- 2P120 アクチン滑り運動中のミオシン頭部コンフォメーションの解析
○片山 栄作¹、木森 義隆¹、上田 太郎²
¹東大・医科研・分子構造解析、²産総研・セルエンジニアリング
- 1aB01 Further characterization of the configuration of actin-attached myosin-crossbridges during *in vitro* sliding
Eisaku Katayama (1), Yoshitaka Kimor (1) and Taro Q.P. Uyeda (2) (1 Div. Biomolecular Imaging, Institute of Medical Science, The Univ of Tpkyo, 2 Div. Cell Engineering, Advanced Institute of Science and Technology

We are studying the conformation of myosin crossbridges during *in vitro* sliding observed by freeze-replica electron microscopy, coupled with a novel image analysis to compare microscopic images with artificial ones produced from the atomic models in various conformations. We previously reported that the major population of crossbridges there took a shape in which lever-arm moiety is kinked as in ATP-bound one but to the opposite side. Similar images were observed for SH1-SH2 crosslinked species. Thus, we built a tentative model in which the lever-arm of ADP/Vi form was rotated to the opposite side only at one Gly (765 in the scallop sequence), within the lever-arm moiety, to mimic such new conformation. According to more precise analyses, however, the matching of the model image to actual EM image was not sufficient, especially at the junction of motor-domain and lever-arm, probably because of the wrong rotation point. Gly695 is between SH1 and 2 that are crosslinked in the observed structure, and is more likely to be the hinge. Since the motor-domain was closest to pPDM-crosslinked structre (Himmel et al., 2002), it was used as a start to generate more realistic atomic model corresponding to sliding intermediate conformation. Assuming rotation around Gly695 with additional one at Gly765, we could generate a new model that nicely explains all the observed images during sliding. The location of putative acto-S1 interface was compatible with various experimental results, confirming the validity of our proposal on the crossbridge cycle.

- 2P122 アクト-S1 キメラ蛋白質フィラメントとミオシンとの相互作用
○和沢 鉄一^{1,2}、Md. Shahjahan P. Siddique²、宮崎 崇^{1,2}、片山 栄作³、上田 太郎⁴、鈴木 誠^{1,2}
¹科技振・CREST、²東北大院・工・マテリアル、³東大院・医科研、⁴産総研・セルエンジニアリング
- Interaction of myosin with the filament of acto-S1 chimera protein
Tetsuichi Wazawa(1,2), Md. Shahjahan P. Siddique(2), Takashi Miyazaki(1,2), Eisaku Katayama(3), Taro Q. P. Uyeda(4) and Makoto Suzuki(1,2). (1: CREST, JST; 2:Dept of Materials Processing, Graduate School of Engineering, Tohoku Univ; 3: Institute of Medical Science, Univ of Tokyo; 4: Research Institute for Cell Engineering, AIST)

ATP hydrolysis by myosin is activated through the interaction with actin, powering its sliding movement along actin filament. During the reaction cycle, myosin and actin filament experience a series of dissociation and association steps, which should work as the key processes for force generation. In this study, we have prepared a recombinant fusion protein of actin and myosin subfragment-1 (S1), and investigated its interaction with myosin. A vector for expressing the fusion protein (Acto-S1 Chimera) was constructed so that full-length actin of Dictyostelium discoidium was inserted into Loop-2 region between 50 kDa and 20 kDa subdomains of Dictyostelium S1 through flexible linkers. Acto-S1 Chimera protein was expressed in Dictyostelium cells. Electron microscopy showed that purified Acto-S1 Chimera protein could polymerize into filament with regular arrow-headed appearance in the absence of ATP. *In vitro* motility assay showed that Acto-S1 Chimera filaments attached firmly to myosin-coated glass surface in the absence of ATP and started to move at a normal speed by adding ATP. We now found by electron microscopy that additional heavy meromyosin bound to Acto-S1 Chimera filament in the absence of ATP. Furthermore, co-sedimentation assay indicated that this unusual interaction was nucleotide-dependent: ATP decreased the interaction. These results suggest that actin could have additional binding site to myosin which is not involved in regular acto-S1 arrow-head formation, but may operate during sliding movement.

