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論　文　内　容　の　要　旨

氏　名　（　山田　有里佳　）	
論文題名	Structural analysis of muscle thin filament by electron cryomicroscopy and image analysis (クライオ電子顕微鏡と画像解析による筋肉の細いフィラメントの構造解析)

論文内容の要旨

Muscle contraction is driven by cyclic interactions of myosin in the thick filament with actin in the thin filament composed of actin, tropomyosin and troponin complex (TnC, TnI, TnT). It is thought that the binding of Ca^{2+} released from sarcoplasmic reticulum to TnC causes a conformational change of tropomyosin on the actin filament to allow actin-myosin interaction. To understand this regulatory mechanism, it is necessary to elucidate the structure of the thin filament at high resolution. First, I developed a method to isolate intact Ca^{2+} free and Ca^{2+} bound thin filament from skeletal muscle of a crab, *Portunus trituberculatus*, at high yield. However, the result of structural analysis by electron cryomicroscopy (cryoEM) showed that only 25% of the isolated thin filaments were fully decorated with tropomyosin and troponin. To increase the number of fully decorated filament, I treated the thin filament by glutaraldehyde to mildly crosslink Tm and Tn with actin filament and obtained a density map of actin filament fully decorated with Tm in the absence of Ca^{2+}.

For visualization of the location of troponin on the thin filament, we established a method of image analysis for 3D reconstruction of the thin filament, where accurate alignment of troponin was very difficult. A cryoEM density map of the thin filament in the absence of Ca^{2+} at around 20 □ resolution showed interesting features of actin-tropomyosin-troponin interactions that have never been seen before.

Chemical crosslinking was necessary to stabilize the actin filament with the Tm and Tn complex for cryoEM image analysis, although it was not necessary for the analysis of negatively stained EM images. The Tm and Tn complex dissociated from the filament possibly by the process of making thin film of solution for quick freezing to prepare frozen-hydrated thin filaments embedded in thin vitreous ice films. To prevent the dissociation of Tm and Tn from the filament without crosslinking, I made an *E. coli* expression system of human cardiac Tn complex and slightly modified Tm, purified them and reconstituted the thin filament using skeletal muscle actin filament. Modification of tropomyosin was designed to increase the binding affinity to actin filament to prevent its dissociation from the filament. However, 3D image reconstructed by cryoEM image analysis still showed only a weak density of tropomyosin. But, an addition of a low concentration of a detergent just before quick freezing prevented the dissociation of tropomyosin. We were thus able to obtain the structure of actin filament in complex with tropomyosin and troponin.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

申請者は、骨格筋の筋収縮制御を司る筋肉の細いフィラメントの構造をクライオ電子顕微鏡と画像解析法を用いて高分解能で解析し、そのメカニズムの解明をめざして研究を行った。筋肉の細いフィラメントは、アクチン繊維のアクチン７分子にトロポミオシンとトロポニン複合体が結合したもので、トロポニンのサブユニットの一つであるトロポニンCにCa^{2+}が結合することでトロポニン複合体の構造が変化し、アクチン繊維上のトロポミオシンの位置をずらしてミオシン頭部のATP加水分解にともなう結合解離を可能にし、筋収縮が起こると考えられている。しかし、半世紀近くにわたる研究によってもその構造的メカニズムは明らかにされていない。申請者はこの困難な問題に取りくみ、様々な工夫により世界で始めて細いフィラメントの構造解析を可能にした。近いうちに原子レベルの分解能を達成し、筋収縮の制御機構を解明すると期待できる成果である。

これら一連の研究は当該分野に新たな進展をもたらしたので、博士号の学位授与にふさわしいと認める。