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Three-State Model for One-Dimensional Brownian Motion of Charged Nanoparticles Along Microtubules

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A variety of motor proteins, such as dynein, KIF1A and MCAK, are known to exhibit one-dimensional (1D) Brownian motion along microtubules. The electrostatic interaction between the proteins and microtubules appears to be crucial for 1D Brownian motion, although the underlying mechanism has not been fully clarified. We examined the interactions of positively-charged nanoparticles composed of polyacrylamide gels with microtubules. These hydrophilic nanoparticles bound to the microtubules and displayed 1D Brownian motion in a charge-dependent manner, which indicates that nonspecific electrostatic interaction is sufficient for 1D Brownian motion. While the diffusion coefficient decreased exponentially with an increasing particle charge (with the exponent being 0.10 $k_BT$per charge), the duration of the interaction increased exponentially (with an exponent of 0.22 k_BT per charge). These results can be consistently explained if one assumes that a particle repeats a cycle of 'binding' and 'diffusion' along a microtubule until it finally 'dissociates' from the microtubule. This entire process can be described by a three-state model analogous to the Michaelis-Menten scheme, in which two parameters - the equilibrium constant between 'binding' and 'diffusion', and the rate of 'dissociation' from the microtubule - are derived as a function of the particle charge density. Further, to understand the molecular basis for this 'binding' and 'diffusion' mechanism, we engineered microtubules with variable charges at the tubulin C-terminal tail (CTT) and measured 1D Brownian motion of the particles along these mutant microtubules. The measurements revealed an unexpected result: the negative charges in the CTT did not significantly affect the diffusion coefficient of the particles, but the equilibrium between 'binding' and 'diffusion' shifted more towards 'diffusion' with increasing charges of the CTT. These results indicate that the negatively-charged CTT provides a field that facilitates the 'diffusion' of charged particles.

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Mechanism of Unidirectional Move of KIF1A Motor Studied by Coarse-Grained Simulations

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KIF1A is a single-headed motor which can move unidirectionally along a microtubule (MT) using the chemical energy produced by ATP hydrolysis. Several experimental studies revealed that KIF1A makes the biased Brownian movements (Okada et al., 2000). Fortunately, two major structures (ATP type and ADP type) are available. However, how KIF1A generates the translational movement from chemical reaction cycle still remains to be elucidated.
To address this question we try to reproduce translational movement of KIF1A by coarse-grained simulation of the multiple-basin model (Okazaki et al., 2006) that realizes conformational change (Kikkawa et al., 2006) during ATP hydrolysis cycle.
With a first set of simulations, ADP-type KIF1A detached from MT, diffused along MT, and attached to MT, but we did not find any forward bias in the stepping. We then found one condition that reproduces the biased Brownian movement. Namely, when a cargo (or a bead) with sufficiently large radius is attached to the C terminus of KIF1A, as in the in vivo situation, KIF1A exhibited the forward-biased Brownian movement along MT, in a consistent manner to experiments. In the presentation, we will also suggest the similarity of stepping mechanism between one-headed KIF1A motor and two-headed conventional kinesin.

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Protein Motors Induced Enhanced Diffusion In Intracellular Transport

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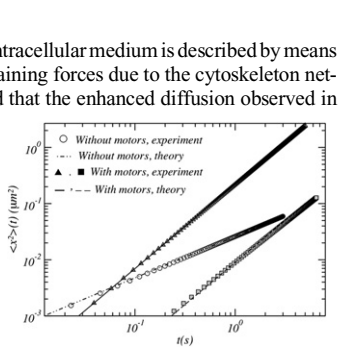
Diffusion of transported particles in the intracellular medium is described by means of a generalized diffusion equation containing forces due to the cytoskeleton network and to the protein motors. We find that the enhanced diffusion observed in experiments depends on the nature of the force exerted by the protein motors and on parameters characterizing the intracellular medium which is described in terms of a generalized Debye spectrum for the noise density of states. The model can be used to account for endocrine exocytosis. Comparison between theory and experiment are in good agreement.



