general > 1 M, we have investigated the effect of urea on the ATPase reaction rate of myosin subfragment-1 (S1) at so low concentration that the proteins were not denatured. Alkyl-ureas and thiourea were also examined to work out how each functional group of urea molecule affected the S1 ATPase reaction. Myosin extracted from chicken pectoral muscle was digested by limited proteolysis using papain to produce S1, which was subsequently purified by gel-filtration chromatography. The S1 Mg-ATPase rate (50 mM KCl, 2 mM Hepes (pH 7.8), 2 mM $MgCl_2$, 2 mM ATP) was almost unchanged in the presence and absence of 0.2 M urea. However, the S1 ATPase rate was observed to increase by 19% with 0.2 M thiourea and by as high as 150% with 0.2 M diethylurea. This result suggests that the step of phosphate release during S1 ATPase reaction, the rate limiting step, is significantly affected by urea derivatives at much lower concentration than that of denaturation condition. The measurements of the ATPase rate and in vitro motility of myosin are to be conducted using several urea derivatives in addition to the present ones.

3M1012 ミオシンフィラメントトラックを乗り換えるアクチンフィラメント滑り運動の観察

Observation of sliding movement of actin filament changing the myosin filament track

Hiroyuki Okubo, Tomoya Shimokawa, Tooru Mogami, Shigeru Chaen
(Department of Integrated Sciences in Physics and Biology, College of Humanities and Sciences, Nihon University)

In vitro motility assays using bipolar myosin thick filaments demonstrated that actin filaments moving on the “mismatched” track of myosin filament increase the speed when its leading end begins to slide along the “matched” track of myosin filament even though most of the actin filament are still on the “mismatched” track (Yamada & Takahashi, 1992). The terms of matched and mismatched track are based on the observation that along myosin thick filament, actin filaments moved at a fast speed towards the central bare zone and at a slower speed away from the bare zone (Yamada et al., 1990; Sellers & Kachar, 1990; Yamada & Wakabayashi, 1993). Recently, we have suggested that the backward movement away from the center causes the myosin heads to be constrained and increase in the energy required for the ADP release step by the findings that the thermal activation energy of the movement away from the center was significantly higher than that of the movement toward the center (Okubo et al 2010). In this study, in order to examine whether ADP release rate changes in accord with the changes in the speed of actin movement when its leading end begins to slide along the “matched” track of myosin filament, we constructed a assay system to estimate the ADP release rate from the displacement of fluorescent nucleotides bound to cross-bridges by flash photolysis of caged ATP.

3M1024 ミオシン分子上を運動するアクチンフィラメントのサブミクロン変形の評価

Evaluation of submicron distortions of actin filaments during sliding movement on myosin molecule

Satoru Kikuchi¹, Kuniyuki Hatori² (¹Univ.Yamagata., Rikou, ²Univ.Yamagata)

Actin filaments are thought to move on myosin molecules as filamental distortions and changes in internal structure of actin constituents take place. We examined the changes in sliding velocity and in fluorescence intensity of a short actin filament ($< 1 \mu m$) labeled with rhodamine-phalloidin during the sliding movement and evaluated these properties by correlation analyses. As a result, the peak fluorescent intensity of the filament increased while the standard deviation measuring the extent of spatial spreading of fluorescent intensity decreased and vice versa, indicating the negative correlation between these values. Thus, it is likely that actin filaments exhibited compressive or extended distortions. In addition, the sliding velocity developed later than 1/30 s after the extent of spatial spreading of intensity increased, indicating the positive correlation with time-delay of 1/30 s. This result shows that the velocity increased when actin filaments expanded, on the other hand, the velocity decreased when they were compressed. Therefore, actin filaments are thought to coordinate the sliding movement depending on changes in the filamental structure.

3M1036 シンブルで強力な生体分子モーターのステップ解析アルゴリズム Simple and Powerful Algorithm for Analyzing Stepping Motion of Biological Molecular Motors

Takeshi Nakagawa, Kazuo Sasaki (Dept. of Applied Physics, Tohoku Univ.)

