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Time-resolved X-ray diffraction studies on skeletal muscle regulation: Intensity changes of the troponin-associated meridional reflections

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Introduction

In order to clarify the structural changes related to the regulation mechanism in skeletal muscle contraction, we have measured the intensity changes of troponin-assosiated meridional reflections by X-ray diffraction experiments. To distinguish the effect from the switch-on process by Ca^{2+}-binding to troponin and that from the myosin interaction process, muscles stretched to the non-overlap length and those with full-overlap length of the filaments were subject to the X-ray experiments.

Experimental

The time-resolved X-ray diffraction experiments were performed at BL15A1 using a CCD-based TV detector. The intensity changes of the meridional reflections were measured at a time-resolution of 15ms at 10 °C. Living frog sartorius and semitendinosus muscles were stimulated electrically for 1.3s under isometric conditions. The sarcomere length of muscles was measured with diffraction of laser light and it was ca. 2.5μm in the full-overlapped muscles and ca. 4.0μm in the non-overlapped muscles. X-ray measurements were repeated 10 - 15 times for each muscle at resting intervals of 90s. The integrated intensities of troponin-associated reflections were calculated in the radial range of $0 \leq R \leq 0.007 nm^{-1}$ by integration of data.

Results and Discussion

Figure 1 shows the intensity changes of the troponin-associated meridional reflections at the full- and non-overlap lengths of muscles. At the full-overlap length, the first order reflection (Tn1) at $1/38.5 nm^{-1}$ increased by ca. 30 % in intensity just after the onset of stimulation (first phase) and then decreased to the level of 40% below the rest value (second phase). The intensity of the second order reflection (Tn2) showed no distinct change in the first phase but decreased in the second phase similarly as the Tn1 did. The third order reflection (Tn3) also showed no intensity change in the first phase, but its intensity increased in the second phase. The latter change of the Tn3 occurred faster than those of the Tn1 or Tn2. At the non-overlap length, the intensity of the Tn1 increased by about 40% immediately after the onset of stimulation and kept its intensity until the cease of stimulation. This intensity increase corresponded mostly to the amount of that first phase change of full-overlapped muscles. The second phase intensity change seems to be caused by the interaction of myosin and actin. Thus the intensity change at the non-overlap length of muscle reflected the first phase change at the full-overlap length.

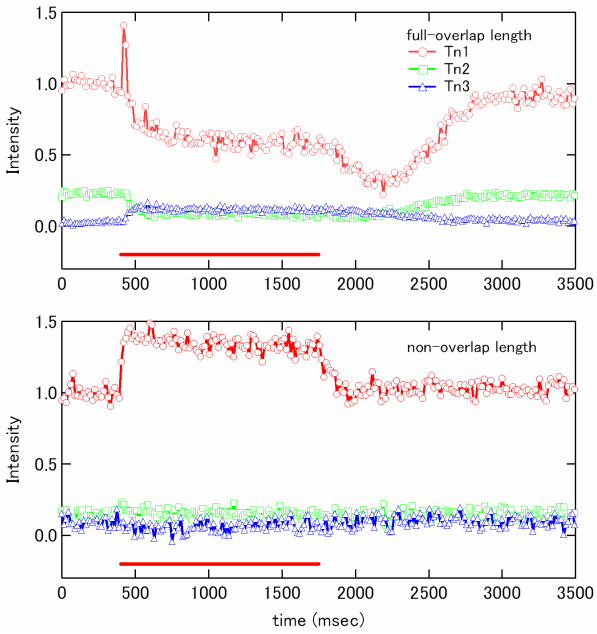

Figure 1. Intensity changes of the first (Tn1), second (Tn2) and third (Tn3) order troponin-associated meridional reflections during activation of frog skeletal muscle at the full- and non-overlap lengths. The horizontal bar shows the duration of the electrical stimulation applied. The intensity of each reflection was normalized to the rest value of the Tn1.

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