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Sarcomere length and tension changes in tetanized frog muscle fibers after quick stretches and releases

(cross-bridge/laser light diffraction/series elasticity/isometric tension transients/mechanical wave propagation)

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ABSTRACT **The sarcomere length changes in tetanized frog muscle fibers in response to quick fiber length changes were examined along the fiber length with a high-sensitivity laser diffraction technique. The experiments were only performed with muscle fibers in which the uniform orientation and sarcomere length of the component myofibrils were well preserved during a tetanus. When the sarcomere length changes were recorded near the fixed fiber end, the delay of the onset of sarcomere length change in response to the applied fiber length change tended to be longer than that of the onset of tension changes recorded at the fixed fiber end. The magnitude of sarcomere length changes was larger near the moving fiber end than near the fixed fiber end. In the case of quick releases, the resulting sarcomere shortening tended to outlast the fiber shortening, so that the quick tension recovery started during the sarcomere shortening. These results indicate (*i*) that the tension changes in response to quick fiber length changes may not give direct information about the cross-bridge properties and (*ii*) that the viscoelastic multisegmental nature of muscle fibers should be taken into consideration in interpreting the tension responses to quick length changes.**

It is generally believed that contraction in striated muscle results from the alternate formation and breaking of crosslinks between the projections on the thick filaments—i.e., the cross-bridges—and the sites on the thin filaments (1, 2). In the contraction model of Huxley and Simmons (3), which has been central in the field of muscle mechanics, each cross-bridge consists of a myosin head and an elastic link extending from the thick filament. This model is based on the experiments, in which quick length changes are applied to isometrically contracting fibers, and the resulting tension changes (isometric tension transient) are examined; the initial tension change coincident with the length change is largely attributed to the cross-bridge elasticity, whereas the subsequent quick tension recovery is explained as being due to the myosin head rotation (3, 4).

Because a muscle fiber is composed of thousands of sarcomeres connected in series, the above interpretation rests on the assumption that the cross-bridges in every sarcomere respond in a similar manner to quick length changes applied at one fiber end. With an ultrahigh-speed cinecamera, Sugi and Tameyasu (5) studied segmental length changes of a tetanized frog muscle fiber in response to a quick release and observed that the resulting fiber shortening was localized near the released fiber end, indicating the nonuniform sarcomere responses along the fiber length. The accuracy of the segmental length measurement (about 1%) was, however, not good enough to exclude the possibility that a quick small sarcomere shortening (less than 1%) took place uniformly along the entire fiber length before the marked segmental shortening localized near the released fiber end became obvious. The present experiments were undertaken to examine the nonuniformity of the sarcomere length changes in response to quick fiber length changes along the fiber length by recording local sarcomere length changes by optical diffraction of He/Ne laser light with a high sensitivity and a high time resolution.

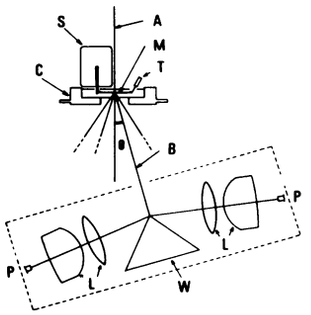

FIG. 1. Diagram of experimental arrangement. The muscle fiber (M) was mounted horizontally in an experimental chamber (C) between the servomotor (S) and the tension transducer (T) and was stimulated tetanically with a multielectrode assembly (not shown). Small sarcomere length changes were recorded as a small shift of the first-order diffraction line of He/Ne laser beam (A) by splitting the beam of the diffraction line (B) with a wedge-shaped mirror (M) into two parts, each of which was focused on a photodiode (P) through the lens system (L). For small sarcomere length changes, the diode voltage difference signal was a linear function of the angle (θ) between the incident and the first-order diffraction beams.

MATERIALS AND METHODS

The experimental arrangement is shown in Fig. 1. Single fast muscle fibers (50–150 μm in diameter) isolated from the semitendinosus or the tibialis anterior muscles of the frog (*Rana japonica*) were mounted horizontally in an acrylic plastic chamber (C in Fig. 1) by tying the tendons to the shaft of a servomotor (S in Fig. 1; General Scanning, G-100-PD) and a tension transducer (T in Fig. 1; Aksjeselskapet, AME-80; natural frequency of oscillation, 5 kHz; compliance, 4 μm/g), respectively, with braided silk thread. Precooled Ringer solution (115 mM NaCl/2.5 mM KCl/1.8 mM $CaCl_2$, pH adjusted to 7.2 by $NaHCO_3$) was constantly circulated through the chamber at a rate of 20 ml/min with a peristaltic pump, the temperature of the solution (2–4°C) being controlled with a thermoelectric device (Yamato, Coolnix) with an accuracy of ±0.1°C. The fiber was held at the slack length (L_o) and tetanized isometrically for 1 sec with supramaximal 1-msec rectangular current pulses at 10–20 Hz given through a multielectrode assembly. When the full iso-

