2P139 F-アクチンの水和状態のミオシン-サブフラグメント 1 密度依存性
Bound-Myosin Density Dependence of Hydration State of Actin-Filament

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Hydration state change of actin filament(F-actin) with bound myosin S1 after ATP hydrolysis were measured by precision microwave dielectric spectroscopy(DRS) at different molar ratio from 0:13 to 3:13 of S1:actin. In the previous study (BBRC 322(2004) 340-346), the hydration state of F-actin with bound S1 exhibited a higher level of hypermobile water(HMW) number per actin than that of F-actin in spite of the absence of HMW around S1. In this study, with increasing the number of bound S1 on F-actin, amount of HMW was found to increase non linearly, exhibiting a cooperative structural change in actin filament.

2P140 ATP 結合アナログを用いたミオシンサブフラグメント 1 の水和研究
Hydration study of myosin subfragment1 with bound ATP analogs

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Hydration properties of myosin subfragment-1 (S1) with bound ADP.Pi analogs were measured by precision microwave dielectric spectroscopy (DRS). In the previous study (BJ, 72 (1997) 18-23), the intermediate state of S1 during ATP hydrolysis reaction, the mixed state of S1.ATP and S1.ADP.Pi, exhibited a lower level of hydration number, which was consistent with the thermodynamic parameter changes in entropy and heat capacity given by Kodama et al (Physiol. Rev. 65 (1985)467-551). However, in the present study, the hydration levels of S1.ADP.AIF4 and S1.ADP.Vi were higher than that of S1.ADP. It suggests that the S1 states with bound ADP.AIF4 and ADP.Vi are different from that in the dynamic ATP-hydrolysis cycle.

2P141 2,3-ブタンジオン 2-モノキシムによるミオシン II ATP 加水分解のケミカルレスキュー
Chemical rescue of myosin II ATP hydrolysis by 2, 3-butandione 2-monoxime

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BDM (2, 3-butandione 2-monoxime) is well characterized as an inhibitor of myosin motor. However, its inhibitory mechanism is not fully understood. We herein demonstrated that BDM elevates skeletal myosin II K⁺, EDTA-ATPase activity. In addition, BDM reactivates the ATPase activity of partially unfolded myosin. Furthermore, computational molecular modeling of docking of BDM on *Dictyostelium* myosin II-nucleotide complexes suggests two putative BDM-binding sites located around the the ATP site. Analogously to oxime compounds such as pralidoxime, which reactivate organophosphate-inactivated acetylcholinesterase, the oxime group of BDM may rescue the ATP hydrolysis of myosin. Based on these results, the mechanism of the inhibition of myosin motor will be discussed.

2P142 F-アクチンの水和状態に及ぼす Mg2+と Ca2+の効果
Effect of Mg2+ and Ca2+ ions on the hydration state of F-actin

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F-actin was reported to have dual hydration shell containing restrained water and hypermobile water (HMW) (BJ 85 (2003)3154-3161) and to exhibit marked increase in HMW upon binding myosin S1 by the previous study (BBRC 322(2004)340-346). In this study, hydration property of F-actin was measured in two buffer solutions at 10°C: (1) 2mM MgCl₂, 50mM KCl, 10mM HEPES, 1mM DTT, 0.1mM ATP at pH 7.8, (2) 2mM CaCl₂, 50mM KCl, 10mM HEPES, 1mM DTT, 0.1mM ATP at pH 7.8. As a result, the HMW signal (fc-fw)δ was markedly stronger in Ca-F-actin solution than in Mg-F-actin solution, indicating a structural change of F-actin.

2P143 Investigating conditions for structure analysis of binding states of formin/mDia1 to the actin filament by electron microscopy

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We investigated suitable conditions for structure analysis of binding states of formin/mDia1 to the actin filament by electron microscopy. We found the mDia1 gradually aggregated and its polymerization activity decreased as time passed after the purification. To solve the problems, we changed several conditions including shortening purification experiments and we achieved a better set of conditions without aggregation and with high activity. We also developed a new device to quantify binding activity to the actin filaments (and other filaments) at a very low concentration, to evaluate formin/mDia1 binding activity.

2P144 高温の心筋細胞内でみられる高速サルコメア振動
High-frequency sarcomeric auto-oscillations in living cardiomyocytes under hyperthermal conditions

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We have developed an experimental system for nano-scale analysis of sarcomere dynamics and Ca²⁺ changes via expression of AcGFP in Z-discs (Shintani et al., J. Gen. Physiol., 2014). By using this system, we found that an increase in temperature by IR laser irradiation generated sarcomeric auto-oscillations with frequencies faster than those of normal Ca²⁺-dependent beating in living cardiomyocytes. The sarcomeric auto-oscillations, termed Hyperthermal Sarcomeric Oscillations (HSOs), were induced independent of Ca transients and exhibited stable oscillation properties (e.g., frequency and amplitude). However, under conditions where normal cardiac beat was blocked, the HSOs were gradually organized. Therefore, the normal cardiac beating may need to induce stable HSOs.