1PS001 アクチンの Tyr143 変異による生化学的性質の変化**Changes of biochemical properties of *Dictyostelium* actin induced by mutation of tyrosine-143**Yuki Gomibuchi¹, Taro Uyeda³, Takeyuki Wakabayashi² (¹*Teikyo Uni. Grad. Science and Engineering*, ²*Teikyo Uni. Dept. Judo Therapy*, ³*AIST*)

The frontal region of actin around Tyr143 has been proposed to be involved in the binding of myosin. It also changes its conformation during the polymerization (Murakami et al., 2010). Thus we introduced mutation to Tyr143 and examined the biochemical properties, such as polymerizability and the activation of myosin ATPase. In polymerization assay, it turned out that actin with Tyr143Phe mutation showed poorer polymerizability in the presence of NaCl compared with the wild-type actin. Tyr143Ile mutation showed, however, only small decrease in polymerizability. In the presence of phalloidin Tyr143Ile-actin showed almost normal polymerizability irrespective of the added monovalent cations, whereas Tyr143Phe showed the somewhat decreased polymerizability. The interaction with myosin moiety was compared with that of the wild-type actin. The rate of hydrolysis of ATP by rabbit skeletal heavy meromyosin (HMM) was determined by measuring the release of inorganic phosphate in the presence of phalloidin and low concentration (4 μ M) of actin. Both of these mutant actins showed weaker ATPase activation. At higher actin concentrations (6-10 μ M), Tyr143Ile-actin showed also poorer activation, whereas Tyr143Phe-actin showed the comparable activation. These data indicate the importance of Tyr143 for the activation of myosin ATPase and for the polymerization.

Reference

Murakami et al. (2010) Cell 143, 275-287.

1PS002 誘電緩和分光法によるアクチンフィラメントの水和特性の温度依存性**Temperature dependence of hydration properties of F-actin by dielectric relaxation spectroscopy**Yuichiro Okazaki, Asato Imao, Noriyoshi Ishida, George Mogami, Tetsuichi Wazawa, Makoto Suzuki (*Grad. Sch. Eng., Univ. Tohoku*)

Polymerization of G-actin into F-actin is known to be endothermic reaction as shown by Oosawa et al., and thus it thought to be entropy driven. Our previous studies by microwave dielectric relaxation spectroscopy (DRS) revealed that F-actin has a dual hydration shell composed of constrained water (CW, dielectric relaxation (DR) frequency $f_c < f_{cw}$ (bulk water)) layer and hyper-mobile water (HMW, $f_c > f_{cw}$) layer which may be formed to a greater extent in an F-actin solution than in a G-actin solution. In this study we examined a temperature dependence of hydration properties of F-actin to understand the mechanism of HMW formation around F-actin. DR spectra of F-actin aqueous solutions were measured at 5, 10, 15 and 20 °C. At every temperature the spectrum was successfully fitted with two DR components, i.e. CW and HMW, in addition to the bulk water component around actin. Assuming that the DR amplitude of the each DR component is proportional to the number of hydration water, the amount of HMW decreased and that of CW increased, with increasing temperature. We estimated the van't Hoff enthalpy of transformation from bulk water to HMW to be +13 kJ/mol, and from bulk water to CW -24 kJ/mol. These results are consistent to the endothermic nature of actin polymerization, and suggest that this is due to the change in the water structure around actin.

1PS003 アクチン重合における水和変化の誘電緩和分光解析**Dielectric analysis of hydration change of actin by polymerization**Asato Imao, Yuichiro Okazaki, Noriyoshi Ishida, Tetsuichi Wazawa, George Mogami, Makoto Suzuki (*Grad. Sch. Eng., Tohoku. Univ*)

Actin is a major protein in cell and plays crucial roles in cellular functions. At low salt concentrations (e.g., less than several mM KCl), actin adopts a monomeric form, G-actin, whereas at higher salt concentrations (e.g., 50 mM KCl) and in the presence of adenosine triphosphate (ATP), actin polymerizes to a filamentous form, F-actin. This polymerization reaction is endothermic and occurs above a critical concentration. These endothermic reactions are believed to involve structural and dynamical changes in the surrounding water.