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Abbreviations: L_o, fiber slack length; ΔL, fiber length change; ΔS, sarcomere length change; V_d, diode voltage difference signal.

metric tension was developed, the fiber length was changed by up to 1% of L_o with variable velocities with the servomotor system, the fiber length changes being sensed by a differential capacitor (demodulation frequency, 2 MHz) incorporated in the servomotor system.

The sarcomere length was measured by optical diffraction with a He/Ne laser beam (A in Fig. 1, 0.3 mm in diameter) directed vertically on the muscle fiber (M in Fig. 1), the diffraction pattern being projected on a horizontal plane at 25 cm from the fiber (not shown). The sarcomere length changes were recorded by the method of Haugen and Sten-Knudsen (6)—i.e., by sensing the position changes of the first-order diffraction line with a wedge-shaped mirror (W in Fig. 1; custom-made by Nikon), which was located at 6.5 cm from the fiber and split the beam of the first-order diffraction line (B in Fig. 1) into two parts. Each part of the split beam was focused through a lens system (L in Fig. 1) to either of two photodiodes (P in Fig. 1; Hamamatsu TV, S-1188-01). The position of the mirror was initially adjusted in such a way that the mirror split the beam into two halves of equal intensities; in this condition, there was no difference in the output voltage signal between the two photodiodes. A small shift of the first-order diffraction line because of a small sarcomere length change (ΔS) produced a difference in the voltage signal between the photodiodes, the diode voltage-difference signal (V_d) being a linear function of the angle between the incident and the first-order diffraction beams (θ) for small values of ΔS. When a series of stretch–release cycles or sinusoidal length changes (20 Hz) of small amplitudes were applied to resting muscle fibers, there was a linear relationship between the fiber length change (ΔL) and V_d (Fig. 2 *Inset*). Thus, the relationship between ΔS and V_d was obtained by the equation: $\Delta S = S\,\Delta L/L$, where S and L are sarcomere length and fiber length, respectively. The ΔS versus V_d relationship was linear within the range of ΔS up to 300 Å (Fig. 2), indicating that V_d can be used as a measure of ΔS up to 300 Å. To compensate for changes in the diffraction line intensity, V_d was divided by the sum of the two diode signals with a fast analog divider (6). With the above system, sarcomere length changes down to less than 1 Å could be recorded with a time resolution of less than 10 μsec, though the actual recording of sarcomere length changes was made with a sensitivity of 100–150 Å/cm.

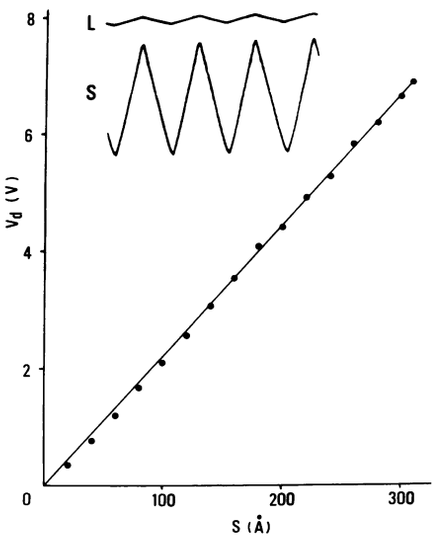

FIG. 2. Relationship between the sarcomere length change (ΔS) and the diode voltage-difference signal (V_d) in resting muscle fibers. (*Inset*) Example of V_d changes in response to a series of stretch–release cycles (ΔL) applied to a resting muscle fiber.

The sarcomere length of resting muscle fibers at L_o was 2.1–2.2 μm; during an isometric tetanus, it shortened to some extent because of internal shortening—i.e., the shortening of the fibers against tendons and the experimental apparatus. Because the internal shortening was reproducible, it was possible to previously adjust the mirror position so that, after the completion of the initial sarcomere shortening during isometric tension development, the beam of the first-order diffraction line was split by the mirror into two halves of almost equal intensities at the plateau of an isometric tetanus.

The changes in fiber length, sarcomere length, and tension were simultaneously displayed on an oscilloscope (Tektronix, 5113) and photographed with a 35-mm camera for analysis.