Many of biological molecular motors move stochastically along a linear track in a stepwise manner. The distributions of step size and dwell time (duration between successive steps) provide important information on the mechanism of how these molecular machines work. One needs to employ a certain algorithm to extract the step sizes and dwell times from the noisy experimental data. Recently the step finding algorithm proposed by Kerssemakers and coworkers [J.W.J. Kerssemakers et al. Nature 442, 709 (2006)] has been used frequently. However as the standard deviation of the noise increases, the performance of the method becomes considerably worse; it misses steps buried in the noise. We propose a new step finding algorithm which improves the performance in the presence of noise. Our method is briefly explained as follows. (i) Adopting the first stage of the Kerssemakers algorithm, a step function is fitted to the data by the least square method. (ii) The confidence of the candidate step is tested by two-sample Student's *t*-test. (iii) If the step passes the test, we adopt it and new step functions are fitted to the two plateaus adjoining it and return to (ii). Otherwise the step is removed. (iv) If a new step is not created in every plateau, the procedure is finished. Our algorithm detects the steps more precisely compared with the Kerssemakers algorithm. We will explain the detail of our method and compare its performance with the Kerssemakers algorithm at the meeting.

3M1048 ミオシンの非線形弾性を考慮した筋収縮の理論

Theory of Muscle Contraction with Nonlinear Elasticity of Myosin Molecules

Keita Miyamoto¹, Kazuo Sasaki² (¹School of Engineering., Univ. Tohoku, ²School of Engineering., Univ. Tohoku)

Contraction of muscle is attributed to the elasticity of myosin molecules interacting with actin filaments in a muscle fiber. It is considered that the contractile force (tension) is generated when myosin is elongated in a process of conformational change. We can estimate this size theoretically by analyzing the transient response of tension to sudden change in the fiber length and the relationship between the constant load force and the contractile velocity. In traditional analyses, researchers have used theories in which myosin is assumed to have a linear elasticity. However, from a recent experiment by Kaya and Higuchi [M. Kaya and H. Higuchi, Science 329, 686 (2010)], it turned out that the elasticity of myosin exhibits strong nonlinearity. In our work, we calculated the tension response and the force-velocity relation using the theory proposed by Duke [T.A.J. Duke, Proc. Natl. Acad. Sci. USA 96, 2770 (1999)] with the linear elasticity of myosin replaced by a nonlinear elasticity. Comparing our calculation to the experimental data [G. Piazzesi and V. Lombardi, Biophys. J. 68, 1966 (1995)], we got about 7 nm as the size of the conformational change of myosin. This value is consistent with the one estimated by Kaya and Higuchi from the movement of a single actin filament produced by several myosin molecules. This result suggests that the contraction of muscle consisting of an enormous number of myosin molecules and actin filaments can be understood from the movement of a single actin filament generated by a few myosin molecules.

3M1112 ミオシンのアクチン繊維へのドッキング過程の MD シミュレーション

Molecular dynamics simulation for the docking process of myosin against an actin filament

Tadashi Masuda (Fukushima Univ.)

I have proposed a theory called “Driven by Detachment” mechanism for the working principle of myosin molecular motors. In this theory, the energy for the power stroke of myosin originates from the attractive force between a detached myosin head and an actin filament. The potential energy associated with the attractive force is converted into elastic energy within a myosin molecule during the docking process, and the elastic energy causes the power stroke. The energy of ATP is used to detach a docked myosin from an actin filament and to restore the potential energy between a myosin and an actin filament.

According to this theory, every step in the myosin working process can be reproduced by the molecular dynamics principle excepting the hydrolysis of ATP. Therefore, as a first step to verify this theory, molecular dynamics (MD) simulations were conducted for the docking process of a myosin S1 fragment against an actin filament consisting of seven monomers using the configuration registered in 1M8Q (PDB ID). The MD software was Gromacs with the OplsAA force field and the Tip4p water model with a step of 2 fs. Initially the myosin is placed close to the actin molecule on the minus side of the filament but about 1.5 nm away from the second actin on the plus side. After 30

ns, in five cases out of ten trials, the myosin made a close contact with the second actin by changing the positions and the configurations as predicted in previous studies. In the remaining five cases, the myosin still moved randomly around the initial position.

