Myosin Heads Do Not Move on Activation in Highly Stretched Vertebrate Striated Muscle

Abstract. *Highly stretched muscles in which thick and thin filaments no longer overlap produce little or no tension on activation. In preparations producing no tension, the 42.9-nanometer myosin layer line shown in x-ray diffraction patterns does not become weaker. This suggests that myosin heads do not move from their resting positions in the absence of actin.*

Myosin heads, which are arranged in a helical manner along each thick filament in resting muscle, move out of their helical positions as the muscle contracts (*1*). There has been a controversy as to whether such movement of the heads from their resting positions requires the presence of actin in their vicinity. Experiments in which a fluorescent dye is attached to the heads (*2*) have suggested that the movement does require actin, since there is no sign of movement either in muscle fibers with no overlap between thick and thin filaments or in isolated synthetic myosin filaments. On the other hand, an x-ray diffraction of muscle (*3*) [in which the movement is detected by a decrease in the intensity of myosin layer lines (*4*)] suggested that the movement may not require actin; the decrease in the layer-line intensity on activation did not change significantly when the extent of overlap between thick and thin filaments was approximately halved by stretching the sarcomeres. This has been interpreted as indicating that an increase in sarcoplasmic calcium concentration may directly cause the movement. However, the interpretation is not conclusive, since the heads in the nonoverlap region may have moved cooperatively with the other heads that moved in the presence of actin in the overlap region (*5*).

An attempt to resolve the controversy was made by x-ray diffraction of muscles stretched beyond the sarcomere length of 3.6 μm to eliminate overlap (*5*). The layer-line intensity of these muscles decreased on activation, indicating movement of the heads. However, this still did not show conclusively that movement occurs in the absence of actin, since some overlap may remain even in overstretched muscles (*5*); indeed, some overlap is suggested by the fact that stretched muscles often produce tension. A large part of this tension is believed to be produced by shorter sarcomeres near the ends of the muscles, but highly stretched sarcomeres in the middle could also produce tension if there were longitudinal dislocation of some thick filaments in the A band or if thick filaments longer than normal were present (*6*). In the present study, we measured the change in the layer-line intensity of overstretched muscles as a function of tension production and found that the intensity remains constant when stimuli do not cause tension. This strongly suggests that the movement of heads out of the helical positions does require the presence of actin.

Overstretched bullfrog muscles with sarcomere lengths of 3.8 to 4.3 μm were prepared by the method described by Huxley (*5*) (see legend to Fig. 1). Each muscle was held isometrically in a specimen chamber by tying one end to a hook and connecting the other to a tension transducer. The chamber had two Mylar windows that allowed x-rays to pass through the middle of the muscle. Diluted Ringer solution at 4°C (see legend to Fig. 1) was continuously passed over the surface of the muscle. The muscle was stimulated tetanically with supramaximal electrical pulses (20 Hz) through a pair of platinum electrodes placed parallel to the muscle axis.

A bent-quartz monochromator (*7*) was used to focus x-rays to a vertical line 55 cm from the center of the quartz. The height of the beam (4 mm at the focus) was defined by a slit on each side of the monochromator. The muscle was set horizontally and the diffraction pattern was recorded on film placed at the focal position (specimen-to-film distance, 46 cm). A series of myosin layer lines was observed at orders of 42.9 nm. Using the recorded pattern as a guide, the film was replaced by a lead mask that passed only the off-meridional parts of the innermost layer lines and that had an aperture of 0.8 mm axially and 5 mm radially in each quadrant of the diffraction pattern. The intensity of the x-rays that passed through the apertures was measured with a scintillation counter connected to a multichannel analyzer; signals occurring at different times were registered in different channels. The multichannel analyzer and the stimuli to the muscle were triggered by the same clock so that, after accumulating signals from a certain number of contractions, the layer-line intensity during presentation of the stimuli could be obtained with reasonable counting statistics $\sqrt{N}/N < 0.005$, where N is the number of counts).