RESULTS

When small sinusoidal fiber length changes (1–5 kHz) were applied at the plateau of an isometric tetanus, the resulting sarcomere length-change signals in many fibers did not follow the time course of the fiber length changes but showed various complex patterns (Fig. 3*A*). This may result from the fact that, in many tetanized muscle fibers, there are different myofibrillar planes of slightly different sarcomere lengths and different orientations with respect to the incident beam; during the applied fiber length changes, different myofibrillar planes would happen to fulfill the Bragg condition to produce the complex sarcomere length signals (7).

In the present study, however, we could obtain muscle fibers in which the sarcomere length signals showed a typical sinusoidal pattern in response to the applied sinusoidal fiber length changes (Fig. 3*B*); the ΔL versus ΔS relationship was linear over the range of ΔS up to 180 Å, irrespective of the region illuminated by the laser beam, indicating that in these fibers the uniform orientation and sarcomere length of the component myofibrils were well preserved during a tetanus. Such "good quality" fibers could be obtained in one out of three to five fibers, and further experiments were only performed with these fibers; consistent results were obtained on 15 tibialis anterior and 20 semitendinous muscle fibers used.

Sarcomere Length and Tension Changes in Response to Fiber Length Changes at Various Velocities. Fig. 4 shows typical changes in fiber length, sarcomere length, and tension when a tetanized fiber was stretched by about 0.7% of L_o at four different velocities, the sarcomere length changes being recorded at the middle of the fiber. In all of the stretch velocities examined, the time course of sarcomere lengthening was almost similar to that of the fiber lengthening, whereas the tension rose to a peak during the fiber lengthening and started to decay

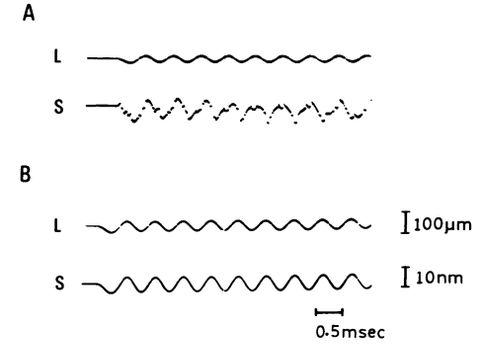

FIG. 3. Examples of sarcomere length-change signals (ΔS) in tetanized muscle fibers in response to sinusoidal fiber length changes (ΔL). Note that the sarcomere length-change signals are complex in *A* and typically sinusoidal in *B*.

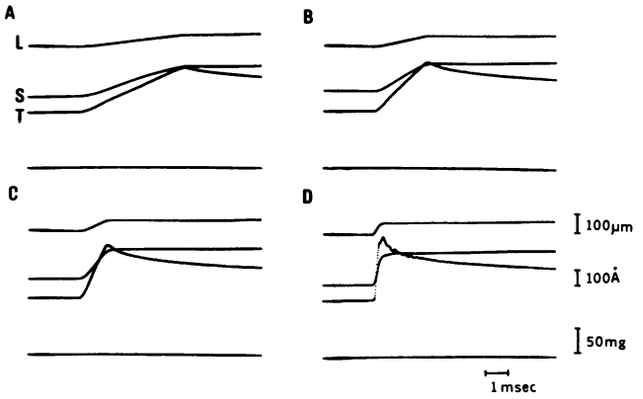

FIG. 4. The changes in fiber length (L), sarcomere length (S), and tension (T) when a tetanized muscle fiber (L_o, 0.7 cm) was stretched by about 0.7% at various velocities. The sarcomere length changes were recorded at the middle of the fiber. The bottom trace shows the zero-tension baseline. The stretch velocity was 1.5 L_o/sec (A), 3 L_o/sec (B), 7 L_o/sec (C), and 20 L_o/sec (D). In this and subsequent figures, oscillations on the tension trace are due to resonance of the tension transducer.