3M1124 ATP 駆動のアロステリック応答から類推されるアクチン-ミオシンの解離における静電相互作用の重要な役割

The critical role of electrostatic interaction in actin-myosin dissociation inferred from ATP-induced allosteric responses of myosin

Takato Sato, Jun Ohnuki, Mitsunori Takano (*Grad. Sch. of Adv. Sci. & Eng., Waseda Univ.*)

In muscle contraction, myosin moves along actin filament repeating the strong and weak binding alternately. Although this strong/weak binding transition is essential for the cyclic energy-conversion of myosin, the atomic-level understanding of this process has not yet been established. The ATP-binding to myosin triggers the dissociation from the actin, and as a possible mechanism, opening of the actin-binding cleft is speculated. However, other structural elements of myosin including loop 2 and surrounding solvents have received little attention. In this study, to clarify the allosteric responses of the structural and the solvation states of myosin to strong/weak binding transition with atomic details, we conducted explicit-solvent molecular dynamics simulation. The crystal structure of scallop myosin was used, and the chemical state was changed from the original ADP-Pi analogue bound to the ATP bound or nucleotide-free state. Then, myosin was immersed in the box containing 33,000 waters and 50 mM KCl. From multiple long MD runs (total over 6 μ s), the anticipated structural responses in the actin-binding cleft and loop 2 were observed. Furthermore, density increase of water and exclusion of anion at around the actin-binding interface upon ATP-binding were observed. It is likely that these responses are explained by the change of electrostatic potential around myosin upon ATP-binding. These "allostery of structure and solvent" of myosin is expected to be deeply involved in the dissociation of myosin from the actin filament.

3M1136 Statistical formulation of fluctuation analysis for generalized stepping motion : Estimation of hidden elementary parameters

Hiroto Tanaka, Hiroaki Kojima (*NICT, Bio ICT Lab.*)

Bio molecular machines generally express macroscopic outputs (MOs) via cyclical and/or multiple microscopic elementary events (MEEs). For example, some bio molecular motors develop sliding movement (as MO) via cyclical unitary stepping events (as MEE). It is important to reveal such MEEs for understanding working mechanism of bio machines. However, such MEEs are often difficult to observe directly, while expressed MOs are often easy to observe. Therefore, a method that enables us to estimate "hidden" parameters (size of unitary step, stepping ratio, rate constants, etc. for molecular motors) of MEEs from expressed "observable" MOs, would be a powerful tool to overcome difficulties of direct observation and help understanding working mechanism of bio nano machines. We propose a widely suitable method to estimate parameters of "hidden" elementary events in this study. We theoretically formulated statistical relation between MEEs and MOs for general "Gaussian numbered random stepper", and propose a fluctuation analysis method extended for general elementary process. As a result of our test for application, our method showed its potential to determine "hidden elementary parameters" precisely. Equations relating "hidden elementary parameters" to "observable expressed outputs" proposed in this study can be powerful tool to tackle hidden more detail characteristics of bio molecular machines, and to improve apparent spatial and time resolutions of measurement system by using statistical analysis.

3M1148 細胞性粘菌ミオシン II の SH1 ヘリックス領域の変異はアクチンの働きを抑える

Mutating the SH1 helix region of Dictyostereum myosin II impairs actin-myosin motility

Tsubasa Koyama¹, Takahiro Maruta¹, Yuta Hukushima¹, Sosuke Iwai², Shigeru Chaen¹ (¹ *College of Humanities and Sciences, Nihon university*, ² *Faculty of Education, Hirosaki university*)

A human myopathy has been reported to be caused by a point mutation in a fast myosin heavy chain gene. The mutation, glutamic acid to lysine at the 706th amino acid, is located at the SH1 helix region of the myosin II motor domain.

