CROSS-BRIDGE MOVEMENTS DURING A SLOW LENGTH CHANGE OF ACTIVE MUSCLE

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ABSTRACT Tension changes caused by slow stretch or release of actively contracting muscle are accompanied by axial displacements of myosin heads (i.e., cross-bridges) from the positions characteristic of isometric contraction. The direction of the axial displacement appears to affect the rate of cross-bridge detachment or reattachment during muscle-length changes.

A slow stretch of contracting muscle causes a continuous tension increase that is followed, after completion of stretch, by stress relaxation (Fig. 1 *a*) (1, 2). A slow shortening of active muscle causes the opposite effect; a continuous tension decrease followed by a recovery (Fig. 1 *b*). On the basis of the cross-bridge model (3), the behavior of myosin heads upon such slow length changes can be predicted as follows (4). When an active muscle is stretched slowly, the myosin heads attached to actin will be shifted towards the Z-line along the filament axis, causing the tension to rise. When the duration of stretch is long enough (e.g., several hundred milliseconds) and when the magnitude of stretch is large enough (e.g., several hundred angstroms per half sarcomere), the myosin heads will undergo cycles of detachment and attachment during the axial shifts. When the stretch is completed, the axial distribution of myosin heads, again through detachment and reattachment, will return towards the original (isometric) pattern; this will be reflected in tension as a stress relaxation. A slow shortening will cause the opposite movements of myosin heads, namely towards the M-line during shortening and the reverse after its completion. These axial shifts caused by slow stretch and shortening will not accompany a significant change in the number of "attached" myosin heads, since muscle stiffness stays constant throughout the tension responses (5).

In the present study we have investigated the axial component of the actual cross-bridge movements during length changes using a time-resolved x-ray diffraction technique. The results suggest that the tension changes are indeed accompanied by changes in the axial alignment of cross-bridges and that the rates of detachment and attachment may depend on the direction of the cross-bridge shifts.

A sartorius muscle of the bullfrog *Rana catesbeiana* was mounted in a chamber perfused with Ringer's solution (2°C). The proximal end of the muscle was fixed to the chamber by clamping the pubic bone; the distal end was connected to an isometric tension transducer (Shinkoh Co., Tokyo, type UT) attached to an ergometer (Akashi Co. Tokyo, V201). The sarcomere length was adjusted to 2.5 μm by the laser diffraction method ($\lambda = 0.6328$ μm). The maximum tetanus, 2 or 4 s in duration, was caused by electrical pulses (20 Hz) applied through parallel electrodes. Each muscle was tetanized 20 times; in 10 tetani the muscle was stretched at a constant speed, and in the other 10 the muscle was released at the same speed. The magnitude of stretches and releases was the same: 7% of the initial muscle length (88 nm per half sarcomere). Stretches and releases were produced alternately; a tetanus in which the muscle was stretched to a longer length was followed, after a 3-min interval, by a tetanus in which the muscle was shortened from the longer length to the initial length.

Intensity changes of a meridional reflection at 1/14.3 nm^{-1} were studied by a method described previously (6); either a position-sensitive x-ray detector or a scintillation counter combined with a mask was used to measure the intensity. These detectors were coupled with a memory circuit which stored the signals as a function of time (7); the time resolution was either 100 or 200 ms. Signals from 10 tetani were averaged to obtain the time course of the intensity during stretch or release. This meridional intensity reflects the axial distribution of myosin heads; more precisely, it reflects the structure of the thick filament projected onto its axis. This intensity was expected to change when the myosin heads move asynchronously along the axis or tilt in the axial direction; azimuthal or radial movements were not expected to affect the intensity. The intensity could be affected also by the structure of the thick-filament backbone. However, this was assumed to be unaltered by stretch and release.

Fig. 1 *a* shows the average response of 12 muscles to stretches at a speed of 2.9% muscle length per second (7% in 2.4 s). During the brief period of steady isometric tension prior to stretch, the meridional intensity was 23 ± 8% (mean ± SEM) greater than the resting value. A similar intensity increase has already been observed

