Lattice Spacing Changes Accompanying Isometric Tension Development in Intact Single Muscle Fibers

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ABSTRACT The myosin lattice spacing of single intact muscle fibers of the frog, *Rana temporaria*, was studied in Ringer's solution (standard osmolarity 230 mOsm) and hyper- and hypotonic salines (1.4 and 0.8 times standard osmolarity respectively) in the relaxed state, during "fixed end" tetani, and during shortening, using synchrotron radiation. At standard tonicity, a tetanus was associated with an initial brief lattice expansion (and a small amount of sarcomere shortening), followed by a slow compression (unaccompanied by sarcomere length changes). In hypertonic saline (myosin lattice compressed by 8.1%), these spacing changes were suppressed, in hypotonic saline (lattice spacing increased by 7.5%), they were enhanced. During unloaded shortening of activated fibers, a rapid lattice expansion occurred at all tonicities, but became larger as tonicity was reduced. This expansion was caused in part by the change in length of the preparation, but also by a recoil of a stressed radial compliance associated with axial force. The lattice spacing during unloaded shortening was equal to or occasionally greater than predicted for a relaxed fiber at that sarcomere length, indicating that the lattice compression associated with activation is rapidly reversed upon loss of axial force. Lattice recompression occurred upon termination of shortening under standard and hypotonic conditions, but was almost absent under hypertonic conditions. These observations indicate that axial cross-bridge tension is associated with a compressive radial force in intact muscle fibers at full overlap; however, this radial force exhibits a much greater sensitivity to lattice spacing than does the axial force.

INTRODUCTION

The quasi-crystalline structure of striated muscle cells permits the use of x-ray diffraction to the study of structural events accompanying tension development. Intact muscle cells are thought to maintain a constant volume in the relaxed state when sarcomere length is varied, based on measurements of fiber cross sectional area (Blinks, 1965) and x-ray diffraction measurements in whole muscle (Huxley, 1953; Rome, 1968; Haselgrove and Huxley, 1973; Millman et al., 1981) and single fibers (Matsubara and Elliott, 1972), changes in fiber length being compensated by an inversely proportional change in its cross sectional area. A uniform change in area requires the radial separation between the surfaces of the myofilaments (actin and myosin) to change, so the molecular force generation mechanism (interaction between actin and myosin molecules) must be capable of operation over a range of filament separations. It has been proposed that the cross-bridges (that portion of the myosin filament known to bind to actin and composed of the S1 portion of the myosin molecule) can move radially from the surface of the myosin filament to reach actin by virtue of a rod-like section of the myosin molecule (S2). This section links S1 to the myosin filament backbone and is thought to be double-hinged and free to swing out from the axis of the myosin filament. One hinge should be located at the junction of S2 with the myosin filament, the other at the junction of S2 and the S1 region (Huxley, 1969).

Such an arrangement should lead to a radial component of cross-bridge force, because the mechanical link (S2) between actin and myosin is disposed at an angle with respect to the axis of the myosin filament. Evidence for such a radial force has been growing over recent years as a result of advances in x-ray diffraction techniques. In skinned fibers of the frog and rabbit at slack length, calcium activation (Brenner and Yu, 1991) or the formation of rigor bridges (Matsubara et al., 1984; Brenner and Yu, 1991; Xu et al., 1993) causes lattice compression, from which an estimate of the magnitude of the radial force acting on the myosin filament may be obtained. Qualitatively, the force varies with initial lattice spacing in the manner expected for changes in the angle between the S2 component and the axis of the myosin filament. However, skinned fibers are not a constant volume system, they experience a large lattice expansion as a result of the skinning procedure, and they are bathed in a medium that differs in composition from the sarcoplasm. Therefore, the behavior of the myosin lattice of a skinned fiber may well differ from that of the intact cell. Recently, it has been shown that during a tetanus, single intact muscle fibers from the frog experience a lattice compression during development of axial tension at full filament overlap (Cecchi et al., 1990), suggesting that a compressive radial force is associated with cross-bridge attachment in the intact cell. Here we report the effects of initial lattice spacing in the intact fiber preparation on the expression of radial force associated with activation. Unlike skinned fiber studies, which have described the static dependence of radial forces on initial lattice spacing, here we show the dynamic behavior of the radial lattice force.

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MATERIALS AND METHODS

Experimental protocol

Single muscle fibers isolated from the tibialis anterior muscle of frogs (*Rana temporaria*) were used. fibers were typically 5.5 to 6.5 mm long. To maximize the intensity of the diffraction pattern, large fibers were selected (maximum diameter typically 200 μm, minimum 70–150 μm). The details of the beamline and mounting procedure have been described elsewhere (Cecchi et al., 1991). For time-resolved experiments, sarcomere length was adjusted to lie at the upper limit of the plateau of the length-tension relationship (2.2–2.25 μm) so that any subsequent shortening would occur without change in the number of cross-bridges capable of interacting with actin. The fibers were electrically stimulated (stimulation frequency 20 Hz) to induce a tetanus of 400 ms duration. In some fibers at the plateau of the tetanus a period of constant velocity (i.e., ramp) shortening was initiated. Sarcomere length was recorded throughout the contraction using a laser diffractometer (Bagni et al., 1985). The laser diffraction pattern was obtained from a HeNe laser beam focused down to a 250-μm spot and passed through the center of the fiber (corresponding to the center of the region exposed to the synchrotron beam). The region of the fiber exposed to synchrotron radiation beam (wavelength = 0.153 nm, height 0.3 mm, width 4 mm) was a 4 mm length of fiber at about the center of its length (i.e., between 60 and 70% of the total fiber volume is exposed to the beam). Equatorial x-ray diffraction patterns were collected on a one-dimensional multiwire proportion gas detector (Hendrix et al., 1982). Counts from 128 output channels were stored (together with sarcomere length, fiber length, beam intensity, and force) in a CAMAC-based data acquisition system controlled by a personal computer. After an experiment, data were dumped to the personal computer for storage and further evaluation. For static patterns from relaxed fibers, a single 1-s exposure was used. For time-resolved patterns and activated fibers, patterns were collected and averaged over 20 cycles at sampling rates of from 2 to 10 ms. All experiments were performed at 4°C

