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# Orientations of Resting Myosin Crossbridges from Skeletal Muscles Determined by X-ray Fiber Diffraction

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## Introduction

The orientation of a crossbridge around the fiber axis was not determined from the intensity analysis of the meridional reflections which was reported in the previous reports [1,2,3,4] because the projected density of a crossbridge onto the fiber axis is identical even if the orientation of a crossbridge is different around the fiber axis. Therefore we used the intensities of the layer-line reflections of diffraction patterns from muscles at full-overlap length and those at non-overlap filament length in the relaxed state to determine the orientation of crossbridge by rotating independently each head of two-headed crossbridges around the fiber axis.

## 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 muscles were recorded with an image plate at the specimen-to-detector distance of ca. 2.4 m. Whether muscles were stretched to the non-overlap length was made by measuring the diffraction periods from the sarcomere of overstretched muscles by diffraction of a laser light.

## Results and Discussion

The myosin-based layer-lines are partially sampled by the hexagonal filament array. In order to remove the sampling effect from the layer-lines, a cylindrically averaged difference-Patterson function ΔQ(r,z) was used. The ΔQ(r,z) of a myosin filament was constructed by using the intensity data from the first to the sixth order myosin-based layer-line reflections in a radial range of $R < 0.157$ nm$^{-1}$ using the equation of

$$\Delta Q(r,z) = \frac{2}{c}\sum_{l=1}^{6}\left\{\int_{0}^{0.157} I_l(R) J_0(2\pi rR) 2\pi R dR\right\}\cos\left(\frac{2\pi lz}{c}\right) \qquad (1)$$

where $I_l(R)$ is the intensity distribution along the $l^{th}$ layer-line reflection of the diffraction pattern and $c$ is the crystallographic period (43.02 nm) and the $J_0(2\pi\gamma R)$ is the zeroth order Bessel function of an argument of $2\pi\gamma R$ (Fig. 1A). In Fig.1A, positive peaks appeared clearly on the ΔQ(r,z) map, corresponding to the vectors between the centers of gravity of crossbridges on a three-stranded helical arrangement. Some weak positive peaks which appeared in the region of $r >$ ~32 nm were interpreted as the inter-filament vectors among thick filaments which came from the lattice sampling. Therefore we removed these peaks (outside the red curve in Fig. 1A) from the ΔQ(r,z) map to recalculate the intensity data of the layer-line reflections from a thick filament without the sampling effect.

The recalculated intensity data of muscles at full and non-overlap lengths were very similar to each other. This indicates that the sampling effect was mostly removed and the intensities of a single myosin filament were obtained. The comparison between the obtained ΔQ(r,z) with the theoretical Q(r,z) of a three-stranded 9/1 helix of two-headed crossbridges showed that the radial position of a crossbridge along a helix was ~12.6 nm from the peak around (z, r)=(0, ~22 nm) and that the two heads of a crossbridge were separated axially. We employed such information for a crossbridge arrangement to carry out the intensity calculation of the layer-line reflections by the Fourier-Bessel transformation.

In the best-fit model, each myosin head of a crossbridge has also a different orientation around the fiber axis. The distal ends of two heads of a crossbridge appear to orient in the same direction when seeing from a top view, making a U-shape structure according to the configuration of the myosin molecule (Fig. 1B).

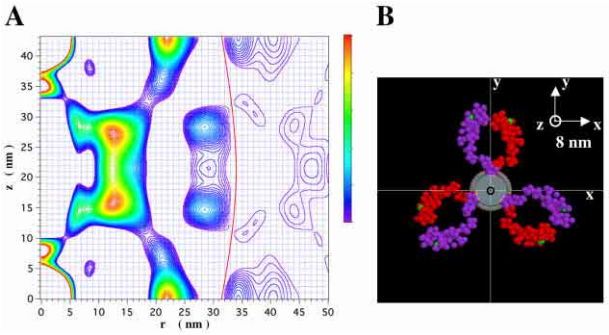


Figure1. A, a cylindrically averaged difference-Patterson map calculated from the layer-line reflections of X-ray diffraction patterns from overstretched muscles. B, orientations of two heads of a myosin crossbrige in the relaxed state. This figure is seen from the Z-band. The z-axis is coincided with the fiber axis. Two heads are shown as a group of red and purple spheres.

## References

[1] Oshima et al., PF Activity Rep #21, 244 (2004).
[2] Oshima et al., Fibre. Dif. Rev. 13, 25 (2005).
[3] Oshima et al., PF Activity Rep #22, 243 (2005).
[4] Oshima et al., PF Activity Rep #22, 244 (2005).

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