- 2P121 ミオシンフィラメント上の計測により見えてきた骨格筋ミオシン1分子の力学特性
○茅 元司¹、樋口 秀男¹
¹東北大 先進医工、²東北大 先進医工
- 1aB02 Mechanical properties of a single skeletal myosin revealed by measurement of force / step of individual myosins assembled into filaments
Motoshi Kaya (1) and Hideo Higuchi (1). (1: Biomedical Engineering Research Organization, Tohoku Univ)

In order to understand the mechanism of muscle contraction, single-molecular studies investigated on the mechanical properties of myosin. However, these results are likely underestimated, since isolated myosins are randomly oriented and are not able to achieve the maximum force capacity due to its non-processivity. Therefore, we investigated on the displacement / force of a single myosin in the assembly of myosins by using the laser trap nanometry, where myosins are properly oriented and can interact with an actin simultaneously, as observed in muscle. The numbers of myosins were estimated by counting the numbers of unbinding events that occur when the rigor actomyosin bonds were ruptured by pulling actin filaments. The step size was virtually constant (5nm) for the tested filaments containing 2-5 myosins. The force-velocity relationship showed that the estimated stall forces were similar for these filaments (13pN), while the observed peak forces dramatically increased with increasing filament lengths (3-10pN). We interpreted that a single myosin can generate force closer to the maximum force capacity in longer filaments, where more numbers of neighboring myosins are likely in the rigor state and prevent the dissociation of actin filament. In conclusion, the peak force of myosin is approximately 10pN and is much higher than those observed in single-molecular studies. Also, the efficiency was found to be 40% similar to that observed in muscle by assuming that single steps are coupled with the hydrolysis of a single ATP molecule.

- 2P123 ミオシンVaのATPase活性と運動活性の相関について
○岩根 敦子^{1,2}、西川 正俊²、小森 智貴²、三宅 孝子³、有賀 隆行²、柳田 敏雄^{1,2,3}
¹Dept. Phys., Grad. Sch. of Medicine, Osaka Univ., ²Lab. for Nanobiology, Grad. Sch. of Frontier, Osaka Univ., ³Soft nano, CREST, JST
- Relationship between actin-activated ATPase activity and motility of Myosin Va.
Atsuko H. Iwane (1)(2), Masatoshi Nishikawa (2), Tomotaka Komori (2), Takako Mimuro (3), Takayuki Ariga (2) and Toshio Yanagida (1)(2)(3)

Myosin V is a molecular motor of about 20 kinds myosin family molecules, participating in transportation of the organelle among the living cells mainly. Myosin V is a composed of a motor domain hydrolyzing ATP at N terminal, a neck domain and a global tail domain binding to the vesicle at C terminal. Furthermore, myosin Va took dimmer structure from the result of the second amino acid structure prediction and was really confirmed by the electron microscope images of an isolated recombinant molecule. On the other hand, it had been understands that a single myosin V molecule can move over an actin filament using hydrolysis energy of ATP processively. Some foreign countries researchers have reported that both the long neck domain and the dimmer structure are required to its processive movement, but the clear conclusion had not be provided yet.

In order to investigate the mechanism of a chemical-mechanical conversion, we prepared myosin Va-HMM (WT) and some myosin Va-HMM mutants of the ATP binding site (P-Loop, Switch I, Switch II) using a baculovirus expression system. Myosin Va-HMM (WT) recombinant had an actin-activated ATPase activity and can move an actin filament at *in vitro* motility system sufficiently. Now, we observed the behavior of recombinants at the single molecule level using these myosin Va recombinants following a biochemical measurement at the multimolecule level. We would like to argue about contribution to the movement of the hydrolysis process from the variety between ATPase activity and motility characteristics.