Data analysis

Lattice spacing was measured using a curve-fitting procedure (Yu et al., 1985) in which six Gaussian reflections (10, 11, 20, 21, 30, and z) and a background intensity function were adjusted to give the best fit to each half of a spectrum. A more detailed description of this procedure has been given in a previous publication (Griffiths et al., 1993). The distance between the positions of the fitted centers of a chosen reflection for the two sides of the spectrum was halved and taken as the reflection spacing at the detector. Lattice spacing (defined here as the center to center distance between nearest neighbor myosin filaments) was then calculated from the 10 and 11 reflection spacings (x_{10} and x_{11}, respectively), using the relation

$$a = \frac{2l}{3^{1/2}x_{10}} = \frac{2l}{x_{11}},$$

where l is the camera constant for the beamline (the product of camera length and the radiation wavelength, usually 5.9×10^{-10} m^2). Because estimates of lattice spacing obtained from the two reflections were very similar, to reduce complexity of figures and reduce noise, the mean of the two values is plotted in all subsequent figures showing lattice spacing and lattice volume. At sarcomere lengths greater than 2.5 μm, the 11 reflection became too small to measure its spacing accurately, and only the 10 reflection was used for fitting. During shortening at the maximum shortening velocity, the relatively slow sampling rate for the x-ray data prevented us from obtaining more than one or two points for spacing at the minimum value for force. To avoid presenting data based on a single lattice spacing value, we decided to average three or four adjacent points about the point(s) obtained at minimum force during shortening. Because the subsequent lattice recompression upon redevelopment of tension was essentially complete before the end of the tetanus and, therefore, was accurately measured, this procedure means that our values for lattice expansion during shortening are somewhat underestimated and so the lattice compression during the redevelopment of tension is an underestimation of the true compression (compared with Cecchi et al. (1990), where peak expansion values were used). The ± values quoted for spacing are the SD of the data points. In time-resolved figures, apparent lattice volume is calculated as the product of sarcomere length and lattice spacing squared. This volume may be referenced to the area of the unit cell upon multiplication by 0.866.

Solutions

The composition of standard Ringer's solution was (mM); NaCl, 115; KCl, 2.5; $CaCl_2$ 1.8; $NaHPO_4$, 0.85; Na_2HPO_4, 2.15, pH 7.00. The tonicity of the bathing medium was changed by either addition or omission of quantities of NaCl from the standard Ringer's composition (Rome, 1968). In the case of hypotonicity, reduction of the NaCl concentration is unavoidable, but for hypertonic conditions an impermeant nonelectrolyte such as sucrose could have been added to the standard Ringer's solution. However, the active extrusion of sodium ions from the sarcoplasm coupled with the low sodium permeability of the cell membrane causes sodium ions to behave as impermeant cations. Furthermore, although the cell membrane is highly permeable to chloride ions, these are effectively excluded by the requirement of charge balance and a constant [K]×[Cl] product on each side of the membrane (Boyle and Conway, 1941), so chloride behaves as an impermeant anion. For a given hypertonicity, the intracellular tonicity and ionic strength should be practically identical whether induced by the presence of an impermeable nonelectrolyte or by elevation of NaCl concentration. Therefore, because of the low atomic weight of NaCl (minimizing scattering), its convenience of use, and consistency with the method of preparation of hypotonic salines, we chose to use addition of NaCl to produce hypertonic media. The osmolarity of salines was checked using a vapor pressure osmometer (Wescor) and a freezing point osmometer (Camlab). The osmolarity of the standard Ringer's solution was 230 mOsm.

RESULTS

The relaxed fiber

Previous studies have indicated that an intact muscle fiber behaves like a "constant volume system" in response to static changes in sarcomere length (Matsubara and Elliott, 1972). This means that the cross sectional area varies in inverse proportion to the fiber length, so the product of sarcomere length (s) and myosin lattice spacing squared (a^2) is a constant (apparent lattice volume, sa^2). The corresponding total volume of the A-band lattice (la^2, where l is the length of the myosin filaments) must consequently increase at shorter sarcomere lengths and decrease at longer lengths, with an accompanying flow of intracellular fluid. If this flow were relatively slow compared with the sampling rate of time-resolved lattice spacing measurements, it might be inappropriate to apply constant volume constraints to dynamic length changes. We first checked whether we could confirm constant volume behavior in the relaxed preparation using synchrotron radiation, where exposure times were only 1 s compared with the 30 min required in the previous single fiber study with a laboratory x-ray source (Matsubara and Elliott, 1972). Sarcomere lengths were varied in the range 2.0–2.8 μm. At sarcomere lengths above 2.8 μm, fibers appeared more susceptible to radiation damage, and lattice spacing was not restored on return to resting sarcomere length. Attempts to reach sarcomere lengths below 2.0 μm resulted in the fibers becoming slack. After adjustment of sarcomere length, about 1 min elapsed before a 1 s exposure was taken from which the lattice spacing was calculated. When sarcomere length was plotted against reciprocal lattice spacing

squared for a relaxed fiber (Fig. 1), the points could be well fitted by a straight line with its intercept close to but not at the origin, as would be expected of a true constant volume system. In five fibers in which this relationship was studied, the mean slope of the fitted line was $0.2094 \pm 0.0766 \times 10^{-3}$ nm^{-2} μm^{-1}, and the mean intercept was $0.1747 \pm 0.1388 \times 10^{-3}$ nm^{-2}. The nonzero intercept of the relationship may indicate the action of restraining forces on the lattice that prevent unlimited expansion and cause a small deviation from the expected constant volume behavior of the whole cell; however, the range of sarcomere lengths studied was small compared with that of other groups (Rome, 1968; Matsubara and Elliott, 1972; Haselgrove and Huxley, 1973; Millman et al., 1981) who have reported true constant volume behavior in intact cells. At least to a first approximation, our measurements of apparent lattice volume suggest that it behaves as a constant volume system.