This paper introduces recent results on the rotational mobility of water around actin filaments (F-actin, a polymerized form of G-actin). Microwave dielectric relaxation spectroscopy (DRS) was employed. The DRS measurements showed that F-actin exhibits dual hydration properties as a structure-maker and a structure-breaker. Thus, in addition to the water molecules with lowered

rotational mobility that form a constrained hydration shell around the F-actin, there exist water molecules with rotational relaxation frequencies higher than those of bulk water, which are here referred to as hyper-mobile water. Thus, the present study revealed a significant increase of hyper-mobile water around F-actin. This could be a clue to understanding the endothermic reactions of actin polymerization and constructing the energetics of adenosine-triphosphate-driven protein functions.

1PS004 アクチンの水和状態に及ぼすハライドイオンの効果**Effects of halide ions on the hydration properties of F-actin**Noriyoshi Ishida, Asato Imao, Yuichiro Okazaki, Tetsuichi Wazawa, George Mogami, Makoto Suzuki (*Grad. Sch. Eng., Tohoku. Univ.*)

Actin is a cytoskeletal protein and polymerizes into filaments (F-actin) in high salt condition. We investigated the effects of potassium halides (KCl, KBr, KI) on the hydration properties of F-actin. Actin was taken from chicken pectoral muscle, and purified by a method of Spudich & Watt (1971). The dielectric relaxation (DR) spectra of F-actin solutions ($c = 6\sim7$ mg/mL, 50mM KX, 2mM $MgCl_2$, 10mM HEPES (pH7.8), 1mM DTT, 0.1mM ATP ; X=Cl, Br and I) by using high-resolution DR spectroscopy in the frequency range from 0.2 GHz to 26 GHz at 20.0 ± 0.01 °C. With a fixed volume fraction analysis at $\phi/c = 0.005$ mL/mg, the extracted spectrum for the fixed volume including an actin filament and a surrounding water layer was obtained by the Wagner mixture theory. The DR spectrum of the fixed volume around an actin filament for every KX was successfully fitted with two Debye components with DR frequencies, 20 GHz and ~ 5 GHz. The total DR amplitude for each salt was similar and around 73 and composed of three parts assigned to be constrained water ($f_c \sim 5$ GHz), hyper-mobile water (HMW, $f_c \sim 20$ GHz), and bulk water ($f_c = 17$ GHz). As a result, the amount of constrained water appeared to be not sensitive to halide ions, and the amount of HMW was found to increase slightly from Cl to I, suggesting some absorption of large halide ions at the surface of actin.

1PS005 ミオシン S1 の水和に及ぼす共溶媒の影響**Cosolvent effects on the hydration state and the enzymatic activity of skeletal myosin**Takuya Nakagawa, Shin-ichiro Yasui, Tetsuichi Wazawa, George Mogami, Makoto Suzuki (*Grad. Sch. Eng., Tohoku. Univ.*)

Cosolvents such as urea and potassium halides are known to affect the hydration state of protein, and in turn cause changes in the conformation and enzymatic activity of protein. Myosin interacts with F-actin and generates mechanical work by ATP hydrolysis reaction. In this study, we have investigated effects of the cosolvents on the hydration state and enzymatic activity of myosin.

Myosin subfragment-1 (S1) and heavy meromyosin (HMM) were prepared from chicken skeletal myosin by papain and chymotrypsin, respectively. By our high-precision dielectric relaxation spectroscopy (DRS), dielectric spectra of aqueous solutions of myosin were measured to derive the hydration properties of myosin. In this study, cosolvents of urea, 1,3-diethylurea (DEU), KBr, or KI were used with a reference aqueous solvent containing 30 -50 mM KCl, 10 mM HEPES (pH 7.8) and 2 mM $MgCl_2$.

The effects of urea at 0.3 M on the sliding speed of F-actin were modest. DEU decreased the sliding speed of F-actin to 1/20 in the gliding assay using myosin, but had little effect on the V_{max} of acto-HMM ATPase, indicating that DEU uncouples the linkage between the ATPase and the mechanical work. DRS of S1 solution showed that the hydration shell of S1 consists of constrained water of a dielectric frequency of 3.8 -5.0 GHz. The content of the constrained water around S1 decreased by $\sim 10\%$, when DEU was added or KCl was substituted for KI in the solvent.

