

TIME-RESOLVED X-RAY DIFFRACTION STUDIES ON SKELETAL MUSCLE REGULATION: INTENSITY CHANGES OF THE TROPONIN-ASSOCIATED MERIDIONAL REFLECTIONS

Yasunobu SUGIMOTO¹, Yasunori TAKEZAWA¹, Shiho MINAKATA¹, Takakazu KOBAYASHI², Hidehiro TANAKA³ and Katsuzo WAKABAYASHI *¹

¹ Division of Biophysical Engineering, Graduate School of Engineering Science, Osaka University, Toyonaka, Osaka 560-8531

² Department of Physiology, School of Medicine, Teikyo University, Itabashi-ku, Tokyo 173-0003

³ Department of Physiology, Teikyo Heisei College of Nurse, Ichihara, Chiba 290-0192, Japan

Introduction

In order to clarify the structural changes related to the regulation mechanism in skeletal muscle contraction, the intensity changes of troponin-associated meridional reflections were investigated by the time-resolved X-ray diffraction technique using a CCD-based TV detector.

Experimental

The experiments were done using the small-angle diffractometer at the BL15A1. Live frog skeletal muscle was stimulated electrically for 1.3 s at 10°C, and the intensity changes of the troponin-associated meridional reflections in the whole process of activation were measured with a CCD-based TV detector. The TV detector was used in a sub-array mode with 64 x 1024 pixels in 250 frames of a time-resolution of 15 ms¹. The measurements were repeated 15 times for each muscle at intervals of 90 s, and all the data were accumulated. Similar measurements were done for 6-7 separate muscles and their data were summed to increase the S/N ratio of X-ray data.

Results and Discussion

Fig. 1 shows the intensity changes of the troponin-associated meridional reflections during isometric contraction. The first order reflection at $1/38.5 \text{ nm}^{-1}$ tended to increase in intensity just after the onset of stimulation (the first phase) and then decreased by about 45% relative to the rest value at the plateau of tension. The second order reflection showed a similar intensity change but the intensity drop at the plateau of tension was about 14%. These results were consistent with the previous ones². However, in the present cases, the initial intensity increase of the first and second order reflections was small. In contrast, the intensity of the third order reflection increased immediately after the onset of stimulation and settled to a value at the plateau of tension which amounted to about 140% of that at rest. Fig. 2 shows time courses of the intensity changes of these three reflections. When an allowance was made for the second phase of the first order reflection, the intensity change run in parallel to the development of tension and kept constant level during the plateau of tension. It was about

120 ms after the onset of stimulation when the intensity reached a half-maximum change. On the other hand, the intensity increase of the third order reflection led the tension change by about 60 ms at the half-maximum change. In the relaxation phase of tension, the intensities of these reflections started to return to the rest levels with biphasic changes.

References

[1] Y. Sugimoto et al., PF Activity Rep., #17B, 299 (2000).

[2] Y. Maeda et al., Biophys. J., 63, 815-822 (1992).

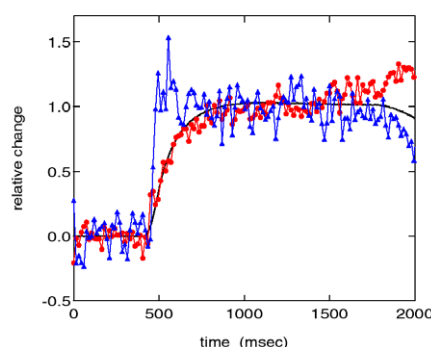
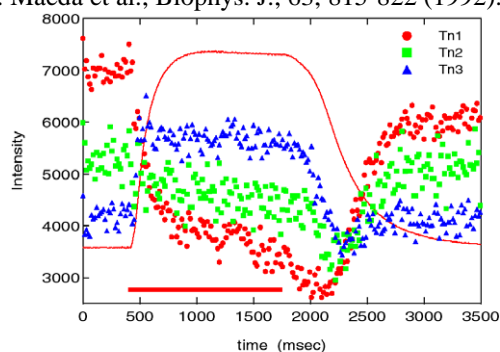


Fig. 1. Intensity changes of the first (Tn1), second (Tn2) and third (Tn3) order troponin-associated meridional reflections during an isometric contraction of frog muscle. The solid curve, tension.

Fig. 2. Time courses of the relative intensity changes of the Tn1 and Tn3 reflections during an isometric contraction. The solid curve, tension.

* third.person@kek.jp