- 1P154 SDSL-EPR 法を用いた心筋トロポニン I リン酸化によるトロポニン C 構造への影響の研究
○植木 正二¹、荒田 敏昭²
1徳島文理大・香川薬、²阪大院・理
- 1pC05 SDSL-EPR study of the effect of troponin I phosphorylation on the structure of cardiac troponin C
Shoji Ueki (1) and Toshiaki Arata (2). (1: Facul Pharm Sci at Kagawa, Tokushima Bunri Univ; 2: Dept Biol Sci, Graduate School of Science, Osaka Univ)

Calcium-induced structural transition in the amino-terminal domain of troponin C (TnC) triggers skeletal and cardiac muscle contraction. Unlike the skeletal TnC that has two sites for regulatory calcium in the N-domain (sites I and II), cardiac TnC (cTnC) has only one active calcium-binding site (site II). We previously revealed the movement of the helices of the N-domain induced by calcium binding and the dimerization with troponin I (TnI) by means of site-directed spin-labeling electron paramagnetic resonance (SDSL-EPR) method. Cardiac TnI (cTnI) has a unique N-terminal extension contains two targets for PKA phosphorylation (Ser23 and Ser24). In cardiac myocytes, this phosphorylation is stimulated by beta-adrenergic agonists and is a regulatory device in cardiac contractility. It is thought that the phosphorylation prevents the interaction between N-terminal extension of cTnI and N-domain of cTnC. In this study, the effect of the phosphorylation on the structure of cTnC complexed with cTnI is examined by SDSL-EPR. We found the distance between C and D helices of cTnC is closer in the phosphorylated complex than that in the non-phosphorylated one. It is suggested that the cTnI N-terminal interaction to cTnC opens the N-domain of cTnC, and the open structure of the N-domain is favorable for calcium binding. The phosphorylation of cTnI prevents the interaction, and decrease the sensitivity of the calcium binding.

- 1P156 ハイパーモバイル水の証拠：F-actin 水溶液中のプロトン拡散係数は純水より大きく myosin-S1 結合でさらに増大
○鈴木 誠¹、Gan Xinying¹、和沢 鉄一¹、宮崎 崇¹
1東北大院・工、²CREST, JST
- 1pC07 Evidence of hyper-mobile water : Enhanced proton diffusion coefficients in F-actin solutions with bound myosin S1 by PFG-SE NMR
Makoto Suzuki(1), Xinying Gan(1), Tetsuichi Wazawa(1) and Takashi Miyazaki(1). (1:Dept Materials Processing, Graduate School of Engineering, Tohoku University)

In the previous studies we reported the presence of hyper-mobile water (HMW) around actin filaments and the volume increase of HMW upon binding with myosin S1 by microwave dielectric spectroscopy. Here, we measured the proton diffusion coefficients (Dh) in chicken F-actin solutions in the concentration range from 2 to 9 mg/ml and in bovine serum albumin (BSA) solutions of 3.0 mg/ml, with KCl from 0 to 200 mM by pulsed field gradient spin echo 1H-NMR using the modified Hahn sequence (90-G-180-G). Actin and S1 solutions were prepared from chicken. The buffer was (4mM Hepes, CaCl2 0.1mM, ATP 0.1mM, DTT 0.1mM, pH 8, 25C). Dh values decreased with increasing KCl up to 100mM of KCl and increased above the concentration. Similar experiments were made with KF instead of KCl, resulting in a monotonic decrease with KF. We conclude that the Dh-decrease below 100mM KCl or KF is due to electrostatic screening effect. The difference above 100 mM between KCl and KF cases can be explained by the effect of Cl⁻ as a water structure breaker. For BSA Dh values were always smaller than that of buffer, consistent with the property of ordinary hydration. As increasing the molar ratio S1:actin the value of (Dh-Dhb)/Dh0, (Dh0:pure water, Dhb:buffer), was +0.41% in F-actin solution, +0.96% in F-actin-S1 solution (actin:S1=13:2), and +1.64% in F-actin-S1 solution at actin:S1=13:4 (for all cases n=3, standard error 0.20%), showing a marked increase in Dh and thus HMW.(Refs) S.R. Kabir, et al. Biophys. J., 85 (2003) 3154; M. Suzuki, et al. BBRC, 322 (2004) 340.

