copolymerization order, pyr-actin blocks were placed at either the barbed or pointed end side of AS1 blocks. The barbed ends of copolymers were capped by R290E actin defective in polymerizing to the barbed-end direction (Murakami et al., 2010), and the pointed ends were capped by a block of unlabeled actin, so that both the AS1 and the pyr-actin blocks were not exposed at the filament ends. When ATP was added to the copolymers in which the pyr-actin block was positioned at the pointed end side of the AS1 block, fluorescence intensity increased by 12%. In contrast, ATP did not increase the pyr fluorescence when the pyr-actin block was at the barbed end side of the AS1 block. These results indicate that the rigor binding within AS1 affects neighbor actin subunits only on the pointed end side to quench the pyr fluorescence. We will discuss possible involvement of this uni-directional cooperative structural change in force generation.

1SG-03 ATP結合とその加水分解によって駆動されるABCトランスポーターの一方向的構造変化

Unidirectional structure changes of ABC transporters driven by binding of ATP and its hydrolysis

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The ATP-binding cassette (ABC) transporters are integral membrane proteins which couple the energy of ATP hydrolysis with the translocation of chemically diverse substrates across the cellular membranes. Generally, ABC transporters consist of a pair of highly hydrophobic trans-membrane domains (TMDs) and two cytoplasmic nucleotide binding domains (NBDs). The TMDs form the permeation pathway for the substrates and the NBDs play indispensable rolls in the catalytic reaction of ATP hydrolysis. It has been hypothesized that an ABC transporter (exporter) undergoes the following unidirectional structural changes: i) upon binding of substrates and ATP, the protein undergoes a large structural change from the inward (cytoplasmic)-facing state to the outward (extracellular)-facing state, during which the substrate is transported to the extracellular side, ii) ATP is hydrolyzed and subsequently ADP and Pi are released from the ATP binding pocket, leading to a conformational change toward the inward-facing state, that is, the initial state. To examine how these structural changes are driven by binding of ATP and its hydrolysis, we here performed MD simulations for ATP-bound and unbound structures in the inward-facing state, and for several structures before and after the ATP hydrolysis in the outward-facing state. In addition, to examine the mechanism of the chemical to mechanical energy coupling, MD simulations were also performed for several mutant proteins in which the so-called coupling helices are modified.

1SG-04 リニアモーターの1分子計測から観た、揺らぎを制御する分子としてのATP

ATP as a fluctuation regulator of linear motor protein revealed by a single molecule measurements

Mitsuhiro Iwaki (*Graduate School of Medicine, Osaka University*)

Myosin is a linear motor protein that converts chemical energy of ATP into mechanical work and unidirectionally moves along cytoskeletal actin filament. Recent single molecule analysis and high-speed AFM focused on dimeric myosins (myosin-V and VI) directly visualized the dynamics of the directional motion coupled with single ATP hydrolysis, where its directional steps are composed of a structural change in the leading motor domain (head) ("lever-arm swing") and a Brownian search of the trailing head ("Brownian search-and-catch"). However, little is known about energetic feature of these processes. Here, we constructed a new assay system that can directly apply force to the Brownian head during the directional motion of myosin-V using DNA handle and optical tweezers, then we found energy bias of the lever-arm swing is at most 3.2 kBT and the Brownian search-and-catch generates 14 kBT work. Thus, the Brownian search-and-catch is the key process for the force generation of myosin-V. The Brownian motion was random and the catch of the Brownian head to the forward actin is coupled to a chemical energy difference of ~12 kBT by Pi release. Therefore, energy of ATP is basically used to strongly catch actin and suppress the thermal fluctuation of the head. However, we recently observed a biased sliding motion of the Brownian head along actin filament by adding a certain amount of disaccharides, sucrose which would affect the hydration of actomyosin. We will discuss the effect of sucrose from a viewpoint of water dynamics.

1SG-05 F1分子モーターの機能発現と非対称構造：理論的考察

Theoretical study on rotational mechanism of F1 molecular motor and asymmetric structures

Mitsunori Ikeguchi (*Grad. Sch. Nanobiosci., Yokohama City Univ.*)

F₁-ATPase is a soluble part of ATP synthase, and is also known as a molecular rotary motor driven by ATP hydrolysis. Here, we present results from molecular dynamics (MD) simulations and statistical mechanics theory for elucidating atomically detailed mechanisms of rotary motions of F₁-ATPase [1-4]. MD simulations for the catalytically active β subunit are in agreement with NMR experiments and provide detailed views of how ATP induces conformational changes of the protein [1,2]. Another MD simulation for the F₁ $\alpha_3\beta_3\gamma$ complex reveals the substantial asymmetry in dynamics and interactions between subunits [3]. Statistical mechanics theory, which is a hybrid method of the angle-dependent integral equation theory and the morphometric approach, indicates that asymmetry in the complex structure is crucially important for the rotational mechanism of F₁-ATPase in terms of hydration, suggesting that water plays key roles in rotary motions of F₁-ATPase [4].

1. Y. Ito and M. Ikeguchi, *Chem. Phys. Lett.*, 490, 80-83 (2010).

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3. Y. Ito and M. Ikeguchi, *J. Comput. Chem.*, 31, 2175-2185 (2010).

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1SG-06 ATP駆動蛋白質の機能発現における水とATPの共同効果

Cooperative Roles of Water and ATP in Functioning of ATP-Driven Proteins

Masahiro Kinoshita (*Institute of Advanced Energy, Kyoto University*)

Upon the biological self-assembly, the number of accessible translational configurations of water in the system increases greatly, leading to a large gain in the water entropy. Using our theoretical methods wherein this effect is treated as the key factor, we have succeeded in reproducing the thermodynamic data of apoplastocyanin folding, elucidating the pressure and cold denaturation of proteins, suggesting a measure of thermal stability of proteins, and developing a free-energy function which is capable of discriminating the native fold from misfolded decoys. The tool is either the three-dimensional integral equation theory or the angle-dependent integral equation theory combined with the morphometric approach. We believe that the water-entropy effect plays critical roles in the functioning of ATP-driven proteins. We have recently made significant progresses in the following subjects: (1) the unidirectional movement of a linear-motor protein along a filament; (2) rotational mechanism of F₁-ATPase; and (3) insertion of an unfoled protein and release of the folded one into and from the chaperonin GroEL. In this talk, I will discuss the roles of water (especially, its translational entropy) coupled with the cycle of the ATP binding, hydrolysis, and release of the products (Pi and ADP) in the functioning of these ATP-driven proteins.

1SG-07 「水とATPがつくる非対称性」シンポジウムのまとめと展望

Summary and Near-Future Perspective of the Symposium "Asymmetry Produced by Water and ATP"

Nobuyuki Matubayasi (*Institute for Chemical Research, Kyoto University*)

The symposium is summarized and is open to free discussion. On the experimental side, state-of-art methods are introduced and molecular-level observations are performed. The role of ATP, which is one of the most important players, is well captured there, and the role of water, which is another, important player and is very often "hidden", is inferred. The theoretical studies disclose the effect of water using (and often combining) large-scale molecular simulation and statistical-mechanical method. The cooperation and competition of a variety of interaction components are discussed, in particular, with emphasis on the water-entropy contribution. In the target systems of interest, the functional groups involved are ordinary, the molecular interactions are nothing special, common laws of equilibrium and rate are employed, and still the asymmetry is produced. The symposium would discuss the link between the microscopic and macroscopic physical chemistry and the asymmetry often observed in biophysics fields.