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A periodicity and an axial disposition of two-headed myosin crossbridges along the thick filaments of frog skeletal muscles in relaxed and contracting states

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Introduction

Intensity analysis of the myosin-based meridional reflections in the X-ray diffraction patterns of live frog skeletal muscles was performed to propose a more precise model for a myosin crown periodicity and an axial disposition of two-headed myosin crossbridges along the thick filaments in a sarcomere. Based on our recent modeling studies [1], we briefly report the alteration of the crossbridge arrangement when muscle goes from the relaxed state to the isometrically contracting state.

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 isometrically contracting muscles were recorded with an image plate at the specimen-to-detector distance of ca. 2.4 m. The intensity of the myosin-based meridional reflections were obtained by integration of the data in the radial range of 0 - 0.0136 nm^{-1}.

Results and Discussion

In the X-ray diffraction patterns with high angular resolution from relaxed muscles, the fine sampling period on the so-called “forbidden meridional reflections”, corresponds to the distance between the centers of two regions in the whole thick filament where the crown levels are systematically perturbed (the perturbed regions). Detailed analysis of the fine splitting of the second and fifth order meridional reflections showed that the average sampling period was ca. 790 nm±90 nm, indicating that the perturbed regions of crossbridge crown levels occupy the central zones of crossbridge arrays. The crowns with a regular repeat of 14.3 nm were assumed to locate both in the proximal and distal parts (the regular region). In the perturbed regions, they form a triplet structure in the 43.0-nm basic period. The intensities of the meridional reflections from the thick filament models were calculated by ten independent parameters: the shifts of the crown distance from the 14.3-nm repeat in the triplets, the width of projected density of each myosin head and its distance between two myosin heads for a crown, the center-to-center distance between two perturbed regions, the number of crown triplets in the perturbed regions. The most probable values of these parameters were determined by searching the best fit of the calculated intensities of the meridional reflections to those observed to minimize the R-factor ($R=(1/N)\sum\{I_{i,obs}-kI_{i,calc}\}/I_{i,obs}$) where k is the scale factor and the summation is performed over the second to eleventh order meridional reflections (N=10) with the 42.9-nm period.

The best fit to the experimental X-ray intensity data gave models for the crossbridge arrangement in the relaxed and contracting states. In the relaxed state, in each crossbridge zone there are ca. 300 nm-long perturbed regions with 7 triplet levels of 42.9 nm. Inside and outside the regions there are ca. 130 nm-long and 290 nm-long regular regions, respectively. In the contracting state, the perturbed regions are ca. 480 nm long and occupy the position from the first to the 33th crossbridge levels while the regular regions occupy only the outside positions containing 17 crossbridge levels.

Figure 1 illustrates the configuration of two-headed myosin crossbridges along the thick filament. Our simulation revealed that weakened forbidden reflections in the contracting state arise from the fact that any displacement of each crown repeat in the triplets becomes relatively smaller and that the projected densities of all crossbridges become more sharper than in the relaxed state. Thus our modeling studies show that not only the configuration of two-headed myosin crossbridges but also the myosin crown periodicity contribute equally to the intensities of myosin-based meridional reflections.

References

[1] Oshima et al., Adv. Exp. Med. Biol. (2003) in press

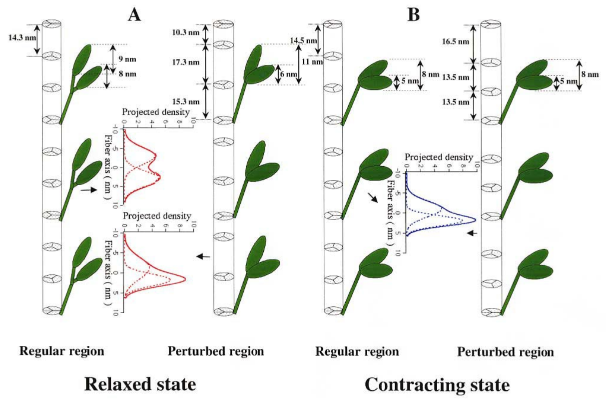

Fig.1 The axial disposition of two-headed myosin crossbridges in the regular and perturbed regions along the thick filament in the best-fit relaxed and contracting models. A; relaxed state, B; contracting state.

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