TIME-RESOLVED X-RAY STUDY OF EFFECT OF SINUSOIDAL LENGTH CHANGE ON TETANIZED FROG MUSCLE

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ABSTRACT Time-resolved x-ray diffraction studies were done on frog skeletal muscles with synchrotron radiation by applying sinusoidal length changes of frequency 10 Hz and amplitude ~1% to isometrically contracting muscles at ~17°C. Distinct periodic intensity changes were observed in the 14.3-nm myosin meridional reflection and the equatorial 1,0 and 1,1 reflections. Response of the 14.3-nm reflection to the sinusoidal length change was nonlinear, as evidenced by a large second harmonic in its oscillatory intensity change, whereas the response of the equatorial 1,1 reflection was closely linear, as evidenced by almost sinusoidal intensity change. Intensity change of the 1,0 reflection was nearly antiphase to that of the 1,1 reflection. Integral widths of the 14.3-nm meridional reflection measured along the meridian and of the equatorial 1,1 reflection remained almost constant during tension development, while that of the 1,0 reflection tended to decrease. The widths of the 14.3-nm meridional reflection perpendicular to the meridian and of the equatorial 1,0 reflection appeared to undergo oscillatory changes in response to the sinusoidal length changes.

INTRODUCTION

Changes in the x-ray diffraction pattern from striated muscle give information about the structural changes during contraction. The use of intense x rays in the synchrotron radiation made it possible to study dynamic structural changes in contracting muscle with a high time resolution as reviewed by Huxley and Faruqi (1983). With this technique, Huxley et al. (1981, 1983) investigated the behavior of the cross-bridges, which are responsible for muscle contraction, by recording the intensity changes of the x-ray reflections from contracting muscles in response to rapid step changes in muscle length. To obtain more detailed information about the dynamic properties of the cross-bridges, we applied sinusoidal length changes to contracting frog skeletal muscles, and found distinct and characteristic intensity changes in both the myosin meridional and the equatorial reflections.

MATERIALS AND METHODS

The sartorius muscle of the bullfrog (*Rana catesbeiana*) was mounted in an experimental chamber with a multi-electrode assembly; the pelvic end was tied to a strain gauge (type UT; Shinkoh Co., Tokyo) while the tibial end was connected to a servo-motor (type 200; Ling Dynamic Systems Ltd., Roxston Herts, England). Muscles were continuously perfused with oxygenated Ringer's solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, pH adjusted to 7.2 with $NaHCO_3$) at ~17°C. The sarcomere length of muscle was adjusted to ~2.4 μm by optical diffraction with He-Ne laser light. The muscle was tetanized isometrically with a 0.7-s train of 3-ms supramaximal current pulses at 30 Hz. Each muscle was tetanized 10 times at intervals of 15 s. We examined the effect of length of the rest time on tension and diffraction patterns and found, for good specimens, no appreciable differences for 15 s, 30 s, and 30 min. Fatigue of muscles during measurement was small, as will be seen later in this paper by the results of two tension curves corresponding to the first and the tenth experiments recorded in Fig. 1 *a*. When the steady tetanic tension was reached, sinusoidal length changes (peak-to-peak amplitude, ~1% of the initial muscle length; frequency 10 Hz) were applied with a servo-motor, the length changes were sensed by a photodiode detector, and controlled with a feedback circuit (Kobayashi and Sugi, 1982). The frequency of 10 Hz was chosen because the memory capacity of data collection and time resolution was limited and because, the results according to Kawai and Brandt (1980), the length-to-tension phase shift is minimal around this frequency. Tension and length changes were recorded with a storage oscilloscope (model 5110; Tektronixs Inc., Beaverton, OR). The storage ring operated at 2.5 GeV and 65 ~105 mA at the Photon Factory (Tsukuba, Japan) was used as a source of high intensity x rays of 0.15 nm. The diffractometer constructed by Amemiya et al. (1983) was used, with a mirror-monochromator focusing optics (for recent performance, see Wakabayashi et al., 1983). Data were taken by a one-dimensional position-sensitive detector of delay-line type (Rigaku Denki Co., Tokyo), and stored as a function of time in a CAMAC memory (3588; LeCroy Research Systems S. A., Geneve, Suisse) linked to a computer (Micro-11/F23; Automation System Research, Tokyo). The memory was divided into 60 time-frames of 256 channels with 10-ms durations. Among many sets of data, we adopted those for which the decay of tension during the plateau phase of tetanus was small. Intensity data presented below are averages of approximately 10 sets of such data from different muscles.