The dynamic behavior of the relaxed fiber lattice was observed using rapid ramp form length changes. When subjected to a quick release at slack length, relaxed fibers merely buckled and the change in sarcomere length was very small; therefore, the dynamic behavior of the lattice in the relaxed cell was studied using stretches, in some cases followed after 600 ms by a release. In this way, the response to stretch and release could be observed, because the initial stretch induced sufficient passive tension to shorten the fiber after the subsequent release. The sarcomere length change accompanying the initial stretch retained a step form, whereas the step release was not accompanied by a step fall in sarcomere length, but instead by a more gradual change (Fig. 2 *A*). When subjected to a quick stretch (3.5–5% of slack length, completed in 1 ms), relaxed fibers showed a lattice compression with a time course similar to that of the imposed length change as shown in the top panel of Fig. 2. To determine whether the lattice spacing change occurred under constant volume conditions, we calculated apparent lattice volume (using the time-resolved sarcomere length record) and plotted this against time as shown in the bottom panel of Fig. 2. The lattice compression accompanying the stretch was slightly smaller than predicted from a constant volume system, as indicated by the increase in apparent lattice volume in Fig. 2 *C*. In six relaxed fibers subjected to a stretch giving a 4.15% increase in length, we observed a 1.48% decrease in lattice spacing, and a 1.58% increase in volume. This increase in volume was observed in all fibers and so is unlikely to be caused by inaccuracies in sarcomere length and spacing estimates. Because spacing changes occurred rapidly with a generally similar form to that of the imposed length change, and were stable over the time course of our measurements with no evidence of any recovery toward the original spacing, and because the observed spacing change is similar to that predicted by the static relationship in Fig. 1, it appears that (at least in relaxed fibers) adjustment of fiber cross sectional area to a change in length is complete within our sampling time of 2–10 ms. As was previously reported by us for static measurements on relaxed fibers, equatorial intensities were substantially unaltered by length changes of this size in the full overlap region of sarcomere length (Griffiths et al., 1993).

Activated fibers

Under time-resolved conditions during a fixed-end tetanus, the lattice spacing of an activated fiber changes in a complex manner. An initial rapid expansion occurs at the onset of electrical stimulation followed by a slow phase of lattice compression. A typical example is shown in Fig. 3. The top panel shows the rise of tetanic tension and the change in lattice spacing calculated from the 10 and 11 reflections. The initial expansion phase is at least partially accounted for by

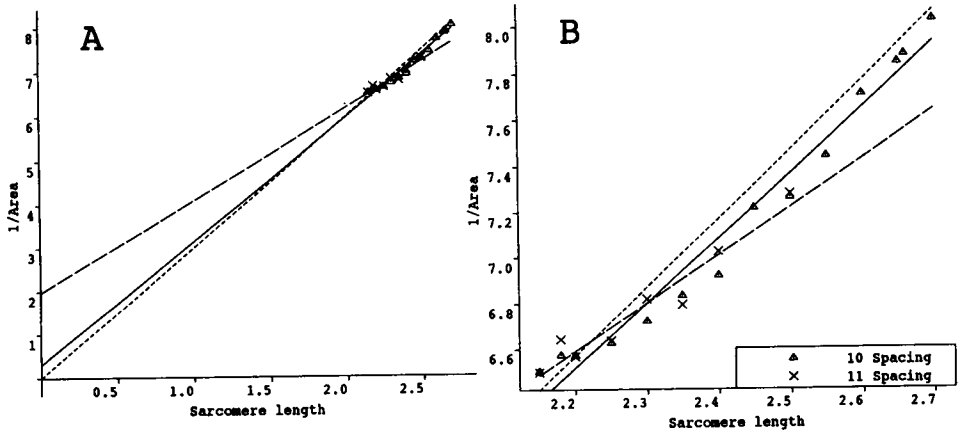

FIGURE 1 Reciprocal lattice spacing squared (area) plotted against sarcomere length in a relaxed fiber (*A*) showing the extrapolated intercept at the ordinate, (*B*) showing the fitted lines over the range of sarcomere lengths where data was collected. Solid line, best fit of a linear regression of reciprocal spacing squared calculated from the 10 reflection (■, slope 2.82×10^{-4} nm^{-2} μm^{-1}, intercept 3.10×10^{-5} nm^{-2}), long-dashed line from the 11 reflection (▽, slope 2.10×10^{-4} nm^{-2} μm^{-1}, intercept 1.98×10^{-4} nm^{-2}). The short-dashed line is the expected constant volume relationship passing through the 10 reflection at a sarcomere length of 2.2 μm. Ordinate units 10^{-4} nm^{-2}, abscissa units μm. All points are obtained from the same relaxed single fiber, using first a series of step increases in sarcomere length, then step decreases. Above 2.4 μm, the 11 reflection became very weak, and only one data point could be determined with confidence in this region.

