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The Orientational Analysis of Resting Myosin Crossbridges in Full-overlapped Skeletal Muscles obtained by X-ray Fiber Diffraction

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Introduction

The azimuthal orientation of a crossbridge around the myosin filament axis in muscle has been obtained using the layer-line intensities from non-overlapped muscles because there were much less lattice sampling effects on the layer lines in their diffraction patterns. However, it is unknown whether the structure of thick filaments is same or not in full- and non-overlapped muscles. We have developed a method for correcting these sampling effects on the layer lines [1,2], elucidating the single filament transform of the myosin filaments. Using this method, we corrected the layer-line intensities and determined the orientations of double-headed myosin crossbridge around the filament axis in muscles at full-overlap length.

Experimental

Live frog sartorius muscles at full-filament overlap length were used for X-ray studies. X-ray diffraction experiments were performed at BL15A1 with the help of T. Kobayashi and H. Tanaka. The 2D-X-ray diffraction patterns from resting muscles were recorded with an image plate at the camera length of ~2.4 m.

Results and Discussion

The myosin-based layer-lines from full-overlapped muscles are partially but strongly sampled by the hexagonal filament array. In order to remove the sampling effects on the layer lines, a cylindrically averaged difference-Patterson function ΔQ(r,z) [3] was used. The ΔQ(r,z) of a myosin filament was calculated by using the intensity data from the first to the eleventh order myosin-based layer lines using the equation

$$\Delta Q(r,z) = \frac{2}{c}\sum_{l=1}^{11}\left\{\int_0^{0.157} I_l(R) J_0(2\pi rR) 2\pi R dR\right\}\cos\left(\frac{2\pi l z}{c}\right)$$

where $I_l(R)$ is the intensity distribution along the l^{th} layer line and c is the crystallographic period of the myosin filament (43.02 nm) and the $J_0(2\pi rR)$ is the zeroth order Bessel function of an argument of $2\pi rR$. Figure 1A shows the ΔQ(r,z) map where positive peaks appear clearly, corresponding to the vectors between the centers of gravity of two heads of crossbridges on a three-stranded helical arrangement. Because positive peaks in the region of r > ~30 nm are interpreted as the inter-crossbridge vectors among thick filaments, we removed these peaks beyond the red line from the ΔQ(r,z) map in Fig. 1A to calculate the sampling-free layer-line intensities. The calculated intensity data of full-overlapped muscles indeed showed little sampling effect, similar to those in the diffraction pattern from non-overlapped muscles. The comparison between the obtained ΔQ(r,z) with the theoretical Q(r,z) of a three-stranded 9/1 helix of double-headed crossbridges showed that the radial position of a crossbridge along a helix was ~12.9 nm from the peak around (z, r)=(0, ~22.4 nm) and that the two heads of a crossbridge were separated axially. We used the mixed structural model of the myosin filament with two regions of different axial periodicities [1] to search for the optimum orientations of two heads around the filament axis in both regions.

In the best-fit model, each myosin head of a crossbridge has also a different orientation around the filament axis in the regular and perturbed regions. In the perturbed region, one head of a crossbridge almost comes in contact with the other head at their lateral sides, making cross-shape structure at the same axial level (Fig.1B). In the regular region, it appears to be in proximity to another head at axially adjacent crown level. Those orientations are similar to those from non-overlapped muscles reported previously [1]. Such interactions between heads represent a general feature, being related to the inhibition mechanism of actomyosin interaction in resting muscles.

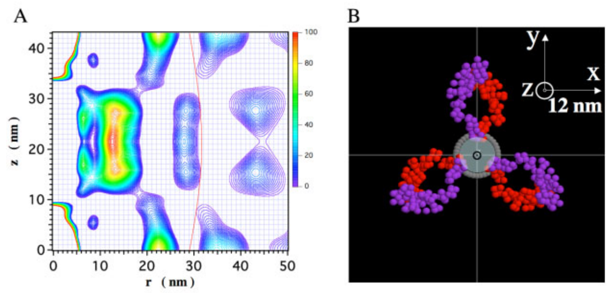

Figure 1. A, a cylindrically averaged difference-Patterson map calculated from the myosin layer-line intensities of X-ray diffraction patterns from full-overlapped muscles. B, orientations of two heads of a myosin crossbrige in the relaxed state. This figure is seen from the Z-band. The z-axis is coincided with the filament axis. Two heads are shown as a group of red and purple spheres.

References

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