To test the sensitivity of our apparatus to changes in the layer-line intensity, we used muscle contracting at a length at which the thick and the thin filaments overlap each other. A muscle was held in Ringer solution (prepared as before) at a sarcomere length of 2.2 μm and was tetanized 40 times (1 second per tetanus). The layer-line intensity decreased

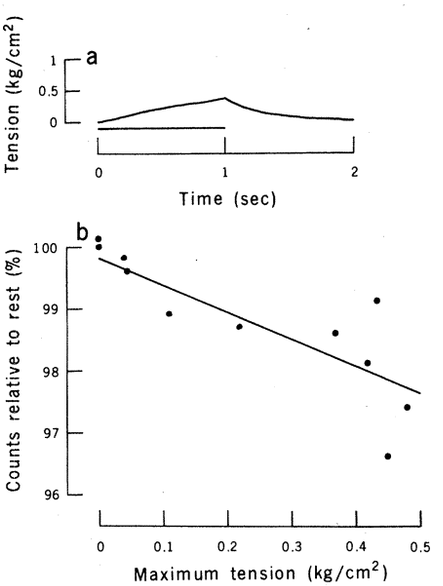

Fig. 1. Tension and the intensity of the 42.9-nm layer line during 1-second stimulation of overstretched muscles. The dorsal branch of a semitendinosus muscle was dissected and kept at the slack length for 24 hours in Ringer solution (4°C) of the following composition (millimolar): NaCl (115), KCl (2.5), $CaCl_2$ (1.8), Na_2HPO_4 (2.15), NaH_2PO_4 (0.85); *p*H 7.2. Then the muscle was transferred to Ringer solution (4°C) diluted to two-thirds the concentration of the first. Several hours after the transfer, the muscle was stretched so that the sarcomeres in the middle of the muscle became 3.8 to 4.3 μm long as measured by the light diffraction method. The stretched muscle was kept in the diluted Ringer solution for 12 hours before use. (a) Typical tension record during 1-second tetanic stimuli at nonoverlap length. The horizontal line represents the period of stimulation. The peak tension depended on how long the tension was allowed to rise. The cross section of the muscle was measured at the nonoverlap length in order to express the tension in grams per square centimeter. (b) Layer-line intensities during 1-second stimulation at nonoverlap lengths. Each muscle was tetanically stimulated 40 times at 2-minute intervals. Each point represents the result obtained from a single muscle. The ordinate indicates the average layer-line intensity during stimulation relative to the resting intensity at the nonoverlap tension during stimulation. The regression line for the points obtained from tension-producing preparations was $y = 4.34 \times 10^{-3}x + 99.8$ ($\pm$ 0.7). Although the data have been fitted by a straight line for simplicity, this is not meant to be a definitive description of the results (*10*).

0036-8075/80/0118-0307$00.50/0

on contraction and stayed at a low level throughout each tetanus (Fig. 2). The average intensity decrease was 12 percent of the resting intensity. After correction for the background intensity (*8*), the real decrease in the layer-line intensity was 67 percent, very similar to that found by Huxley and Brown (70 percent) (*1*). Therefore our technique was sensitive enough for the present purpose; the intensity change reported to occur at nonoverlap lengths is about half of that at normal overlap (*5*), and we would be able to detect such a change.

In overstretched muscles at rest, the intensity of the x-rays behind the mask was approximately 1200 count/sec. Most preparations produced tension. The tension, when it occurred, developed slowly and reached a maximum of 500 g/cm^2 at the end of the 1-second stimulus (Fig. 1). The size and the time course of tension in each preparation did not change appreciably over the 40 tetanic contractions required for recording the active layer-line intensity. The average intensity in each preparation during stimulation, relative to the resting value at the nonoverlap length, was plotted against the maximum tension (Fig. 1). In the two preparations that produce no tension, the layer-line intensity did not decrease (*9*). In the other preparations, the intensity seemed to be greater with a larger tension development (r = .77); extrapolation of the regression line for the points obtained from tension-producing preparations to zero tension gave an intensity very close to the resting value (see legend to Fig. 1) (*10*). Thus we can conclude that when there is no tension development in overstretched preparations there is no decrease in the layer-line intensity.

There are two possible mechanisms that would account for the intensity decrease accompanied by tension production at muscle lengths at which no overlap would be expected. First, the decrease may be due to a stretching of the middle part of the muscle caused by contraction of sarcomeres retaining some overlap near both ends of the muscle; such stretching would decrease the intensity, either by disordering the helical arrangement of myosin heads or by reducing the amount of x-ray scattering material in the beam that passes through the middle part of the muscle. This, however, is an unlikely possibility since an intensity decrease occurred in a previous study (*5*) in which the overstretched muscle was made to contract isotonically so that little stretch took place in the middle part. A second possibility is that the decrease may be due to some overlap in the middle part of the muscle (*5*). Such an overlap could be produced by a shift of some thick filaments toward one side of the sarcomere (*11*), and would decrease the layer-line intensity through movements of myosin heads on activation of the muscle; the overlap might also contribute to tension generation *12*). We prefer the second explanation, but cannot rule out the first possibility entirely.

Whatever the actual mechanism may be, our observation that no decrease in the intensity occurs at zero tension strongly suggests that the myosin heads require the presence of actin in their vicinity to move out of their helical positions on activation. This leads us to conclude that this displacement may be caused by actin-myosin interaction rather than directly by an increase in sarcoplasmic calcium.