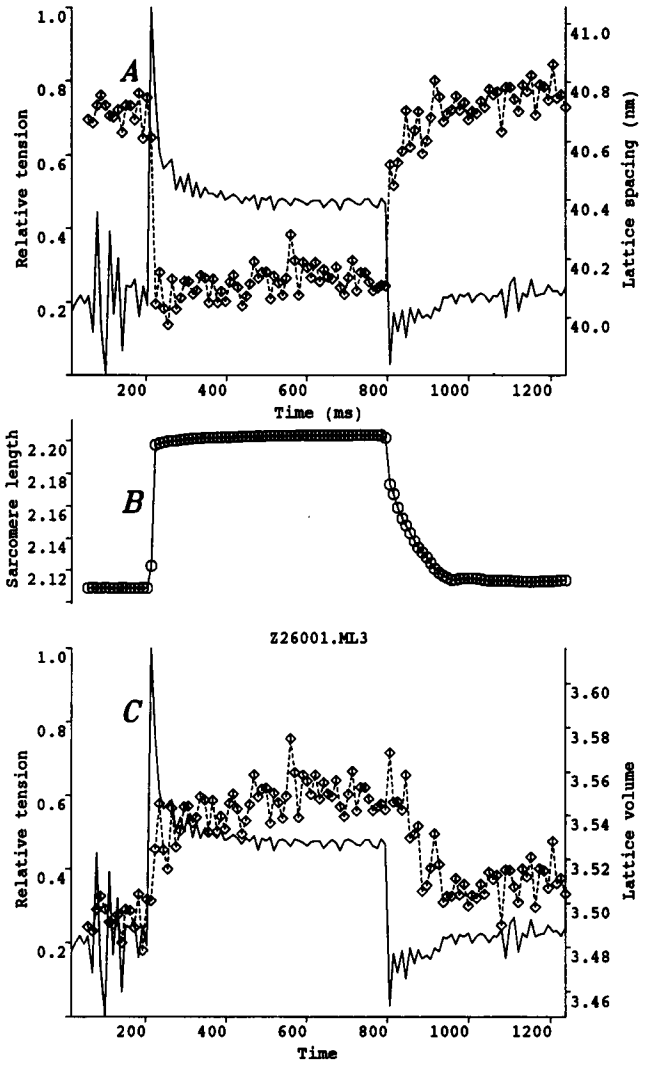

FIGURE 2 Dynamic lattice spacing changes accompanying a quick stretch and release of a relaxed fiber, averaged over 26 cycles. Spacing calculated from the 10 and 11 reflections (--◇--) and force (——) are plotted in the upper panel, sarcomere length (μm) on the middle panel. Apparent lattice volume and force are plotted in the lower panel, volume in units of 10^6 nm^3. Time resolution 10 ms.

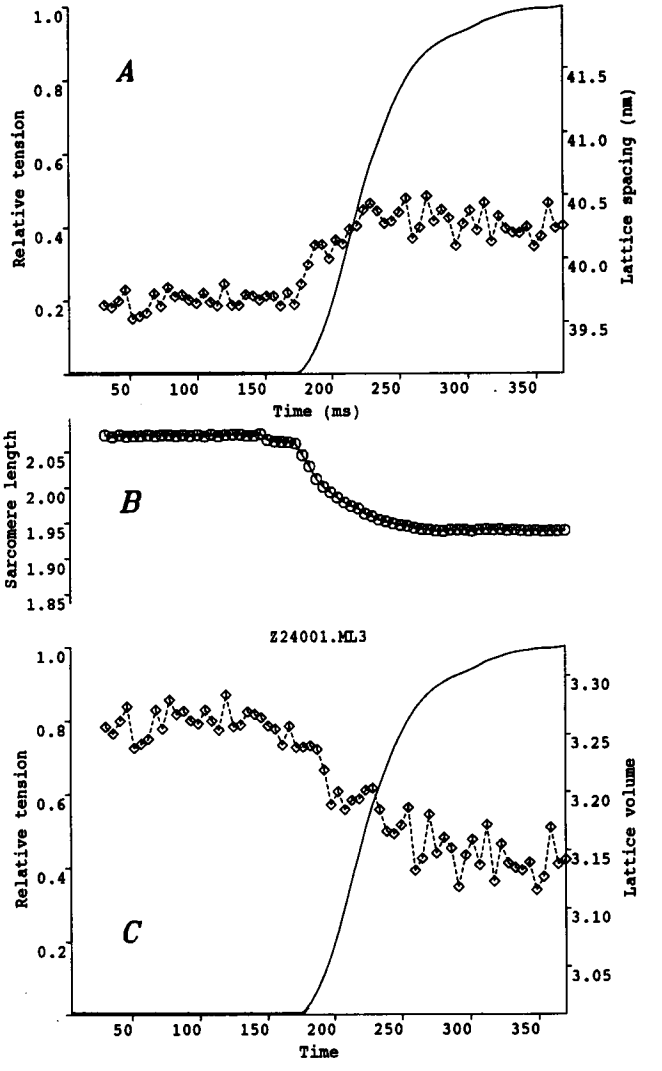

FIGURE 3 Dynamic lattice spacing changes in an intact single fiber during the onset of tetanic tension. The exceptionally large degree of shortening accompanying the rise of tetanic tension (6.4% of initial sarcomere length) is caused by series compliance, and not by any imposed motion of the stretcher. Tetanic tension (P_o 258 kN m^{-2}). Panels and symbols as for the previous figure. Averaged over 30 cycles, time resolution 5 ms.

fiber shortening against series compliance. This is indicated in the lower panel of Fig. 3 in which apparent lattice volume is plotted during the contraction. The expansion phase is then reduced in amplitude compared with the subsequent compression because area changes that are inversely proportional to sarcomere length changes disappear in such a plot. In 35% of fibers used in this study, the initial expansion could be explained completely in this way. Furthermore, when activated under sarcomere length-clamped control, this initial expansion phase became much smaller or vanished because initial sarcomere length changes were greatly reduced. Under length-clamped control, in 78% of fibers the initial expansion was absent or could be explained entirely by residual initial shortening (Fig. 4).

Relaxation in intact fibers from a fixed-end tetanus occurs in two phases, the first a slow phase during which sarcomere length is maintained almost constant, the second more rapid and associated with quite large changes in sarcomere length. Lattice spacing increases during the first (isometric) phase, as shown in Fig. 5, until the point at which the rapid relaxation phase begins, after which its behavior is unpredictable. During the isometric phase, the change in spacing follows a similar form and time course to that of axial force relaxation.