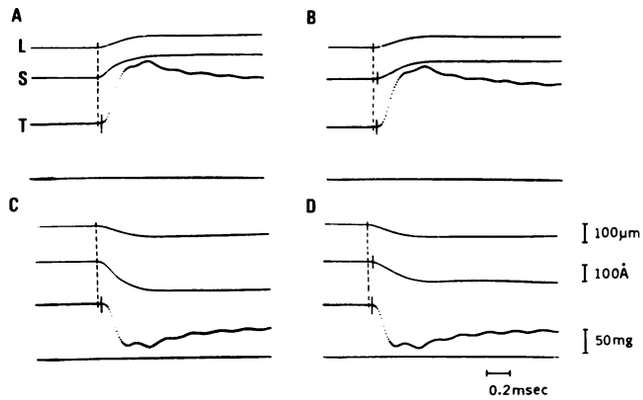

FIG. 6. The changes in fiber length (L), sarcomere length (S), and tension (T) when a tetanized muscle fiber (L_o, 0.7 cm) was stretched (A and B) or released (C and D) quickly by about 0.7% at 20 L_o/sec. The sarcomere length changes were recorded near the moving fiber end in A and C and near the fixed fiber end in B and D. The vertical broken line indicates the time of onset of fiber length change, whereas the onset of sarcomere length change and that of tension change are indicated by short vertical lines on each trace.

at the completion of the fiber lengthening. The increase in tension above the initial isometric level for a given amount of stretch was larger, the larger the stretch velocity (8).

Fig. 5 shows typical changes in fiber length, sarcomere length, and tension when a tetanized fiber was released by about 0.7% at four different velocities, the sarcomere length changes being recorded also at the middle of the fiber. With slow release velocities (Fig. 5 A and B), the sarcomere shortening took place almost in parallel with the fiber shortening, while the tension continued to fall during the fiber shortening and started to rise at the completion of the fiber shortening. With faster release velocities, on the other hand, the sarcomere shortening tended to outlast the fiber shortening and was followed by small oscillatory changes (Fig. 5 C and D), whereas the tension started to rise at the completion of the fiber shortening. This phenomenon will be described later in more detail. The fall in tension below the initial isometric level for a given amount of release was larger, the larger the release velocity.

Propagation of Sarcomere Length and Tension Changes Along the Length of Tetanized Muscle Fibers. In order to study the propagation of sarcomere length and tension changes along the length of tetanized muscle fibers, the sarcomere length changes in response to quick fiber length changes (up to 1% of L_o, complete in 0.2–0.4 msec) were recorded at various regions along the fiber length. Typical examples of the records obtained from the tibialis anterior muscle fibers (L_o, 0.5–0.7 cm) are shown in Fig. 6. The tension changes in response to quick fiber length changes consisted of the initial rapid tension change and the subsequent quick tension recovery as has been reported by Huxley and Simmons (3, 4).* When the sarcomere length changes were recorded near the moving fiber end, both the fiber and the sarcomere length started to change simultaneously, while the tension began to change with a definite delay (Fig. 6 A and C). The delay of the tension change in response to the applied fiber length change is due to the time required for the propagation of the mechanical wave, which starts at the moving fiber end and propagates along the fiber length to be sensed with the tension transducer at the fixed fiber end, the propagation velocity being estimated to be about 180 m/sec (10).

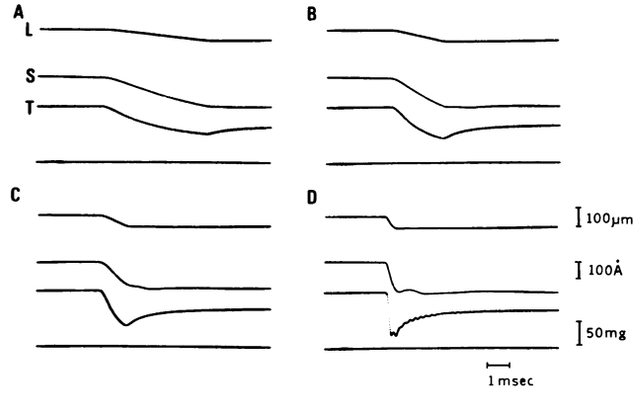

FIG. 5. The changes in fiber length (L), sarcomere length (S), and tension (T) when a tetanized muscle fiber (L_o, 0.7 cm) was released by about 0.7% at various velocities. The sarcomere length changes were recorded at the middle of the fiber. The release velocity was 1.5 L_o/sec (A), 3 L_o/sec (B), 7 L_o/sec (C), and 20 L_o/sec (D).

When the sarcomere length changes were recorded near the fixed fiber end, the sarcomere length started to change with a definite delay in response to the applied fiber length changes (Fig. 6 B and D); the delay of the onset of sarcomere length changes was as long as, or appeared to be longer than, that of the onset of tension changes. In the case of the semitendinous muscle fibers (L_o, 0.9–1.2 cm), the sarcomere length changes near the fixed fiber end always started definitely after the onset of the tension changes as shown in Fig. 7. These results indicate that the longitudinal propagation of the mechanical wave producing the onset of tension changes is not identical with that of the sarcomere length changes; the former takes place faster than the latter. In both stretches and releases, the magnitude of sarcomere length changes was 20–40% larger near the moving fiber end than near the fixed fiber end (Fig. 6), indicating the decremental propagation of sarcomere length changes along the fiber.

