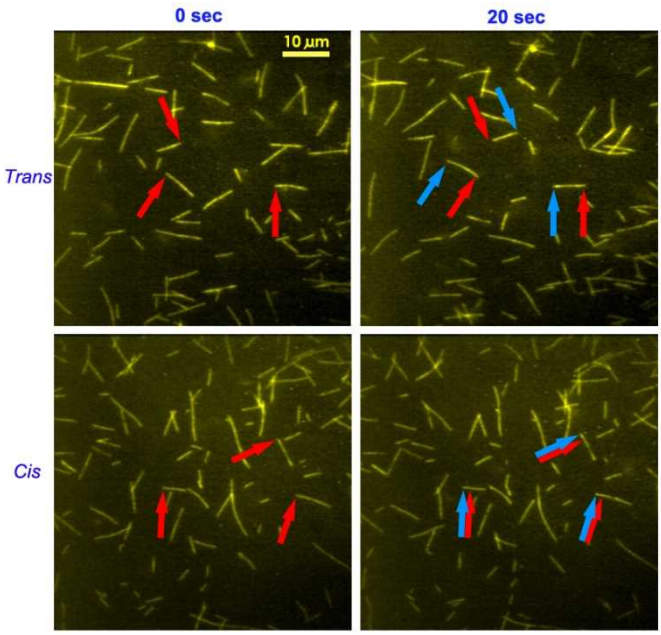

Fig.3 Fluorescence images of the gliding microtubules driven by AzoTP (3 mM) on the kinesin-adsorbed glass surface. (Top) Non-irradiated state at 0 s (left) and 20 s (right), and (bottom) 366 nm irradiated state at 0 s and 20 s in the flow cell. Red arrows indicate the leading points of microtubules at 0 sec and blue arrows indicate the leading points of the same microtubules at 20 sec.

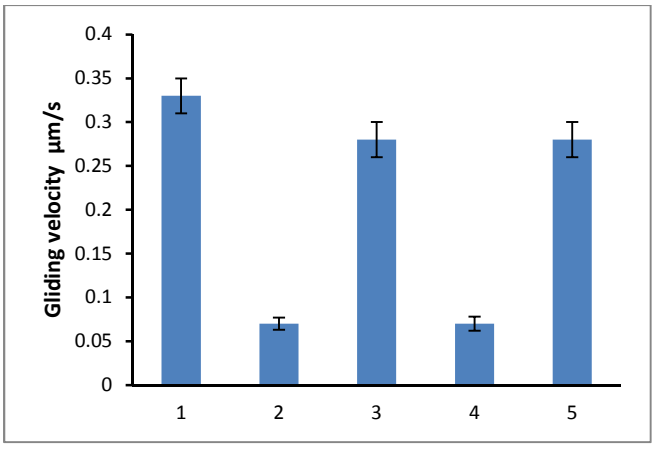

Fig.4 Repeatable changes in the gliding velocity of microtubules in the presence of AzoTP (3 mM) by the alternative UV and visible light irradiations. 1) Before irradiation. 2) After UV (366nm) irradiation for 10 s. 3) After subsequent irradiation at 436 nm for 30 s. 4) After subsequent UV irradiation for 10 s. 5) After subsequent irradiation at 436 nm for 30 s. Error bars represent standard deviations for 10 microtubules.

Motility experiment shows that all the three compounds are capable of driving microtubule motility. NPhAETP and NPhAdMTP showed the saturated gliding velocity of the microtubule with only 0.03 μ m/s and 0.04 μ m/s, corresponding to 4 and 6%, respectively, of the saturated velocity driven by 1 mM of ATP (0.67 μ m/s). In case of kinesin, NPhAETP works but with limited efficiency comparing to myosin. And it was confirmed that the change in the linkage between the aromatic ring and triphosphate from amine to amide does not largely affect to the activity. In contrast to these two nitro derivatives, AzoTP shows high microtubule gliding motility (0.33 μ m/s) at the concentration of 3 mM,[16] corresponding to 49% of velocity of 1 mM ATP-driven motility. These results indicate that aromatic group in non-nucleotides largely affect to the activity as a substrate of kinesin.

Fig. 2 shows the change in the absorption spectra of an aqueous solution of AzoTP upon UV and visible light irradiations. By the 366 nm light irradiation, AzoTP solution reaches its *cis*-rich photostationary state, and subsequent irradiation at 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 μ m/s. Subsequent visible light irradiation induces the recovery of velocity of microtubule to 0.28 μ m/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 photoirradiation 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).

Fig. 5 shows the results of motility experiments with different concentrations of AzoTP at non-irradiated and irradiated states. 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[5,6] and

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various nucleoside triphosphates containing different base units[17] such as guanine, thymine, uracil, cytosine and inosine. With 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.

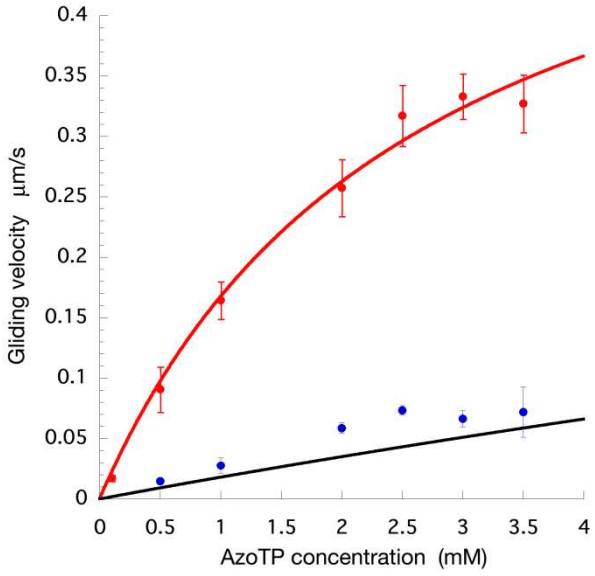

Fig.5 Concentration dependence curves of AzoTP on the gliding velocities of microtubules. Red circles are corresponding to observed gliding velocity for non-irradiated state. Red line represents the fitting curve by Michaelis-Menten equation (V_{max} = 0.61 μm/s and K_m = 2.6 mM). Blue circles are the observed gliding velocity for *cis*-rich states after 366 nm light irradiation. Black line is a theoretical curve derived from the red line for 8% of *trans* AzoTP. Error bars indicate standard deviations for 10 microtubules.

In sharp contrast to the photo-responsive ATP derivatives substituted at the ribose with a 4-*tert*-butylazobenzene,[12] the present AzoTP showed a quite large change in the activity upon photoisomerization 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.[18] 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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Notes and references

Research Institute for Electronic Science, Hokkaido University, N20, W10, Kita-ku, Sapporo, Hokkaido, Japan.
Fax: +81-11-706-9357; Tel: +81-11-706-9356;
E-mail: tamaoki@es.hokudai.ac.jp.

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