When tetanic tension was stable, some fibers were permitted to shorten at a velocity close to the velocity of unloaded shortening (V_{max}). The resulting fall in axial tension was accompanied by a rapid recoil of lattice spacing to a value close to its relaxed spacing at that sarcomere length. This is shown in Fig. 6. In the lower panel of Fig. 6, it can be seen that lattice volume also increases during the release and shortening period, but the increase is less marked than

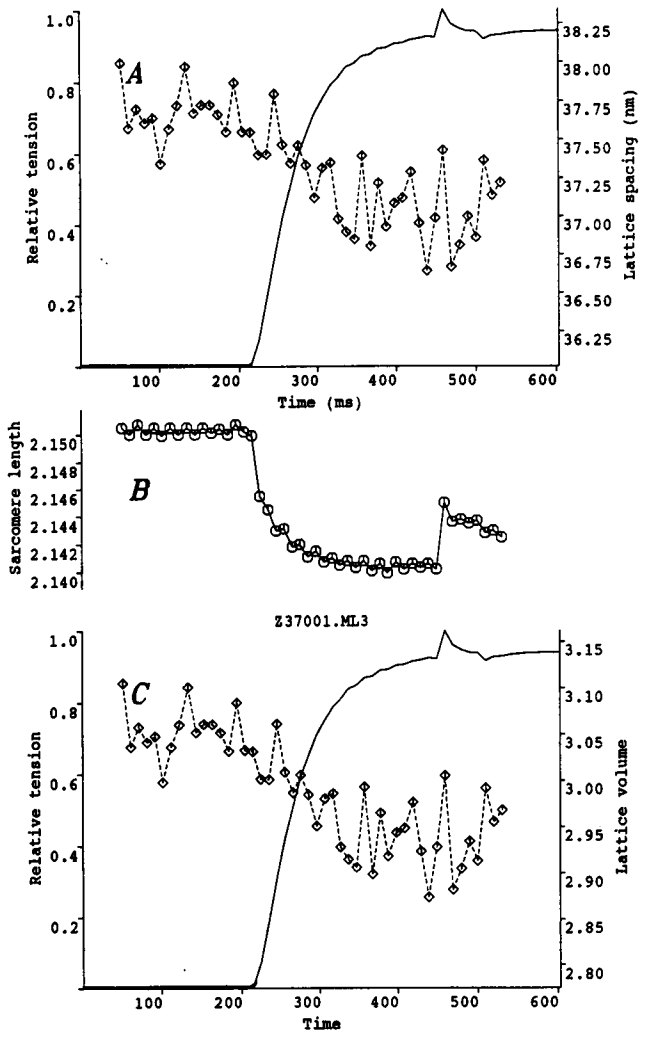

FIGURE 4 Lattice spacing (*top*) and apparent volume (*bottom*) during the rise of tension in a length-clamped tetanus. Symbols and lines as for Fig. 2. Despite the length clamp feedback, some sarcomere shortening was unavoidable, amounting to 0.1–0.5% of the initial sarcomere length, depending on the fiber. Note that no expansion phase is now detectable in lattice spacing or volume. The small stretch evident at 450 ms from the start of the trace was the result of the action of electrical noise at the beamline on the length clamp feedback, and not a deliberately applied change in fiber length. Sum of 49 tetani. Time resolution 10 ms.

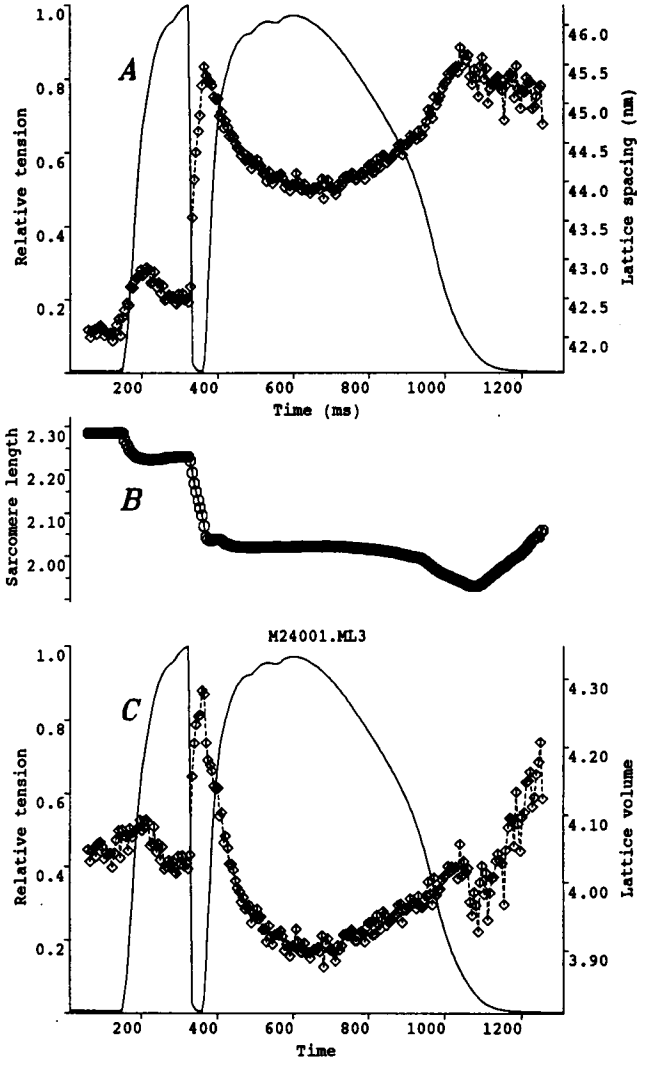

FIGURE 5 Lattice spacing changes during an entire tetanus, including a period of shortening at close to V_{max}. Panels and symbols are as for Fig. 2. For convenience, a whole tetanic response is shown, including a period of shortening which occurred just after the tetanus rise. Note that in this fiber, lattice expansion during the rise of tension and shortening is not completely accounted for by the sarcomere length record, as seen in the lower panel. P_o 197 kN m^{-2}. Average of 28 tetani, time resolution 5 ms.

