1P150 トロポニンの遺伝性心筋症型変異が筋原繊維の機能に及ぼす影響
○松本 富美子¹、前田 佳代²、似内 靖²、小田 俊郎²、前田 雄一郎²、藤原 悟¹
¹原子力機構、²ERATO 前田プロジェクト
Effects of the cardiomyopathy type mutations of troponin on the ATPase activity and Ca²⁺ sensitization in myofibrils.
Fumiko Matsumoto(1), Kayo Maeda(2), Yasushi Nitani(2), Toshiro Oda(2), Yuichiro Maeda(2), Satoru Fujiwara(1). (1:Quantum Beam Science Directorate, Japan Atomic Energy Agency ;2:ERATO Actin Filament Dynamics Project)

Familial hypertrophic cardiomyopathy (HMC) has been reported to be caused by mutations in a regulatory protein, troponin (Tn). Tn is a complex of three subunits, TnC, TnI, and TnT. HMC is characterized by functional aberration on the force-pCa relationship. Only a few cardiomyopathy-causing mutations have been mapped on the coiled-coil region (IT-arm) in the Tn core domain. Here we focus on two mutations in the IT-arm, E244D and K247R of TnT. Whereas E244D has been reported to show an increase of the maximum level of ATPase activity (Yanaga et. al., 1999), the functional consequence of K247R mutation has not been analyzed. In order to understand how these mutations cause functional aberrations, we measured ATPase activity of myofibrils containing various mutants of these residues (E244: D, M, A, K and K247: R, E, A). The maximum level of ATPase activity was found to increase in K247R, without changing the Ca²⁺ sensitivity, as found in E244D. A close inspection of the crystal structure showed that the side chains of E244 and K247 form the triplet with that of E110 of TnI on the outside of the hydrophobic core of the coiled-coil. This triplet is thus likely to introduce flexibility into the IT-arm at this position. The mutations at the residues 244 and 247 could alter this flexibility. The results obtained here suggested that the "proper" flexibility of the IT-arm is important for the correct function of the myofibrils. The relationship between the IT-arm flexibility and functional aberration in the myofibril will be discussed.

1P152 蛍光消光測定による筋肉細いフィラメント上のトロポミオシン分子の動的性質研究
斎藤 隆弘¹、松田 陽介¹、○三木 正雄¹
¹福井大院・工・生物応用化学
Study of the dynamic structure of the tropomyosin molecule in muscle thin filaments by fluorescence quenching measurements.
Takahiro Saitoh, Yousuke Matsuda and Masao Miki (Applied Chemistry and Biotechnology, graduate School of Engineering, Univ of Fukui)

Tropomyosin (Tm) in association with troponin (Tn) plays a central role in the calcium regulation of striated muscle contraction. To study the dynamic structure of the Tm molecule in the reconstituted muscle thin filament, acrylamide quenching of the fluorescence of IAEDANS attached to the single cysteine residue of mutant Tms have been carried out under a variety of conditions (alone, complexed with actin or Tn, complexed with actin and Tn in the presence and absence of Ca²⁺, and complexed with actin, Tn and myosin subfragment 1 in the presence and absence of Ca²⁺). We generated mutant Tms containing single-cysteine residues at different positions along the coiled-coil structure. The fluorescence lifetimes of IAEDANS-Tm at various concentrations of acrylamide (0 - 0.4 M) were measured by single photon counting methods. From their Stern-Volmer plots, the collisional quenching constants were calculated. At most sites the values of the quenching constant largely decreased by the addition of F-actin and further decreased by the addition of F-actin and Tn. However, they did not significantly change in response to a change in the Ca²⁺ concentrations, suggesting that the Ca²⁺-induced rolling of Tm does not occur.

1P151 家族性心筋症を引き起こす変異トロポニンの分子動力学解析
○山口 真紀¹、大塚 由美子¹
¹東京慈恵会医科大学
Structural Change of Mutant Troponin Related to Cardiomyopathy
Yamaguchi, M, Otsuka Y, Department of Physiology, Jikei University School of Medicine

Many of the troponin mutants which can cause familial cardiomyopathy have been reported so far. To elucidate the molecular mechanism of the cardiomyopathy related to troponin, we performed molecular dynamics study on the structure of troponin mutants which relate to familial cardiomyopathy. Two different troponin T mutant related to hypertrophic cardiomyopathy, Glu244Asp and Lys247Arg, and a troponin C mutant related to dilated cardiomyopathy, were studied. Dynamics was calculated by the use of software Amber (vers. 9). Iteration was done in TIP3 water sphere with 0.5 or 1 fs time step in periodic condition at constant temperature (310 K) and pressure. Model structures of troponin mutants were constructed by introducing the mutation to the crystal structure of human cardiac troponin (core region of TIC complex) obtained from Protein Data Bank (ID number 1J1E). More than 9 trajectories of 1ns were obtained for wild and mutant structure. It was observed that electrostatic interaction between troponin I and troponin T which linked alpha helix of troponin T and troponin I in the wild was lost in the two troponin T mutant. Furthermore, when a terminal residue of troponin I was pulled toward an actin molecule mimicking the intra-molecular force on activation, different structural change was observed in troponin T mutant compared with wild. The involvement of this difference between the wild and mutant in the tension regulation will be discussed.

1P153 細いフィラメントへのミオシン頭部結合により生じる協同性のスピラバル化トロポミオシンからの解析
○植田 啓介¹、木邑 智恵子²、相原 朋樹²、植木 正二¹、三木 正雄³、荒田 敏昭¹
¹阪大院・理・生物科学、²ERATO・前田プロジェクト、³福井大・工・生物応用化学
Cooperative effect of myosin binding to spin-labeled tropomyosin on muscle thin filament
Keisuke Ueda (1), Chieko Kimura (2), Tomoki Aihara (2), Shoji Ueki (1), Masao Miki (3) and Toshiaki Arata (1). (1: Dept. of Biol. Sci. Osaka Univ., 2: ERATO-JST, 3: Dept.of Applied Chem. and Biotech. Fukui Univ.)

Ca²⁺ and S-1 induced positional or flexibility change of tropomyosin (Tm) on the thin filament, which facilitates the interaction of the myosin heads with actin filament (F-actin). The Tm-Tm polymer is continuous rather than disjointed, and Tm affects myosin:actin binding by shifting among three positions as in recent structural studies. We have applied site-directed spin labeling (SDSL) ESR to see whether such structural change occurs. We have labeled rabbit skeletal Tm at its native cysteine residues and at several genetically engineered cysteine residues along a Tm molecule with MSL and MTSL, and then reconstituted the complex consist of Tm, troponin (Tn), myosin head S-1, and F-actin. It was found that the strongly immobilized component markedly appears on the spectrum of Cys160 by addition of myosin head S-1. This strongly immobilized component appears regardless of the presence of Tn or Ca²⁺. The ratio of peak heights corresponding to strongly/weakly immobilized components, markedly changed at the molar ratio of S-1/actin (~1/7), but did not increase any more as the ratio of S-1/actin from 1/7. Therefore, conformational changes of Tm at Cys160 residue occurred when a S-1 bound to actin subunit distant from this residue. This suggested that conformational changes of Tm or F-actin propagated along the thin filament. In order to see whether Tm moves on the thin filament in response to Ca²⁺ elevation and S-1 binding, we are now planning to investigate the distance between Tm and F-actin with CW-ESR and pulsed-ELDOR.