1PS006 蛍光性カルモジュリンを用いた血管平滑筋ミオシン I および足場タンパク質によるアクチン繊維の組織化メカニズム**Actin filament organization of smooth muscle and possible roles of myosin I and scaffolding protein visualized by fluorescent calmodulin**Yoh Okamoto, Kai Sunada, Syougo Tokuda (*Muroran Institute of Technology, Division of Applied Science*)

In contrast to the architecture of contractile apparatus of striated muscle, smooth muscle has dynamic nature with respect to both of actin and myosin filaments. It should be noticed that a significant decrease of G-actin pool was observed upon the contractile stimulation of vascular smooth muscle according to Zang, W. et al.(2005). We have identified the presence of myosin I, a single headed myosin

with lipid binding short tail in both porcine aorta smooth muscle and A10 cell. An appreciable proportion of the myosin I existed in the cytosol of the A10. (Hasegawa, Y. et al; 1996, 1998) We have attempted to find out the role of the cytosolic myosin I with respect to the actin filament organization in vascular smooth muscle cell. During the study of binding between the aorta myosin I and F-actin, an appreciable proportions of F-actin has been sedimented with small amounts of myosin I under physiological ionic strength. This phenomena was attributed to the occurrence of myosin I induced F-actin bundling. During the studies on myosin I, we have also found IQGAP1 in aorta smooth muscle. IQGAP1 has been known as a scaffolding protein of F-actin along with small GTPase, calmodulin. Both myosin I and IQGAP1 in aorta smooth muscle can be key players for actin reorganization during contraction and relaxation cycles of vascular smooth muscle. We have made a fluorescently labeled calmodulin for further characterization of F-actin bundling with myosin I or F-actin gel forming with IQGAP1.

1PS007 ホッキ牽引筋 NAM Mg-ATPase 活性に対する 2 種類の TM (TM1, TM2) の影響

The effects of two kinds TM(TM1, TM2) to Mg-ATPase activity of hokki retractor muscle NAM

Yoichi Yazawa

We have studied Hokki clam, *spisula sachalinensis*, protein components of muscle natural actomyosin (NAM) and Mg-ATPase activities (= Ca-sensitivities).

In autumn(october and november), Ca sensitivity of hokki clam retractor muscle NAM ATPase activities showed low values (15~50%). Them of NAM prepared in other three seasons were higher than autumn(88~97%).

SDS-PAGE patterns of two kinds of NAM.

We was different from TM(TM1 and TM2). The difference between TM1 and TM2 is the first report about two types of TM function to Mg-ATPase activity.

1PS008 クライオ電子顕微鏡法を用いたアクチン-ミオシン硬直複合体の高分解能構造への試み

Approach to higher-resolution structure of actin-myosin rigor complex by electron cryo-microscopy

Norihiro Shimizu¹, Yoshihiro Tsukada¹, Yakuo Yasunaga^{1,2} (¹Kyushu Inst. of Tech., ²JST)

Actin-myosin interaction is involved in muscle contraction, cell motion, and intercellular transport, and the interaction mechanism in detail is not figured out yet. We convince it necessary to obtain high-resolution structure of the rigor complexes to elucidate the mechanism to produce strong binding between actin and myosin. The previous studies have reported their 13 Å resolution density maps but their resolution isn't good enough to discuss the rigor structure at the atomic level. We also have been challenging to obtain high resolution maps beyond 4 Å by cryo-electron microscopy and helical particle analysis. The obtained filaments formed arrowhead every 36 nm. It is due to the behavior of actin-myosin rigor complex: actin filaments take double-helical architecture with a half pitch of 36 nm, and actin molecule bind to myosin molecule in pairs. Therefore, those filaments were obtained as full-decoration. This time, we could get to about 30 filaments (~2000 units) in addition to existing 5500 units from earlier study (Tsukada, 2010), but need 10000 units or more number of units in order to get high-resolution structure beyond 4 Å. We calculated that it is important to increase the number of better quality complex filament.

1PS009 In vivo マウス心臓におけるサルコメア長のリアルタイム計測 Real-time measurement of sarcomere length in the mouse heart in vivo by using α-actinin-GFP

Akari Mizuno¹, Fuyu Kobirumaki-Shimozawa², Kotaro Oyama¹, Takako Terui², Erisa Hirokawa², Togo Simozawa³, Shin'ichi Ishiwata¹, Norio Fukuda² (¹Department of Physics, Waseda University, ²Department of Cell Physiology, The Jikei University School of Medicine, ³Department of Physics, Gakushuin University)