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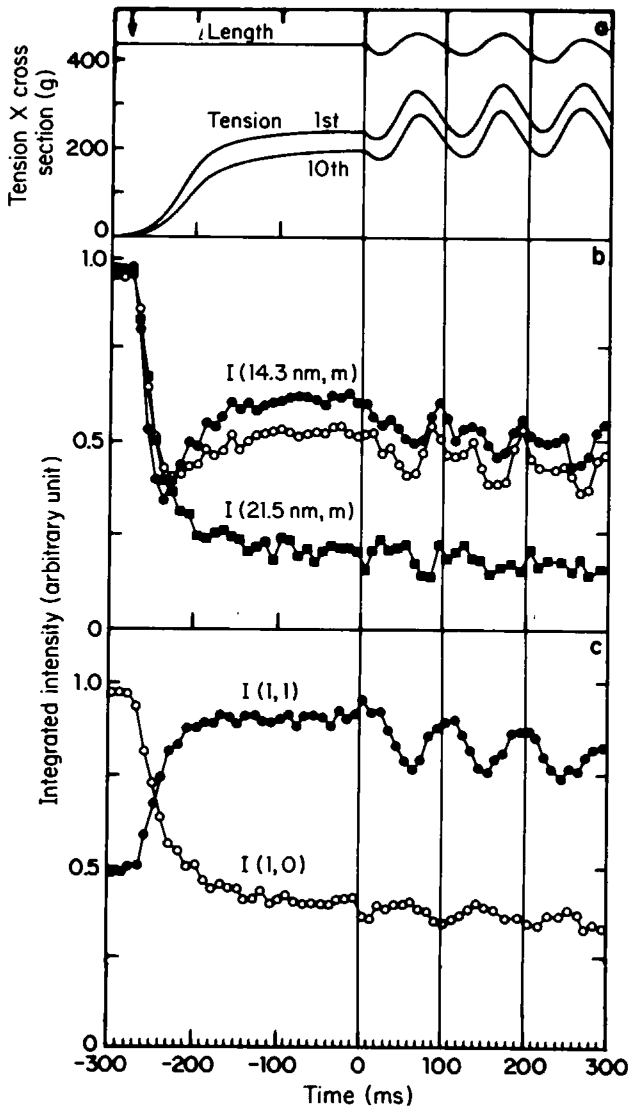

FIGURE 1 Effect of sinusoidal length changes on the intensities of the myosin meridional and the equatorial reflections from tetanized frog sartorius muscle at ~17°C. (*a*) Changes of muscle length and tension. Two curves of tension correspond to the first and the tenth experiments. At the tenth tetanus, the plateau tension decreased by 17% relative to the value of the first tetanus. Data of tension decrease >20% were not adopted. Arrow indicates the onset of stimulation ($t = -275$ ms). (*b*) Changes in integrated intensity of the 14.3-nm meridional reflections measured perpendicular (○) and parallel (●) to the meridian and of the 21.5-nm meridional reflection (■) measured parallel to the meridian. The muscle is mounted vertically. For the intensity measurement of these reflections perpendicular to the meridian, a lead mask with an aperture 3.5 mm high by 170 mm long was placed at the position of this layer line. For the measurement parallel to the meridian, a lead mask with an aperture 198 mm long by 8 mm wide was placed in front of the detector. (*c*) Changes in integrated intensity of the equatorial 1,0 (○) and 1,1 (●) reflections. The muscle is mounted horizontally. For the intensity measurements, a lead mask with an aperture 100 mm long by 8 mm wide was placed in front of the detector. In *b* and *c*, the maximum values of intensity data for each reflection are normalized as ~1.