The SH1 helix region acts as a linker for transmitting the structural changes of ATP-binding site in the catalysis domain to the lever arm. To investigate the effect of the mutation on the actin-myosin motility, we have introduced a corresponding mutation into the SH1 helix of Dictyostereum myosin II (E683K). The mutation resulted in a decrease in the actin-myosin sliding velocity (about 1/8 of the wild type). This suggests that the SH1 helix has an important function in the motor activity. We are currently measuring the actin-activated MgATPase activity and also the thermal stability of the mutated myosin.

13:10~16:10 M 会場 / Room M

分子モーター 5

Molecular motor 5

3M1322 カーボンナノチューブを用いたミオシン運動の局所加熱

Local heating of myosin motors using single carbon nanotubes

Mitsunori Nagata², Hiroshi Matsutaka², **Yuichi Inoue**¹, Takeru Okada³, Akihiko Ishijima¹ (¹ *IMRAM, Tohoku Univ.*, ² *Grad. Sch. Life Sci., Tohoku Univ.*, ³ *Inst. Fluid Sci., Tohoku Univ.*)

Since carbon nanotube (CNT) has highest thermal conductivity (~6000 W/m/K), CNT is a potential candidate to explore heat transfer of proteins at a single molecular level. For the application, however, there are many difficulties including water insolubility of CNTs, denature of proteins on CNTs, and sensitivity of thermal detectors. As a start toward understanding the energetic mechanism of motor proteins, we tried to use CNTs as a heat conductor for the ATP-driven motility of myosin motors. Multiwall CNTs (~170 nm diameter, ~10 μ m length) were coated with skeletal myosin to observe sliding of fluorescently-labeled actin filaments in the presence of 2 mM ATP. During the sliding, an endpoint of the CNT was temporarily heated with a laser irradiation (637 nm, ϕ ~2 μ m, 2 mW) to supply external heat.

Actin filaments moved along the myosin-coated CNTs at a speed of ~2.9 μ m/s. By the local heating with laser, the speed increased to ~5.9 μ m/s, indicating temperature change of myosin from 19°C to 24°C. However, temperature change of actin filament, which was examined from the change in fluorescence intensity, was negligible, suggesting that the heating was not due to thermal diffusion of water.

Our results show that CNTs work as a heat conductor to induce local temperature change for myosin. Possibility of the local temperature and future applications would be discussed in the meeting.

3M1334 生体外でのアクトミオシン駆動型リボソーム

Actomyosin-driven liposomes in vitro

Satoshi Iwabuchi, Kuniyuki Hatori (*Grad. Sch. Sci. Eng., Yamagata Univ.*)

We intended to add a function of active motion to liposomes by actomyosin motor proteins. Using a hydration method, positively-charged liposomes were prepared from a equimolar mixture of dioleoyl-phosphatidylcholine (DOPC) such as a neutral lipid with dioleoyl-trimethyl ammonium propane (DOTAP), which is a cationic lipid. When the mixture of the liposomes and rhodamine-phalloidin-bound actin filaments was observed under a fluorescent microscope, it was found that actin filaments were bound to the surface of liposomes. It is likely that the binding of actin filaments to liposome surface was caused by electrostatic interaction. Under an in vitro motility assay, we observed that the actin-bound liposomes moved at about 1.2 μ m/s of velocity on heavy meromyosin (HMM) molecules fixed on glass surface in the present of ATP. This motion was different from Brownian motion, and was slower than sliding movement of actin filaments without liposome on HMM (about 5 μ m/s). We achieved introduction of actomyosin system into liposomes for active transport.

3M1346 Characterization of photochromic ATP analogue as a substrate for myosin

Takeshi Itaba, Shinsaku Maruta (*Div. of Bioinfo., Grad. Sch. of Eng., Soka Univ.*)

Azobenzene is a photochromic molecule that undergoes rapid and reversible isomerization between the *cis*- and *trans*-forms in response to ultraviolet and visible light irradiation, respectively. Previously, we have cross-linked reactive cysteine SH1 and SH2 of skeletal muscle myosin subfragment-1(S1) with the sulphydryl-reactive bifunctional azobenzene derivative, ABDM and succeeded to induce lever arm swinging reversibly by photo-irradiation. The results suggested that it may be possible to control motor proteins by light irradiation.