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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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Abstract of Doctoral Dissertation

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

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 µm/s, 1.50
µm/s, 1.24 µm/s, 0.73 µm/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 365nm UV and 436nm 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 cisform 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.

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.

Fig. 1. Repeatability of the complete and reversible photoregulation of F-actin gliding velocity induced by AzoethoxyTP at saturated concentration (0.25 mM). (BI: before irradiation; UV: after irradiation with 365 nm light; Vis: after irradiation with 436 nm light). Error bars: standard deviation for 10 actin filaments

Fig. 2. AzoTP induces and photoregulates the shortening of glycerinated muscle fibre.