RESULTS AND DISCUSSION

Fig. 1 shows the time course of the length and tension changes of the muscle (*a*), the changes in integrated intensity of the 14.3- and 21.5-nm myosin meridional reflection (*b*), and of the equatorial 1,0 and 1,1 reflections (*c*). Intensities of these reflections are denoted as I(14.3 nm, m), I(21.5 nm, m), I(1,0), and I(1,1), respectively. As seen in Fig. 1 *a*, there was no appreciable phase shift between the length and tension changes. I(14.3 nm, m) first decreased on stimulation and then increased to reach a steady level during the plateau of a tetanus, as reported by Huxley et al. (1982). When the sinusoidal length change was applied to the tetanized muscle, I(14.3 nm, m) exhibited periodic changes consisting of higher and lower peaks and two troughs within each cycle of the applied length changes. Similar results were obtained with the detector set either parallel or perpendicular to the meridian (Fig. 1 *b*). The higher peak was seen around the midpoint from the maximum to the minimum tension, while the lower peak was seen around the midpoint from the minimum to the maximum tension. Meanwhile, the larger and the smaller troughs were observed around the maximum and the minimum tension, respectively. The fact that the intensity decrease was greater in the stretch phase than in the release phase might be connected to the observation of Yagi and Matsubara (1984) and Matsubara and Yagi (1985), that slow stretch causes a greater decrease in the intensity of this reflection than slow release at low speeds. I(21.5 nm, m) decreased during the tension development as reported by Huxley et al. (1982). Its change in response to the length change could not be observed with good signal-to-background ratio. No appreciable changes were observed in I(14.3 nm, m) and I(21.5 nm, m) when the sinusoidal length change of 1% was applied to muscles in the rigor and the relaxed states, respectively.

I(1,0) decreased while I(1,1) increased on stimulation by a factor for each of about 2, and both reached steady levels during the plateau of an isometric tetanus, as reported by Haselgrove and Huxley (1973). Subsequent application of sinusoidal length changes produced distinct oscillatory changes in both I(1,0) and I(1,1) (Fig. 1 *c*). Approximately, I(1,1) was maximum when the tension was minimum, and was minimum when the tension was maximum. The change of I(1,0) was nearly in antiphase with that of I(1,1). These changes were much more marked than those taking place in response to slow length changes (Sugi et al., 1977; Yagi and Matsubara, 1977) or quick length changes (Amemiya et al., 1980; Huxley et al., 1981, 1983). These oscillatory changes of I(1,0) and I(1,1) do not seem to be caused by the filament translation, since such effects are very small as shown by Sugi et al. (1978) for isometric contraction at different muscle lengths and by Podolsky et al. (1976) and Tanaka et al. (1984) for isotonic shortening during tetanus.

Fig. 2 shows the changes of integral widths of the

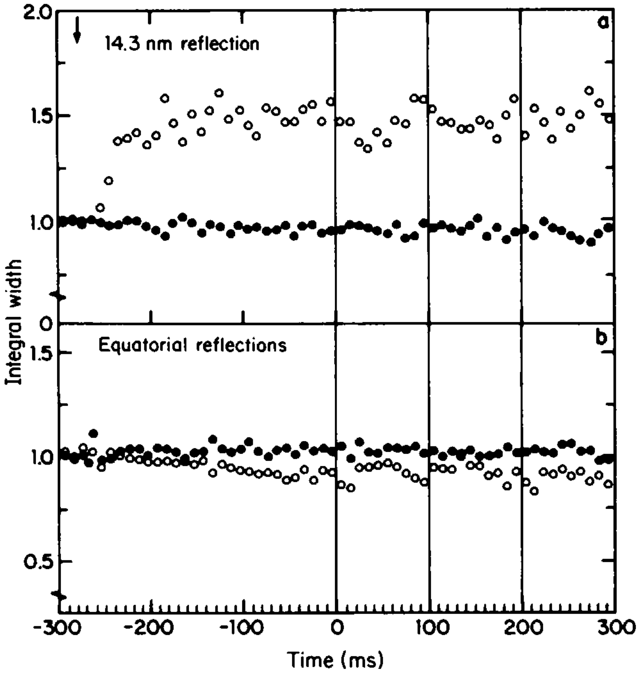

FIGURE 2 Changes in integral widths of the diffraction peaks. (*a*) The widths of the 14.3-nm meridional reflection measured perpendicular (○) and parallel (●) to the meridian. (*b*) The widths of the equatorial 1,0 (○) and 1,1 (●) reflections. In *a* and *b*, average values of the integral widths in the resting phase are normalized as 1 for each reflection.