in the lattice spacing plot and restores the apparent lattice volume to the value that existed before stimulation. This suggests that in the activated state at low axial tension, the relation between sarcomere length and spacing of the relaxed fiber is restored. However, because this restoration involves an increase in apparent lattice volume of the activated lattice, part of the observed spacing change corresponds to this change in apparent lattice volume and is associated with the loss of axial tension. It cannot be explained by the small deviation of the relaxed lattice behavior from a constant volume system because this would have tended to reduce lattice expansion during a release rather than causing it to be overestimated. Further evidence of a lattice spacing change unrelated to sarcomere length changes is seen during the redevelopment of isometric tension. Upon termination of unloaded shortening, the recovery of isometric tension occurred at constant sarcomere length and was accompanied by a gradual recompression of the lattice (average value 0.771 ± 0.338 nm, 13 fibers). In general, this recompression was slower than the recovery of axial tension (half-time for force 21.6 ms, for spacing 67.5 ms). Because it occurs in the absence of sarcomere length changes, it represents a lattice spacing change in contravention of the constraints of constant apparent lattice volume.

In Fig. 7, a fiber has been allowed to shorten at a lower velocity than V_{max}, which has allowed us to prolong the period of shortening. Because of the lower velocity, the loss of tension during shortening is smaller than in Fig. 6. In the

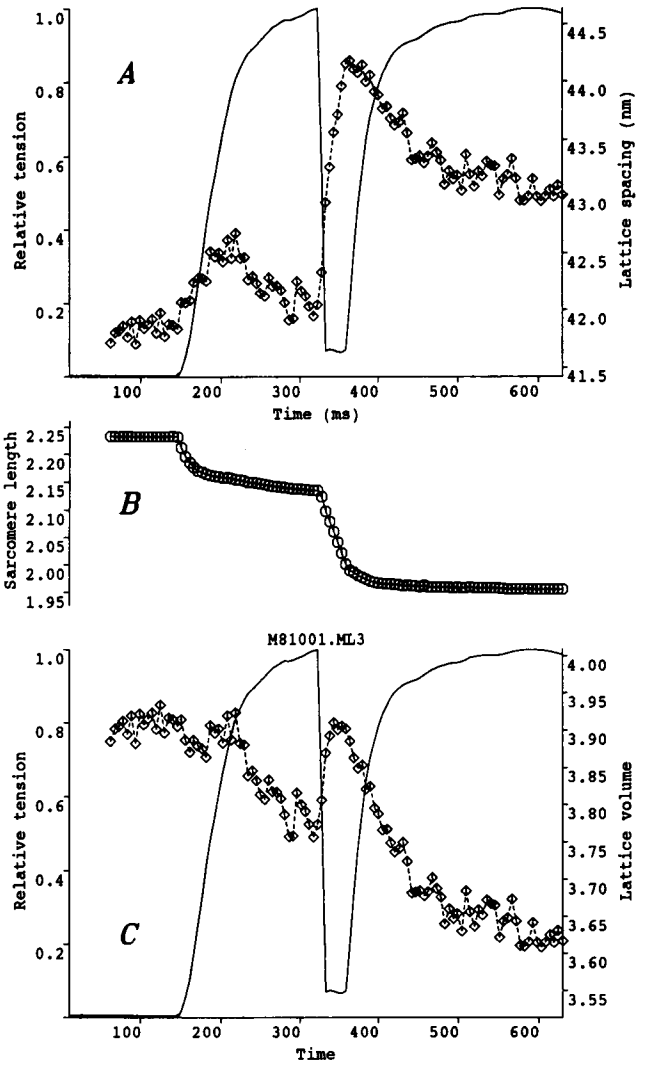

FIGURE 6 Lattice spacing changes during activation and shortening at standard tonicity. Panels and symbols are as in the previous figures. P_o 290 kN m^{-2}. Velocity of shortening 3.89 μm/half sarcomere s^{-1}. Average of 22 tetani, time resolution 5 ms.

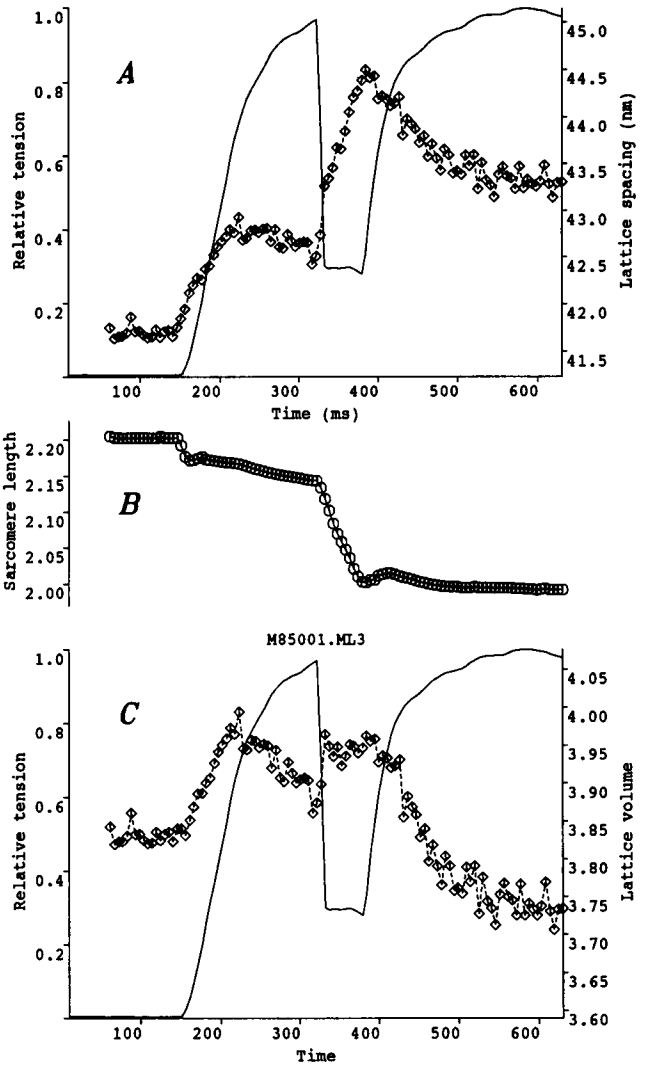

FIGURE 7 Lattice spacing changes at standard tonicity during shortening at a velocity less than V_{max}. Panels and symbols are as in the previous figures. As in Fig. 5, the expansion of the lattice accompanying the rise of tetanic tension is not completely accounted for by the sarcomere length changes. P_o 298 kN m^{-2}. Velocity of shortening 2.67 μm/half sarcomere s^{-1}. Average of 25 tetani, time resolution 5 ms.

