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Title	Driving and photo-regulation of myosin-actin motor at molecular and macroscopic level by photo-responsive high energy molecules [an abstract of dissertation and a summary of dissertation review]
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Degree Grantor	北海道大学
Degree Name	博士(生命科学)
Dissertation Number	甲第12952号
Issue Date	2017-12-25
Doc URL	https://hdl.handle.net/2115/68140
Rights(URL)	https://creativecommons.org/licenses/by-nc-sa/2.1/jp/
Type	doctoral thesis
File Information	Halley_Mariet_Menezes_abstract.pdf, 論文内容の要旨



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 : Halley Mariet Menezes

Degree requested: Doctor of Life Science

Applicant name: Halley Mariet Menezes

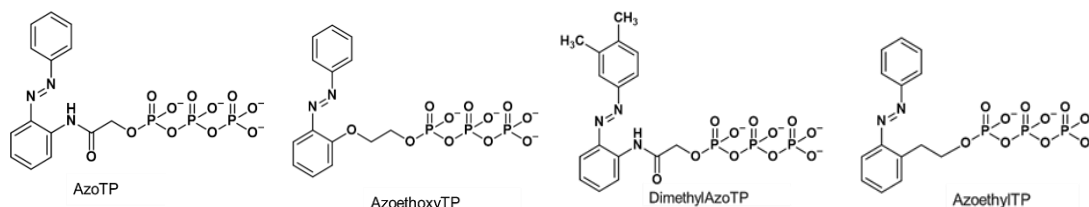
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Driving and photo-regulation of myosin-actin motor at molecular and macroscopic level by photo-responsive high energy molecules

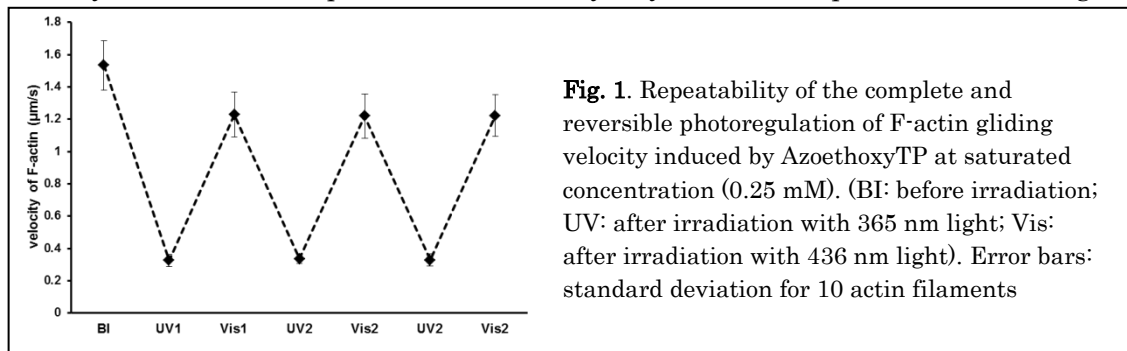
(光応答性高エネルギー分子による分子およびマクロレベルでのミオシンーアクチンモーターの駆動と光制御)

The free energy derived from the hydrolysis of adenosine triphosphate (ATP) acts as the energy fuel for biomolecular motors' motile functions. Harnessing these robust and versatile molecular motors for nanotechnology involves the dynamic control over their motile properties including velocity, direction of motion, processivity and on/off switching. Light stimuli are the most favorable approach as the photochromic molecules can reversibly control the motile functions by virtue of photoisomerization. Our group reported the photochromic azobenzene-based non-nucleoside triphosphate (AzoTP) analog of ATP for reversibly photo-controlling the motility of kinesin-microtubule motor system *in-vitro*. In the present study we have developed three new derivatives of AzoTP by modifying the bridging group between azobenzene and triphosphate and by substitution on azobenzene moiety of parent AzoTP. Substituting the amide linkage with ether and ethyl linkage resulted in AzoethoxyTP and AzoethylTP respectively, while the substitution of methyl groups at *meta* and *para* on azobenzene moiety resulted in DimethylAzoTP. All the four AzoTP molecules were employed in myosin-actin motile system and demonstrated their efficiency as energy molecules to drive and photo-regulate the myosin-actin motile function at macroscopic and molecular level.