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Synthesis of Photochromic ATP Analogue and its Interaction with Motor Proteins

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Azobenzene is one of the photochromic molecules, which undergoes rapid and reversible transitions between the cis isomer and trans isomer by visible and ultra-violet light irradiation. We have been trying to control the activities of motor proteins using the photochromic molecules as photo-regulatory devices. We have recently demonstrated that microtubules dependent ATPase activity of the kinesin modified by azobenzene derivative was regulated by UV-VIS light irradiation. However, it was not so easy to incorporate the photochromic molecules into the functional site of motor proteins without altering the native enzymatic properties.
In the present study, we have design the ATP analogues consist photochromic molecules in order to photo-regulate the motor proteins without their chemical modification. It is expected that the ATP analogues induce the reversible conformational change in the active site by alternate UV-VIS light irradiation. We have synthesized non-nucleotide ATP analogue composed of azobenzene derivative, Phenylazobenzoic-aminoethyl -triphosphate (PABATP). PABATP showed UV/VIS light absorption spectral change accompanied by transition between cis and trans similar to that seen with azobenzene. Cis isomer of PABATP was hydrolyzed by skeletal muscle myosin in the presence of Mg2+, Ca2+ or EDTA-K+ much faster than trans isomer. It has been demonstrated that the cis isomer and trans isomer perform differently as a substrate of myosin. Currently we are studying the conformational changes of myosin head induced by cis isomer and trans isomer of PABATP.

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Synthesis of Novel Fluorescent ATP Analogue and its Interaction with Nucleotide Dependent Motor Proteins

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Previously several kind of fluorescent ATP analogues have been synthesized for the application to the kinetic studies of ATPases. However, some of the ATP analogues exist as a mixture of isomers. For instance, 2′(3′)-*O*-Mant-ATP has isomer of 2′ and 3′ in its ribose moiety and each isomer performs differently as substrate for the ATPases. In the present study, we have tried to synthesize novel fluorescent ATP analogues that have no isomer. The fluorescent ATP analogue 6-(N- (7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino) ethyl triphosphate (NBDTP) and N-methylanthraniloyl amino ethyl triphosphate (MANTTP) have been designed and synthesized, which are similar to non-nucleotide ATP analogue 2-[(4-azido-2-nitrophenyl) amino] ethyl triphosphate (NANTP). It is known that NANTP are good substrate for skeletal myosin and induces actin gliding in vitro motility assay. Excitation and emission maximums in the fluorescence spectrum of the ATP analogues were 474nm and 533nm for NBDTP, and 374nm and 430nm for MANTTP, respectively. In addition, molar absorbance coefficients of ATP analogues were 40274 $M^{-1}cm^{-1}$ for NBDTP in 478nm, and 3730 $M^{-1}cm^{-1}$ for MANTTP, respectively. The analogues showed ATP hydrolysis for conventional kinesin and skeletal myosin at the almost same level to that of ATP. Moreover, the analogues induced dissociation of acto-myosin. The ADP form of the fluorescent ATP analogues showed the formation of skeletal muscle myosin/ADP analogues/BeFn complexes which mimic the transient state in ATPase cycle.

Ion Motive ATPases

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Nonequilibrium Energetics of a Single F_1-ATPase Molecule

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Energetics of a rotary molecular motor F_1-ATPase was studied by applying recent developments in nonequilibrium physics. Since molecular motors are engines that transduce chemical energy to mechanical motions, it is essential to focus on their energetics. Here, for a single F_1-ATPase molecule, we have evaluated the amount of heat dissipation Q_{rot} through its rotational degree of freedom as well as the work W against external load. Q_{rot} was estimated using a new nonequilibrium equality connecting the heat dissipation to the violation of the fluctuation dissipation theorem. External torque was applied using the electrorotation method. We found a nontrivial energy balance relation that $W+Q_{rot}$ per 120° rotation was almost equal to the free energy change in a single ATP hydrolysis $\in''\in 1/4$ under various conditions. This implies that F_1-ATPase focuses the free energy consumption toward rotations with an efficiency of nearly 100%.