- 1P155 トロポミオシン・ジャンクションのスイベルはアクチンフィラメント制御に必須である
村上 健次¹、Stewart Murray²、○若林 健之¹
1帝京大学・理工学部・バイオサイエンス、²MRC Lab. Mol. Biol
- 1pC06 A Swivel between Successive Tropomyosin Moecules is Crucial for Actin Filament Regulation
Kenji Murakami(1), Murray Stewart (2) and Takeyuki Wakabayashi(1). (1: Dept Biosciences, School of Science and Engineering, Teikyo University; 2: MRC Lab. Mol. Biol)

The head-to-tail polymerization of tropomyosin is crucial for its function in actin filament assembly and actin-myosin contraction in cytoskeleton. We describe the 2.1 Angstrom resolution structure of crystals containing overlapping tropomyosin N- and C-termini of tropomyosin (TM-N and TM-C) and also the 2.9 Angstrom resolution structure of crystals containing these fragments together with a fragment of troponin-T (TN-T). A crucial water molecule breaks the local 2-fold symmetry in TM-C coiled coil. Generated asymmetry is essential for binding only a single TnT to the tropomyosin homodimer and enabling formation of a 4-helix bundle with TnT. The junction can function as a novel molecular swivel that accommodates irregularities in the coiled coil and enables successive tropomyosin molecules to interact quasi-equivalently with actin. Generation of a pseudo-atomic thin filament model by docking the crystal structures into a cryo-EM map and annealing using molecular dynamics identifies actin-tropomyosin interfaces that indicate how the swivel regulates the tropomyosin-based inhibition of actin-activated myosin mechanochemistry. We thank collaboration of Drs N. Kudou, N. Igarashi, Y. Shirakihara, S. Wakatsuki, and T. Yasunaga.

- 1P157 X線繊維回折による骨格筋ミオシンクロスブリッジの構造と動態
○大島 勘二¹、武澤 康範²、杉本 泰伸²、Thomas Irving³、若林 克三²
1大阪大学臨床医工学融合研究教育センター、²大阪大学大学院基礎工学研究科、³Illinois Institute of Technology
- Dispositional and Conformational Changes of Myosin Crossbridges in Skeletal Muscle Contraction and Regulation by X-ray Fiber Diffraction
Kanji Oshima (1), Yasunori Takezawa (2), Yasunobu Sugimoto (2), Thomas C. Irving (3) and Katsuzo Wakabayashi (2) (1: Cent. of Adv. Med. Eng. and Info., Osaka Univ; 2 Div. of Biophys. Eng., Graduate School of Engineering Science, Osaka Univ; 3 Dept. of Biol. Chem. Phys. Sci., Illinois Inst. of Tech., USA)

The dispositional and conformational changes of the myosin crossbridges along the thick myosin filament were investigated by X-ray fiber diffraction. Detailed analysis of the axial sampling periods on the myosin-based meridional reflections due to the mirror-image structure of myosin filaments across the M-line in the sarcomere showed that there exists a mixed population of crossbridge arrangement along the myosin filament, called "regular" and "perturbed" regions. The modeling studies using the crystallographic structure of a myosin head revealed that the perturbed region positioned the central part in the half of the myosin filaments, occupying about 70 % of the length of the crossbridge region in both states and that the two heads of a crossbridge were axially tilted in opposite directions along the three-fold helical tracks of myosin filaments. In the relaxed state, one head of a crossbridge appeared to be in close proximity to the head of the adjacent crossbridge in the regular region. This approach occurred between narrower inter crossbridge levels in the perturbed region. Thus head-head interactions of myosin crossbridges occur and could provide an additional switching off mechanism for the actomyosin interaction. During the contraction, one head of a crossbridge appeared to be flared axially and the partner head oriented more perpendicularly to the actin filament in the perturbed region. The results lead to the concept that two heads of a myosin crossbridge could play alternate roles for the generation of contractile force.