X-ray Diffraction from Skeletal Muscle on Shortening with no Load

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Introduction

The shortening is one of the important physiological states of active muscles. When muscle is stimulated electrically under the isometric condition, it generates the maximum tension via an actomyosin interaction. X-ray diffraction studies on isometrically contracting muscle have extensively been performed. It has been suggested that there are differences in the actomyosin interaction mode between shortening muscle and isometrically contracting muscle. We have made X-ray diffraction studies on shortening muscles under no load in order to obtain some evidence about the different actomyosin interaction.

Experiments

Live frog sartorius muscles were used for studies. Time-resolved X-ray diffraction experiments were performed at the BL15A1. The 2D X-ray diffraction patterns from muscle were recorded with the XII-CCD camera (Hamamatsu Photonics) at the specimen-todetector distance of ~1300 mm. The length of muscle was fixed and stimulated electrically. After the tension reached the plateau level, the hook that fixed the muscle length was released to shorten muscle under no load. Xray diffraction patterns were recorded at an exposure time of 20 ms for each image frame, and they were recorded at 200, 500, 800 and 1100 ms after the stimulation, corresponding to the isometric contracting phase, the free shortening phase and two frames of the tension recovering phase. X-ray measurements were repeated 10 times for each muscle.

Results

Figure 1 shows a comparison of X-ray diffraction patterns during an isometric contraction and a shortening under no load with their vertical axis coincident. Figure 2 shows typical meridional intensity profiles near the myosin third order reflection (M3) at $1/14.5 \text{ nm}^{-1}$ during the isometric, shortening and tension recovering phases. Release of the muscle length from the isometric value made the shortening of muscle by ~20% of the initial length to drop the tension to the zero level. The axial spacing of the M3 reflection decreased toward the value at rest during the shortening phase. After the shortening, the tension recovered gradually. The intensity traces along the meridian during the tension recovering phase

showed an increase in the background scattering, higher than the level of isometric phase and the shortening phase. The integrated intensity of the M3 reflection increased during the shortening but remained in the lower level than the isometric level. The M3 reflection also exhibited a broader profile in the shortening phase than in the isometric or tension recovering phase. The axial periodicity of myosin crossbridges seemed to be less during the tension recovering phase. The M3 reflection showed no distinct difference at 800 ms- and 1100 msflames during the recovering phase.



Figure 1 Comparison of X-ray diffraction patterns during isometric contraction and shortening



Figure 2 Meridional intensity profiles near M3 meridional reflection

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