upper panel of Fig. 7, it can be seen that the initial fall in tension caused by shortening is accompanied by a rapid increase in lattice spacing. This increase in spacing is also less pronounced than in Fig. 6 because the component caused by the loss of axial tension has not been completely removed. As shortening proceeds, there is a more gradual, ramp-like change in the spacing. In the lower panel, it can be seen that the apparent lattice volume changes only at the time of the fall in axial tension and remains relatively stable during the shortening period. This behavior is again consistent with two components of the lattice expansion during shortening; the first rapid change in spacing is caused by the fall in axial tension and reduced at this slower shortening velocity, and the second stable section is the lattice response to a changing sarcomere length at constant force.

If the radial force causing compression during activation or recovery of isometric tension resulted from the disposition of S2, changes in lattice spacing should affect the radial component. Lattice spacing in intact cells can be varied by adjustment of the tonicity of the bathing medium. fibers were equilibrated in the chosen saline for 30 min. At standard osmolarity, lattice spacing in relaxed fibers was 41.75 ± 1.55 nm at 2.1 μm sarcomere length. When the osmolarity of the bathing medium was increased at constant sarcomere length to 1.4× standard osmolarity, the lattice spacing decreased to 38.36 ± 0.57 nm. Axial tetanic force was reduced by 23.6%. Reduction of osmolarity to 0.8× standard osmolarity increased the spacing to 44.88 ± 1.33 nm, and axial force increased by 11.7%. Under hypertonic conditions (1.4×), the lattice spacing expansion accompanying activation was greatly reduced. The slow compression phase was also much smaller, and in some fibers was completely absent. During unloaded shortening, lattice spacing increased, but less than

was observed at standard tonicity. It can be seen in the lower panel of Fig. 8 that when apparent lattice volume is plotted, the lattice expansion accompanying the fall of force at the onset of shortening is virtually abolished. This is consistent with the absence of any pronounced compression of the lattice during the onset of tetanic tension, and suggests that at this initial lattice spacing the radial forces have become very small. Redevelopment of isometric tension was accompanied by little or no lattice compression, which is as expected if radial forces have been reduced (average value 0.191 ± 0.223 nm, 6 fibers, Fig. 8). By contrast, in hypotonic media (0.8×) the lattice spacing changes seen at standard tonicity became more pronounced. The compression of the lattice during the development and redevelopment of axial tension (1.703 ± 0.534 nm, 7 fibers, Fig. 9) became larger, and the recoil of the lattice after discharge of axial tension also increased. The apparent lattice volume plot shows quite large changes in lattice volume accompanying the onset and termination of unloaded shortening, and the maximum expansion of the lattice during shortening approximately restores the original, relaxed apparent lattice volume.

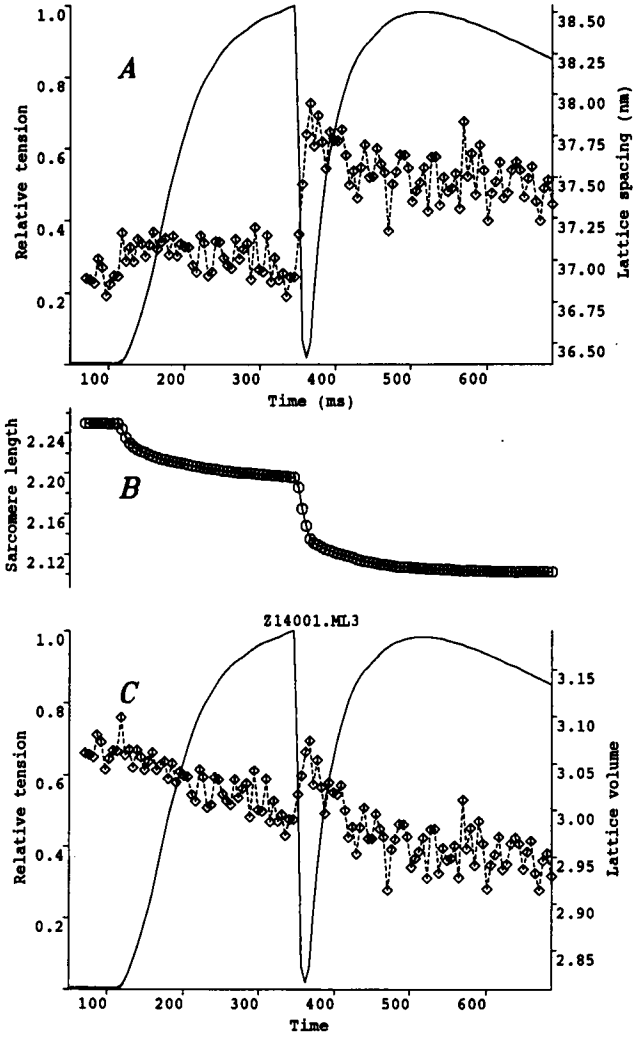

FIGURE 8 Lattice spacing changes during shortening of a single fiber in hypertonic saline (1.4 × standard osmolarity). Note the increased noise level on the lattice spacing. This is caused by a marked reduction of equatorial intensities in hypertonic saline. P_o 274 kN m^{-2}. On average, tetanic tension was reduced by 23.6% in hypertonic Ringer's solution. Velocity of shortening 3.40 μm/half sarcomere s^{-1}. Average of 21 tetani, time resolution 5 ms.

