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Head-head interaction of myosin crossbridges from frog skeletal muscle thick filaments obtained by X-ray fiber diffraction

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Introduction

Interaction between myosin heads is responsible for switching off mechanism in various types of myosin molecules such as vertebrate smooth and invertebrate striated muscles (Tarantula and Limulus) with phosphorylation-dependent regulation in resting state. It is unknown whether or not such head-head interaction is occurred in live resting skeletal muscles, which are not intrinsically regulated by the phosphorylation. X-ray fiber diffraction is one of useful techniques to obtain the structural information about the thick myosin filaments in resting frog skeletal muscles. We developed a novel method using cylindrically averaged difference Patterson function ($\Delta Q(r,z)$) [1] to correct the partial sampling effect due to the hexagonal lattice of myofilaments on the myosin based layer lines in the X-ray diffraction pattern from full-filament overlapped muscles. We report the result of the modeling of the azimuthal orientation of two heads of myosin crossbridges along the thick filament in resting state.

Experimental and Modeling

X-ray diffraction experiments were performed at BL15A1. X-ray patterns were taken from live resting frog skeletal muscles with full-filament overlap. In modelling, we used a mixed structural model of a thick filament with two different axial periodicities [2] to search for an optimum orientation of two heads of a myosin crossbridge.

Results and Discussion

In our best-fit models, each head of a myosin crossbridge has a different orientation around the filament axis in the perturbed and regular regions of a crown repeat. In the perturbed region, two heads of a myosin crossbridge showed a cross shape structure when seen from the top of the thick filament reported previously [3]. Myosin crossbridge seems to form intramolecular head-to-head interaction. In the regular region the myosin crossbriges form intermolecular head-head interaction between adjacent crown levels. Interestingly, the interface between two myosin heads is similar to that of intramolecular interaction between two heads of regulated myosin molecules reported previously [4]. One head faces toward the converter domain of the other head (Figure 1). The electrostatic potentials on the molecular surface of myosin heads are shown in Figure 2 by using eF-surf (http://ef-site.hgc.jp/eF-surf); the converter domain is positively charged and an interaction area of the partner head is negatively charged, suggesting that the intramolecular head-head interaction in the regular region is caused by the electrostatic interaction.

The head-to-head interaction possibly by the electrostatic interaction is one of common features of resting muscles and may be related to the inhibition mechanism of actomyosin interaction in resting skeletal muscles as well as in vertebrate smooth and invertebrate striated muscles.

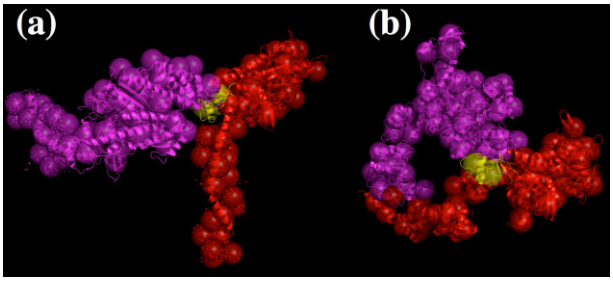

Figure 1. The head-head interaction between myosin heads. (a) Intermolecular head-head interaction in the regular region. (b) Atomic structure of a myosin crossbridge (3dtp) without a subfragment-2 structure from talantula muscles. The converter domain is shown by yellow color. The blocked head is shown by purple color and the free head is shown by red color.

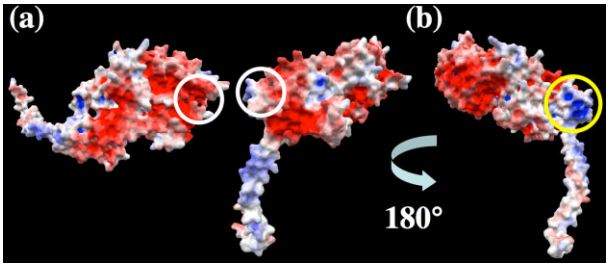

Figure 2. (a) Electrostatic potentials on the molecular surface of myosin heads in the regular region. (b) The myosin head which is given by a rotation of the right head in (a) around the vertical axis by 180 degrees. An interaction area is shown by white circle in (a) and the converter domain by yellow circle in (b).

References

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