In the case of quick releases, the resulting sarcomere shortening tended to outlast the fiber shortening especially near the fixed fiber end; as a result, the rapid tension recovery tended

* According to Ford *et al.* (9), the tension responses to quick fiber length changes are essentially the same irrespective of whether the releases are applied to the fiber with the feed-back from the spot follower signal or without the feed-back as in the present study (9).

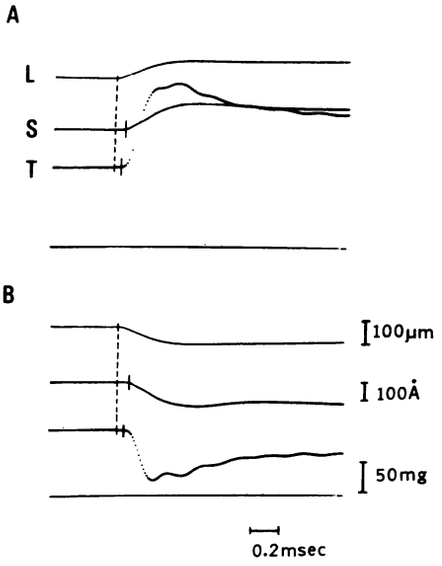

FIG. 7. The changes in fiber length (L), sarcomere length (S), and tension (T) when a tetanized muscle fiber (L_o, 0.9 cm) was stretched (A) or released (B) quickly by about 0.7% at 22 L_o/sec. The sarcomere length changes were recorded near the fixed fiber end. Note that the delay of the onset of sarcomere length changes is definitely longer than that of tension changes in both A and B and that the quick tension recovery starts when the sarcomere shortening is only about 50% complete in B.

to start while the sarcomere shortening was still in progress (Figs. 5*D*, 6*D*, and 7*B*). The onset of the quick tension recovery during the sarcomere shortening was most clearly observed near the fixed fiber end of the semitendinous fibers; it can be seen in Fig. 7*B* that the initial rapid fall in tension is followed by the quick tension recovery starting when the initial sarcomere shortening is only about 50% complete.

DISCUSSION

In the present experiments, the sarcomere length changes in tetanized frog muscle fibers after quick fiber length changes have been studied in detail by using muscle fibers in which the uniform orientation and sarcomere length of the component myofibrils are well preserved during a tetanus (Fig. 3). The results showed that the sarcomere length changes are not uniform along the fiber length; both the magnitude and time course of sarcomere-length changes were different between the moving and fixed fiber ends, especially in the case of quick releases (Figs. 6 and 7). In addition, the onset of tension change recorded at the fixed fiber end tended to precede that of sarcomere-length change recorded near the fixed fiber end especially in long fibers (Figs. 6 and 7), indicating that the mechanical wave producing the onset of tension change can propagate along the fiber without any appreciable sarcomere length change, at least with the sensitivity of the measurement used. These findings may lead to a conclusion that the initial rapid tension change coincident with the applied stretch or release is a complex phenomenon arising from the multisegmental nature of the muscle fiber, but not simply results form the elastic extension or recoil of the cross-bridges in every sarcomere. Another interesting feature brought about by the present study is that, after a quick release, the resulting sarcomere shortening near the fixed fiber end outlasted the fiber shortening, so that the quick tension recovery started before the completion of sarcomere shortening (Figs. 5*D*, 6*D*, and 7). This also seems to be inconsistent with the idea that the quick tension recovery is due to the myosin head rotation taking place immediately after the completion of relative sliding between the thick and thin filaments (3, 4).

In an attempt to simulate the sarcomere length and tension changes observed in the present study, we recently have reached a viscoelastic multisegment model consisting of many segments connected in series. Each segment representing a sarcomere contains a viscous and an elastic element in parallel with the contractile component and another elastic element in series with the contractile component. The contractile component is based on a two-state model described elsewhere (11). By giving appropriate values for the viscous and elastic elements, all the sarcomere length and tension responses to quick stretches and releases, including the onset of the quick tension recovery before the completion of sarcomere shortening (Fig. 7), can well be simulated (unpublished data). For the successful computer simulation, the presence of the series elastic component in each sarcomere is essential, being consistent with the finding that both the thick and thin filaments have appreciable compliance (12). In conclusion, we emphasize that the multisegmental nature of muscle fibers should be taken into consideration in interpreting the transient mechanical responses to quick length changes.

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