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The load dependent kinetics of the Chara myosin motor domain

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Chara myosin is the fastest motor among myosin superfamily identified so far. To investigate the molecular mechanism of this fast movement, the biochemical measurements and the gliding assay were performed by using the *Chara corallina* myosin motor domain constructs (Ito et al. 2003). One construct containing a motor domain with two α -actinin repeats as a lever arm shows high ATPase activity with V_{max} of 500 Pi/s/head. Ensemble of these molecules drives actin filaments at 16 μ m/s, much faster than obtained with other myosins. When working in ensemble, each myosin molecule should detach from an actin filament on sustaining a negative load to avoid interference with the active sliding generated by other molecules. Here, we have characterized effects of the load on kinetics of Chara myosin by using this construct. In the measurement, the constructs were attached on a 200-nm ϕ bead, which was then brought into contact with an actin filament fixed on glass surface by an optical trap. In the presence of 5 μ M ATP, the movement of the bead was not processive but showed on-off interactions. The life time of the bound state was binned according to load and averaged at each bin. The average life time was 170 ms at 0.12 pN but shortened to 100 ms when myosin sustained small negative load of -0.04 pN. The results indicate that even the slight change of the load affects the kinetics of Chara myosin and that such load dependence would play an important role for the efficient movement of actin filaments driven by the fast Chara myosin ensemble.

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How Does the Energy Released by ATP Hydrolysis Spread throughout the Myosin Head?: Molecular Dynamics Simulations

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We have carried out 10-ns all atom molecular dynamics simulations in order to examine how the energy released by ATP hydrolysis in the nucleotide-binding site of a myosin molecule spreads throughout the motor domain. From the structure data of myosin S-1 (PDB code 1kk7), we cut out the motor domain consisting of the N-terminal 785 residues and immersed it in a spheroidal water droplet. After a thermal equilibration at 300 K for 300 ps, the trajectories of each atom were calculated for 10 ns with a time-step of 2 fs, starting from two initial conditions: 1) without giving any disturbance to the system, 2) an energy of 8.60×10^{-20} J corresponding to the hydrolysis energy of one ATP molecule was distributed to 80 backbone atoms around the ATP pocket as kinetic energies. We used a software SANDER module in AMBER 8 and a computer equipped with a special purpose board MDGRAPE-3 to calculate the electrostatic interactions without using the cut-off method. The difference between trajectories of each atom obtained in the two initial conditions 1) and 2) indicates the presence of conformational deviations due to the ATP hydrolysis from thermal equilibrium configurations. The result of simulations exhibited that the root mean square of the conformational deviations was enhanced at the actin-binding site and the junction with the neck, which are both relevant to the movement of the myosin head along the actin filament. Further, its residue number dependence was found to have a strong similarity to that of thermal fluctuations themselves.

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Intramolecular Correlation and Structural Change of Myosin Motor Domain

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We investigate the structural relaxation of myosin motor domain from the pre-powerstroke state to the near-rigor state by molecular dynamics simulation. In order to describe the structural change, we propose "dual Go-model", a variant of Go-like model, in which two states are embedded in the Go-interaction. Nucleotide dissociation process is also investigated with a coarse-grained nucleotide as well as a coarse-grained protein included in the simulation. So far we obtained following results: (1) Structural relaxation follows or is coincident with nucleotide dissociation, (2) Delay of structural relaxation from nucleotide dissociation depends on binding strength of protein-nucleotide interaction, (3) When the structural fluctuation of some part is suppressed (e.g. 50kDa cleft, converter), nucleotide dissociation rate is changed. To clarify the coupling between nucleotide binding site and other parts, in this study we investigate structural fluctuation during the relaxation in detail by calculating the correlation of inter-residue distances.

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Three dimensional nanometry of moving vesicles in a living cell

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Nanometries of vesicle transports are critical issue to understand the molecular mechanisms of motor proteins in a living cell. Development of three dimensional (3D) measurement system has been necessary to achieve this, because the vesicles in cells move cubically on the cytoskeletal networks. A 3D observation with a confocal microscopy is too slow to pursue the moving vesicle with high velocity ($\sim 1 \mu$ m/s).

In this study, we developed a new imaging method, which enable us to pursue individual fluorophore of quantum dots with the spatial and temporal resolution of 4 nm and 2 ms in 3D. Anti HER2 (Human Epidermal factor Receptor 2) antibody labeled with a quantum dot (antiHER2-QD) was endocytosed inside the cell. The vesicles including antiHER2-QD were transported toward the nucleus on GFP-microtubules in KPL-4 cell line. The vesicles were moved with successive 8 nm steps without rotating around the microtubule, and along a protofilament at most time. Sometime, we observed the vesicles transfer among protofilaments. Dynein, which is minus-end motor, plays a role in the inside transport of the endocytic vesicle on a microtubule. The results in this study suggests that dynein transports the vesicles with 8 nm step along a protofilament and transfers from one protofilament to the other when the vesicle meets obstacle. The 3D single particle tracking in this study can be applied to wide field in Cell biology and Biophysics.