The heart is a unique organ in which cardiomyocytes repeat contraction and relaxation in response to electrical stimulation, thereby ejecting blood. Despite numerous studies conducted thus far under various experimental settings, the molecular mechanisms of contraction and relaxation of cardiomyocytes till remain elusive in vivo. In the present study, we expressed GFP at sarcomeric Z-discs (α-actinin) by using the adenovirus vector system in adult mice, and conducted real-time imaging of the movement of single sarcomeres in

cardiomyocytes in the left ventricle under fluorescence microscopy at 10 nm precision (at 100 fps). First, we found that sarcomere length (SL) was ~2.0 μm in the isolated heart when perfused with 30 mM BDM (hence during diastole). This value is close to what was obtained previously by other investigators in rats using various experimental techniques. When perfused with Tyrode's solution containing 1 mM Ca²⁺, the heart started to exhibit beating with the diastolic and systolic SLs ~2.2 and ~1.7 μm in the left ventricle, respectively. Finally, we attempted to visualize single sarcomeres in vivo in open chest mice under anesthesia. We found that SL was ~2.0 and ~1.7 μm during diastole and systole, respectively, but varied by ~300 nm even in the same left ventricular cell. We also found that sarcomere contraction occurred at the T-wave end point on the electrocardiogram, followed by an increase in the left ventricular pressure. At the meeting, we discuss these findings at the molecular level.

1PS010 チャネルロドプシンを発現した骨格筋細胞における筋収縮と成熟の光操作

Optogenetic manipulation of myogenic contraction and maturation in channelrhodopsin-expressing skeletal muscle myotubes

Toshifumi Asano^{1,2}, Toru Ishizuka^{1,3}, Hiromu Yawo^{1,3,4} (¹Grad. Sch. of Life Sci., Tohoku Univ., ²JSPS, ³JST, CREST, ⁴Center for Neurosci., Tohoku Univ. Grad. Sch. of Med.)

Traditionally, neuron and muscle have been electrically stimulated to be induced the artificial activity and examined the specific functions. Although, this technique is simple and convenient, it is often nonuniform, nonspecific and invasive with the electrical field stimulation using metal electrodes placed in the extracellular space. In this study, to improve the spatiotemporal resolution and to reduce the invasiveness, mouse muscle cell lines, C2C12 myoblasts were genetically introduced a variant of light-gated ion channel, channelrhodopsin-green receiver (ChRGR), and subsequently induced into multi-nucleated myotubes according to the standard differentiation conditions. The LED light pulses depolarized the membrane potential of a ChRGR-expressing myotube and eventually evoked action potentials dependent on the intensity and duration of the illumination. The light-activated contraction of the photosensitive myotube was obvious in synchronous to the light pulses of the given frequency and the pattern (1-10 Hz, 20-100 ms). We also found that the optical stimulation using pulsed flash light promoted the sarcomere assembly and the contractile ability of the ChRGR-expressing myotubes. It is suggested that the myogenic contraction and the maturation of skeletal muscle cells can be manipulated by light using optogenetics. This technique could facilitate the studies such as the fundamental biological processes during myogenic development, the stem cell therapy for muscular disorder, and the bioengineering using the muscle-powered actuator.

1PS011 軟体動物横紋筋のコネクチン様タンパク質 Connectin-like protein in molluscan striated muscle

Yulong Bao¹, Akira Hanashima¹, Fumiaki Sodeyama², Sumiko Kimura¹ (¹Department of Biology, Graduate School of Science, Chiba University, ²Department of Biology, Graduate School of Science, University of Tokyo)

Connectin is an elastic muscle protein in vertebrate striated muscle. Invertebrate striated muscle is also known to possess several connectin-like proteins. Because the results of our SDS PAGE and immunoblot analyses have suggested the presence of a connectin-like protein in molluscan muscle, we examined its primary structure and compared it with those of some known connectin and connectin-like proteins.

Using the genome information of *Lottia gigantea*, we searched for a connectin-like protein and determined its sequence by RT-PCR. As a result, the connectin-like protein of the limpet was estimated to be 54 kbp and have a molecular weight of 2,020,000. The domain structure of this protein was similar to that of vertebrate connectin in that it possessed tandem Ig domains, a PEVK region at the N-terminus and a kinase domain at the C-terminus. However, the protein had characteristics substantially different from those of the already known connectin and connectin-like proteins. In particular, it was found to possess a tandem Fn region consisting of 45 repeated Fn domains at the C-terminus and to lack a super-repeat region.

Immunoblot analysis using a polyclonal antibody for the recombinant protein showed that the antibody against the PEVK region reacted with a 3000-kDa band of the limpet heart muscle. Furthermore, the observation with fluorescence microscopy revealed that the PEVK region of limpet connectin-like protein is localized in the I-band of the striated muscle sarcomere.