14.3-nm meridional reflection (*a*) and of the equatorial 1,0 and 1,1 reflections (*b*). As reported by Huxley et al. (1982, 1983), the width of the 14.3-nm meridional reflection measured parallel to the meridian remained nearly constant during the course of the experiments, while that measured perpendicular to the meridian increased during the isometric tension development and appeared to undergo oscillatory changes in response to the length changes. The integral widths of the equatorial 1,0 and 1,1 reflections did not change appreciably during the course of the experiments, although there was a tendency for the width of the 1,0 reflection to decrease during the tension development and to oscillate during the length change.

For the data shown in Figs. 1 and 2, the sinusoidal length change started from the release phase at $t = 0$ (Fig. 1 *a*), so that the change was proportional to $-\sin 2\pi\,(t/T)$, where t is time and $T = 100$ ms. Experiments were done also for the length change starting from the stretch phase so that its change was proportional to $\sin 2\pi\,(t/T) = -\sin 2\pi\,\{(t/T) - 0.5\}$. All intensity changes observed for this length change were roughly expressed by shifting the scale of abscissa by $0.5\,T = 50$ ms in Fig. 1.

Fig. 1 *a* suggests that there was no appreciable phase shift between the length and tension changes. We checked it further by Lissajous figures and observed almost straight lines, which agrees with the results reported by Kawai and Brandt (1980) for frog semitendinosus muscle.

Fourier series analysis was made for the time courses of I(14.3 nm, m) measured by the detector setting perpendicular to the meridian and of I(1,1), which were determined with fairly high signal-to-background ratio. The analysis proved that, referring to the length change proportional to $-\sin 2\pi(t/T)$, approximately $\mathrm{I}(14.3\ \mathrm{nm, m}) \propto -[0.56 \sin 2\pi\{(t/T) - 0.30\} + 0.44 \sin 4\pi\{(t/T) - 0.01\}]$ and $\mathrm{I}(1,1) \propto -[0.95 \sin 2\pi\{(t/T) - 0.31\} + 0.02 \sin 4\pi\{(t/T) - 0.39\}]$. Here two points are noticeable. (*a*) The second harmonic is large in I(14.3 nm, m), whereas higher harmonics are small in I(1,1). (*b*) The phase shifts in the fundamental frequency components are similar for I(14.3 nm, m) (0.30) and I(1,1) (0.31). The Fourier series analyses were made for several sets of intensity data other than those shown in Fig. 1 and for "corrected" I(14.3 nm, m), which was I(14.3 nm, m) multiplied by the integral width measured perpendicularly to the meridian (cf. Fig. 17 of Huxley et al. [1982]). Results obtained were similar concerning the above two points.

Huxley et al. (1983) observed that I(14.3 nm, m) decreased both for quick release and rapid stretch of the muscle length during tetanus. Yagi and Matsubara (1984) observed that it also decreased for both slow release and stretch. This even-functional character of I(14.3 nm, m) seems to be the origin of the large second harmonic in its oscillatory change. Huxley et al. (1983) reported that I(1,1) remained almost constant for quick release and stretch. Presumably, the fact that there was almost no even-functional response in the equatorial reflections is in close relation with our result that I(1,1) has a very small second harmonic in response to the sinusoidal length change. As seen in Fig. 1 *c*, the oscillatory changes of I(1,0) and I(1,1) are evident. In this connection it might be worthwhile to mention that Yagi and Matsubara (1977) reported that there was no change in intensity of these equatorial reflections during slow stretch and release, but recently Tanaka et al. (1984) have shown that I(1,1) from a tetanized muscle decreases during the tension rise by slow stretch.

Finally, it is tempting to speculate about the behavior of cross-bridges during the sinusoidal length changes on the basis of the present results, especially large second harmonic in the intensity change of the 14.3-nm meridional reflection. A possible explanation for this may be that during the applied sinusoidal length changes the distal portion of the cross-bridge (attached to actin) moves to and fro, while its proximal portion remains fixed in position. Another possibility may be that, if one has the two-headed nature of myosin molecule in mind, one head firmly attached to actin remains fixed in position, while the other head less firmly attached to actin moves to and fro around the former. Much more experimental works are needed to clarify the actual behavior of the cross-bridges responsible for muscle contraction.

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WAKABAYASHI ET AL. *Sinusoidal Length Change on Tetanized Frog Muscle* 583

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