15A/2003G323

## Modeling Analysis of Myosin-based Meridional Reflections from Skeletal Muscles in Relaxed and Contracting States. II. Revised Models

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## **Introduction**

The modeling analysis of the myosin-based meridional reflections in the X-ray diffraction patterns of live frog skeletal muscles was reported in the previous report [1,2]. The intensities of the meridional reflections were corrected rigorously because they were modulated by the lateral filament arrays. Using the corrected data, we reinvestigated to propose a more precise model for the crossbridge structures of myosin filaments in the relaxed and contracting states.

## **Experimental**

Live frog sartorius muscles were used for X-ray studies. X-ray diffraction experiments were performed at BL15A1. The 2D-X-ray diffraction patterns from relaxed and contracting muscles were recorded with an image plate at the specimen-to-detector distance of ca. 2.4 m. The intensities were obtained by integration of the data in the radial narrow range of 0 - 0.00085 nm<sup>-1</sup>. The meridional intensities were corrected for the effect of the filament disordering. The measured intensities were multiplied twice by the radial widths of the reflections so that their reflection volume becomes constant.

## **Results and Discussion**

Detailed analysis of the axial fine splitting periods of the second and fifth order meridional reflections showed that the average period was ca.  $820\pm40$  nm in the relaxed state, while it was ca.  $1010\pm40$  nm in the contracting state. The result was obtained from the experiments done at the BioCAT beamline, APS.

For modeling the crossbridges structure along the thick filament in the relaxed and contracting states, we used the observed intensities of the second to eleventh order meridional reflections. In the model, the crown regions with a regular repeat of 14.3 nm (the regular regions) were assumed to be located on both sides of the perturbed The intensities of the assumed models were region. calculated by using eleven independent parameters; the shifts of the crown level from the 14.3-nm repeat in the triplets, each width of two myosin heads of a crossbridge projected onto the fiber axis, the distance between two myosin heads and each number of the crossbridges in the regular and perturbed regions. The most probable values of these parameters were determined by searching the best fit of the calculated intensities to those observed.

In the relaxed state, the perturbed regions are ca. 560nm long with 13 triplet levels of the 42.9-nm repeat. The lengths of the inner (towards the M-line) and outer (towards the Z-band) regular regions are ca. 57 nm and ca. 86 nm, respectively. In the contracting state, the perturbed regions become shorter than in the relaxed state and the lengths are ca. 480 nm with 11 triplet levels of the 43.5-nm repeat. The inner regular regions are ca. 160-nm long and the outer regular regions are ca. 73-nm long.

Figure 1 illustrates the configuration of two-headed myosin crossbridges along the thick filament in the relaxed and contracting states. Our simulation shows that the intensities of forbidden meridional reflections are caused to be weakened in the contracting state by the fact that each displacement of the crown repeats in the triplets becomes relatively smaller and that the projected densities of crossbridges become sharper than in the relaxed state. Thus our modeling studies suggest that not only the structural change of two-headed myosin crossbridges but also the change of myosin crown periodicity make contributions to the intensity change of myosin-based meridional reflections.

We acknowledge Dr. T.C. Irving for the experiments at the BioCAT beamline, APS.

References
[1] Oshima et.al., Adv. Exp. Med. Biol. (2003)
[2] Oshima et.al., PF Activity Rep #20 (2003)



*Relaxed state Contracting state* Fig.1 The axial disposition of the two-headed myosin crossbridges along the thick filament in the regular and perturbed regions in the relaxed (A) and contracting states (B).

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