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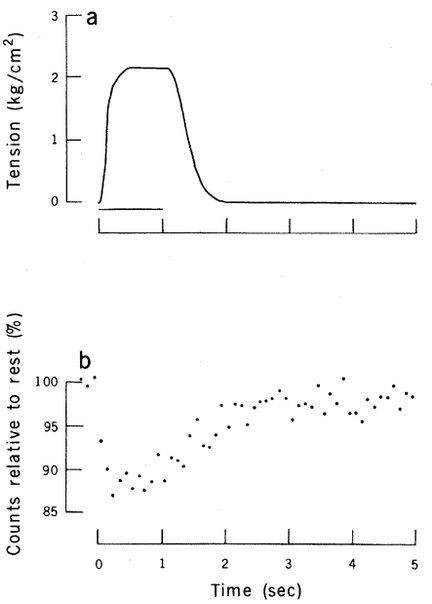

Fig. 2. Tension and the intensity of the 42.9-nm layer line during 1-second tetanus at the sarcomere length of 2.2 μm. (a) Tension record averaged over the 40 tetanic contractions required for obtaining the time course of the layer-line intensity. A sartorius muscle was dissected from *Rana catesbeiana* and tetanized for 1 second at 2-minute intervals. The horizontal line represents the period of stimulation. Tension was recorded with an isometric tension transducer (Shinkoh, type UL). (b) Intensity of the first-order myosin layer line at 42.9 nm. The x-ray source was a rotating-anode generator (Rigaku FR) with a fine focus (1.0 by 0.1 mm) on a copper target. This was operated at 50 kV with a tube current of 70 mA; such a high power was possible with an anode of a large diameter (30 cm) rotating at a high speed (9000 rev/min). A bent-crystal monochromator was used at a source-to-crystal distance of 25 cm with a viewing angle of 6°. The intensity of the myosin layer line was measured with a scintillation counter combined with a mask; the mask had apertures at the positions of the off-meridional parts of the first-order layer line. The meridional reflection at 14.3 nm is known to be slightly displaced during contraction, suggesting a minute change in the myosin periodicity (*1, 3*). It is, therefore, possible that the 42.9-nm layer line is also slightly displaced. However, the possible displacement (14 μm at the position of the mask) would be insignificant compared with the width of each aperture (0.8 mm). The intensity measured at the resting state was 1400 count/sec. The intensities during and after tetanus were expressed as percentages of the resting intensity and plotted against time after the first stimulus of each set of stimuli. Each point represents the intensity averaged over a 100-msec period. The first three points represent the measurements made before stimulation.

References and Notes

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4. The decrease in the layer-line intensity during contraction has been interpreted to be caused by movements of myosin heads out of the helical positions. An alternative interpretation, that the decrease is caused mainly by disorder in the longitudinal alignment of the thick filaments, was shown by Huxley and Brown (*1*) to be most unlikely.
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6. A. M. Gordon, A. F. Huxley, F. J. Julian, *J. Physiol. (London)* **184**, 143 (1966).
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8. Changes in the background intensity during contraction were studied in separate experiments by using a mask with narrower apertures (0.3 by 5.0 mm) on both sides of the 42.9-nm layer line. The background intensity thus measured did not change significantly during tetanic contractions.
9. It is unlikely that the activation mechanism was failing in these preparations, since, when they were allowed to shorten to a sarcomere length of 3.2 to 3.5 μm after the experiment, they produced a tension of 400 to 500 g/cm^2.
10. Since the layer-line intensity is supposed to be proportional to the square of the number of myosin heads in the helical positions (*3*) while the tension is linearly related to the number of activated heads, it may be more appropriate to plot the layer-line intensity against the square of the tension. The regression line in such a graph also gave an intensity close to the resting value at zero tension.
11. A shift of an entire A band to one side of a sarcomere can often be seen in overstretched muscles by electron microscopy (R. Natori, personal communication).
12. Various mechanisms can be proposed as to how the force produced by such an overlap would be transmitted to both ends of the muscle. For example, the force could be transmitted by a network of connectin stretching from one end of each muscle fiber to the other [K. Maruyama, R. Natori, Y. Nonomura, *Nature (London)* **262**, 58 (1976)], and might be attached to Z and M lines.
13. We thank M. Endo for encouragement and P. M. Bennett, Y. E. Goldman, G. W. Offer, and R. M. Simmons for helpful comments. Supported by the Toray Science Foundation and the Muscular Dystrophy Association of America.
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8 June 1979; revised 13 August 1979