The four AzoTPs served as substrates for myosin by driving HMM induced gliding motility of F-actin on HMM immobilized glass surface and the average velocity of F-actin triggered by AzoTP, AzoethoxyTP, DimethylAzoTP, AzoethylTP is 1.25 $\mu\text{m/s}$, 1.50

$\mu\text{m/s}$, $1.24 \mu\text{m/s}$, $0.73 \mu\text{m/s}$ respectively at saturated concentration of 0.5 mM for AzoTP, DimethylazoTP, AzoethylTP and 0.25 mM for AzoethoxyTP. We observed the reversible photo-switching of *in-vitro* gliding velocity of actin filaments upon alternative irradiation with 365 nm UV and 436 nm Visible light triggered by the reversible photoisomerization of AzoTP molecules. The velocity decreased remarkably after irradiation with 365 nm light corresponding to *cis*-rich state, the subsequent irradiation with 436 nm recovered the velocity, comparable to that of initial velocity before irradiation. This phenomenon of reversible switching between the faster and slower velocity could be repeated over many cycles as represented in Fig. 1.



To probe the efficiency of AzoTP molecules at macroscopic level we applied them in glycerinated muscle fibre shortening experiments. The parent AzoTP in its *trans* state induced the shortening of muscle fibre accounting for $40 - 45\%$ shortening of muscle fibre's initial length. Pre-generated *cis* isomer of AzoTP didn't induce any significant shortening, thus affirming the poor activity of *cis*-isomer as evidenced in our molecular *in-vitro* motility experiments (Fig.2a). When the *cis* isomer infused muscle fibre was irradiated with 510 nm , remarkable shortening of about 40% of its initial length was observed, thus confirming the efficiency of AzoTP to drive and photocontrol the myosin motor function in the macroscopic system (Fig.2b). Further, the efficiency of all three newly synthesized AzoTP derivatives was investigated. Shortening of muscle fibre with respect to time increases with the order of AzoTP ~ AzoethoxyTP > DimethylazoTP > AzoethylTP, where the substrates AzoTP and AzoethoxyTP induce the shortening swiftly with larger magnitude of length change than DimethylazoTP and AzoethylTP over the time range. Similar to AzoTP, the *cis*-form of its three derivatives was unable to induce any significant shortening and the 510 nm light irradiation of *cis* infused muscle fibres induces the shortening except AzoethylTP.

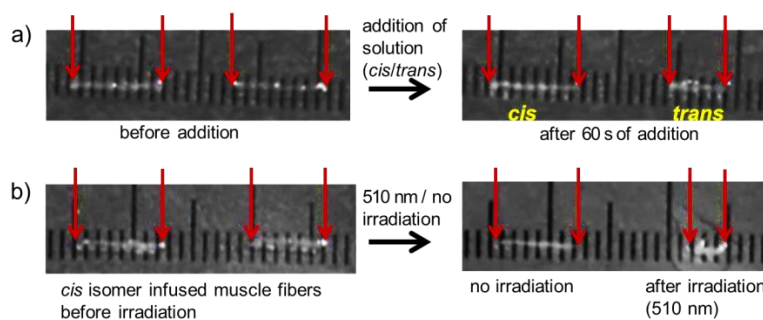


Fig. 2. AzoTP induces and photo-controls the shortening of glycerinated muscle fibre.

The newly designed and synthesized three AzoTP derivatives along with the parent AzoTP drive the myosin motor and photoregulate the its motile functions at molecular as well as macroscopic level with high efficiency.