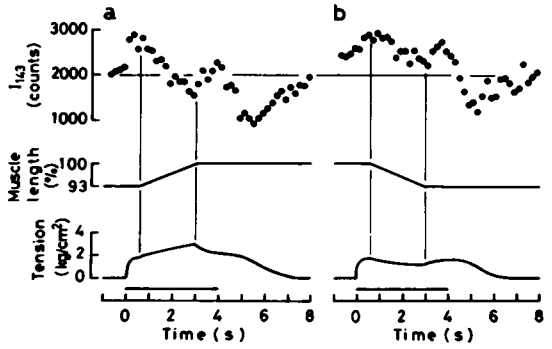

FIGURE 1 Effects of changing muscle length on tension and the intensity of the 14.3-nm meridional reflection (I_{143}). The muscle was (*a*) stretched or (*b*) released at a constant speed (7% muscle length in 2.4 s) during 4-s tetani. The intensity plot (*top*) represents an average of data obtained from 12 muscles; each muscle was stretched and released 10 times (see text). The change in muscle length (*middle*) corresponded to a sarcomere-length change from 2.33 to 2.50 μm on stretch and the reverse on release. Each tension curve (*bottom*) represents an average of 10 tetani of a typical preparation. The x-ray generator was a rotating-anode type (Rigaku Co., Tokyo, type FR) with a fine focus (0.1 × 1.0 mm) on a copper target. This was operated at 50 kV with a tube current of 70 mA. A mirror-monochromator camera was used at a specimen-to-counter distance of 46 cm. Both point and line-focused beams were produced and superimposed (6). The chamber containing the muscle (see text) had mylar windows to allow x-rays to pass through the middle of the muscle. The composition of Ringer's solution perfusing the chamber was 115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 2.15 mM Na_2HPO_4, and 0.85 mM NaH_2PO_4; the pH was 7.2. The intensity of the meridional reflection was measured with a scintillation counter combined with a lead mask (6) that had apertures (0.8 mm horizontally × 4 mm vertically) at the positions of the reflection. The meridional periodicity of 14.3 nm has been known to change by 1% on activation of muscle (11). The resultant displacement of the reflection (48 μm, or 6% of the aperture width, at the mask) did not significantly affect the intensity measurement (6). The measured intensity included the background; the latter was estimated by shifting the mask along the meridian by 0.8 mm. Changes in the background intensity during stretch and release were studied in separate experiments on seven muscles; it changed by up to 3% of the initial background level. The intensities (I_{143}) shown above have been corrected for the background and its change. Similar time courses of I_{143} were obtained with a position sensitive x-ray detector. The isometric tension at the beginning of each experiment was 2.39 ± 0.08 kg/cm² (mean ± SEM, n = 12) at a sarcomere length of 2.5 μm. The tension decreased gradually during each experiment; the tension averaged over 20 tetani was 79.1 ± 1.3% of the initial value. The horizontal bars indicate the periods of stimulation.

(6, 8, 9) and attributed to a rearrangement of the myosin helix occurring on activation (6, 10). The increased intensity suggests also that the myosin heads maintain their own axial periodicity while interacting with actin during isometric contraction (8, 9). When the muscles were stretched the intensity decreased, suggesting axial movements of crossbridges.[1] In the middle of stretch the intensity was 21 ± 3% less than that during isometric contraction. During stress relaxation the intensity returned gradually towards the isometric level. When the same muscles were released at the same speed, the intensity changed in a similar manner (Fig. 1 *b*); it decreased during shortening and recovered after completion of release. The amount of decrease in the middle of the release was 13 ± 6% of the isometric value; this was significantly smaller than the decrease caused by stretch (paired comparison, $P < 0.01$).

On termination of the stimuli after stretch or release there were further intensity changes accompanying relaxation (Fig. 1 *a*, *b*). Possible origins of such changes were discussed in a previous paper (6).

When the speed of length change was increased to 8.7% muscle length per second (7% in 0.8 s), the reductions in the intensity during stretch and release were not affected appreciably. However, when the speed was further increased to 23% muscle length per second (7% in 0.3 s), the reductions became significantly greater ($P < 0.05$); that is, intensity reductions of 37 ± 6% in the middle of the stretch and 36 ± 5% in the middle of the release. At this high speed, the difference between the effects of stretch and release was no longer significant.

The reduction in intensity caused by stretch or release can be attributed to two types of cross-bridge movements. First, asynchronous shifts of cross-bridges in the axial direction will reduce the intensity by disordering the myosin periodicity. Secondly, axial tilts of cross-bridges, without shifts of the centers of individual cross-bridges' mass, can cause an intensity decrease (9, 12). In either case we can conclude that cross-bridge movements in the axial direction do accompany the tension changes caused by stretch or release, verifying the prediction made at the beginning of this paper.

The two types of movements may be distinguished by observing the equatorial x-ray reflections. Because the equatorial intensities reflect the filament structure projected axially onto the plane perpendicular to the muscle axis, tilting of cross-bridges that are known to have an elongated shape (13) would affect the intensities (12, 14). On the other hand, axial shifts of cross-bridges without tilting would not affect the intensities. In practice, Podolsky et al. (15) have shown that a slow shortening of active muscle does not affect the intensities of the 1,0 and 1,1 equatorial reflections. Yagi and Matsubara (16) have shown that, during stress relaxation after a slow stretch, the 1,0 and 1,1 intensities stay at the same level as those in isometric tetanus. Therefore, it is unlikely that tilting is taking place on a significant scale; this suggests that the major cause of the decrease in the meridional intensity is likely to be axial shifts of cross-bridges.

It may seem possible to attribute the observed decrease

[1]A decrease in the meridional intensity could also be caused by a disorder in the relative axial positions of the thick filaments (11). An intensity decrease of this origin is actually observed when a point-focused x-ray beam is used; the disorder broadens the reflection parallel to the equator, decreasing the intensity on the meridian (9). In the present experiments, however, the measured intensity was almost insensitive to this type of disorder, since the beam was linearly focused parallel to the equator, and the intensity was integrated over the whole length of the reflection (see Discussion in reference 6).

in the meridional intensity to a change in the number of myosin heads bound to actin. However, such a change is expected to affect also the equatorial intensities (17); the absence of equatorial changes (15, 16) makes this interpretation unlikely.

The present results have shown that, at slow speeds, stretch causes a greater decrease in the intensity than release. A possible explanation for the difference is as follows. During stretch the rate of cross-bridge detachment may be smaller, or the rate of reattachment may be greater, than during release. Then the average duration of attachment for each myosin head would be longer during stretch. Therefore, a sliding of thick and thin filaments would cause a greater amount of axial shift during stretch. This would lead to a greater amount of axial disorder during stretch, causing a greater intensity fall. When the speed of length change is increased, an extensive disorder would occur even during release so that it could no longer be distinguished from the disorder caused by stretch. Thus the present results could be explained by assuming that the rate of detachment, or attachment, of cross-bridge is affected by the direction of shift from its isometric position.

After stress relaxation following a stretch, the tension is known to settle to a level higher than the isometric level (1). Fig. 2 shows the average data from 15 muscles in which contraction was continued for as long as 3.5 s after the end of each length change in order to study this phenomenon. After a stretch at a speed of 70% muscle length per second (7% in 0.1 s), the tension settled to a value 48 ± 4% greater than the isometric tension (at the longer length) (Fig. 2 *a*). This extra tension was accompanied by a sustained depression of the meridional intensity; the final intensity was 24 ± 10% smaller than the isometric value. On the other hand after a release at the same speed, the tension returned to the isometric tension (at the shorter length) (Fig. 2 *b*). This was accompanied by a return of the meridional intensity to the isometric level.

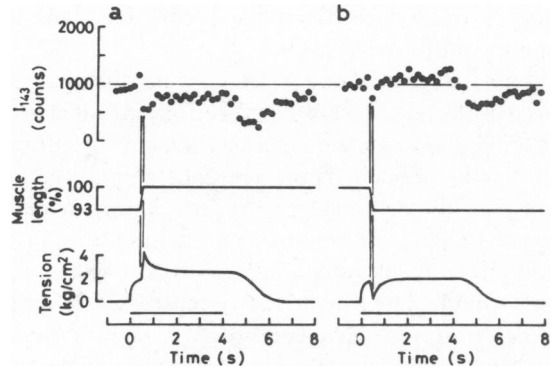

FIGURE 2 The intensity of the 14.3-nm meridional reflection (I_{143}) in relation to a residual tension after stretch. The muscle was (*a*) stretched or (*b*) released at a constant speed (7% muscle length in 0.1 seconds) during 4-s tetani. The intensity plot (*top*) represents an average of data obtained from 15 muscles; each muscle was stretched and released 10 times. The intensity was corrected for the background in the same manner as in Fig. 1. The length changes (*middle*) corresponded to sarcomere-length changes from 2.33 to 2.50 μm and the reverse. Each tension curve (*bottom*) represents an average of 10 tetani of a typical preparation. Note that the extra tension remaining after stretch accompanied a sustained decrease of I_{143}. The initial isometric tension at a sarcomere length of 2.5 μm was 2.41 ± 0.09 kg/cm² (mean ± SEM, n = 15). The tension averaged over 20 tetani was 78.1 ± 1.3% of the initial value. The horizontal bars indicate the periods of stimulation.

Three explanations have been proposed for the mechanism underlying the extra tension. In one (5), the extra tension is attributed to inhomogeneity of sarcomere lengths. In another (18), it is attributed to an elastic structure, other than the cross-bridge, recruited or formed during activation. In the third explanation (19), cross-bridges displaced from their original positions produce the extra tension. Our study supports this third explanation; that is, the present results, combined with the previous observation that the intensities of the principal equatorial reflections during extra tension are the same as in the isometric contraction (16), suggest that the extra tension may be due to axial shifts of cross-bridges.

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