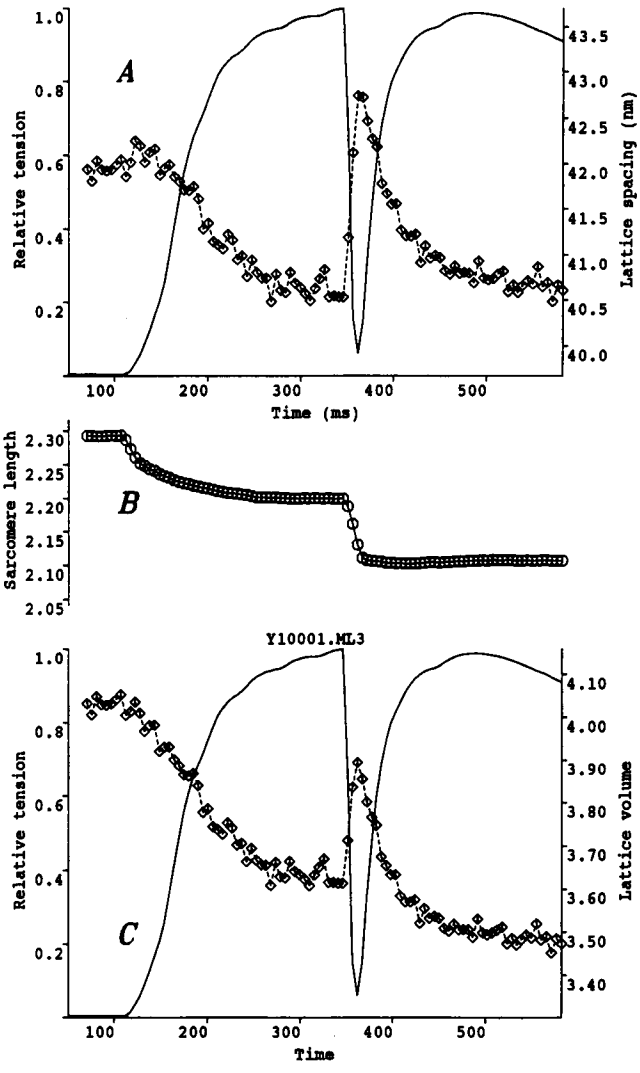

FIGURE 9 Lattice spacing changes during shortening of a single fiber in hypotonic saline (0.8 × standard osmolarity). P_o 355 kN m^{-2}. Same fiber as Fig. 8. On average, tetanic tension was increased by 11.7% in hypotonic Ringer's solution. Velocity of shortening 5.09 μm/half sarcomere s^{-1}. Average of 10 tetani, time resolution 5 ms.

Because the initial lattice expansion during the rise of tetanic tension was not always eliminated completely by length clamp or plotting as lattice volume (see Figs. 5 and 7), and because the lattice recoil accompanying unloaded shortening was sometimes greater than expected from the relaxed fiber apparent lattice volume, we considered the possibility that activation would give rise to a repulsive force between filaments independent of cross-bridge attachment, detectable only before the compressive force of the cross-bridges was not yet fully developed, or when the cross-bridge force was

discharged by shortening. Therefore, we exposed three fibers to 8 mM 2,3-butanedione monoxime (BDM), which blocks the formation of cycling cross-bridges and thus axial force, while leaving the calcium transient associated with activation largely unaffected (Horiuti et al., 1988). Therefore, a cross-bridge independent lattice expansion associated with activation, if present, should remain, whereas the compressive radial force caused by the cycling cross-bridges would be lost. BDM caused an average increase in lattice spacing of unstimulated fibers of 2.65%, accompanied by an intensity increase of the 10 reflection of 29.91% and of 23.57% in the 11 reflection. The general appearance of the pattern remained that of a typical relaxed cell. When electrically stimulated, fibers developed no detectable axial tension, and no lattice spacing changes were observed. The sarcomere length record showed a displacement during the period of stimulation, suggesting that calcium activation was still occurring and had given rise to a small proportion of cycling bridges. The response of the fiber to rapid stretches was identical to that of the relaxed preparation. Upon removal of BDM, fibers recovered their ability to develop tetanic tension. The absence of an expansion phase in the presence of BDM suggests it is unlikely that the processes associated with activation can give rise to a repulsive force between filaments capable of accounting for the early lattice expansion seen in contracting fibers.

DISCUSSION

We find that the dependence of relaxed intact muscle fiber myosin lattice spacing on sarcomere length approximates to constant volume system, i.e., static changes in sarcomere length produce almost inversely proportional changes in cross sectional area as measured by the myosin lattice spacing. However, in all fibers we examined, we found the fitted line to data points plotted as in Fig. 1 failed to pass through the origin, suggesting that some constraint exists on the freedom of the lattice to undergo unlimited expansion. The cell membrane is thought to provide little mechanical resistance to changes in fiber cross sectional area (Blinks, 1965), so it is to be expected that fiber cross sectional area adjusts to tonicity and sarcomere length changes as a near-perfect osmometer. If fiber volume remains constant, changes in sarcomere length should cause redistribution of sarcoplasm, because the true myosin lattice volume must be changing, which could influence the time course of spacing changes. Time-resolved dynamic sarcomere length changes (stretches) in the relaxed intact fiber seem to cause virtually instantaneous adjustment in lattice spacing within the time resolution of our measurements (2–10 ms). The accompanying decrease in lattice spacing does not show any indication of a recovery phase, as observed during stretches and releases in activated fibers. Changes in spacing during a subsequent release were more complex, because fiber shortening occurred passively and, therefore, with a time course quite divorced from that of the imposed motor movement. However, the original apparent lattice volume before the stretch was restored within 200 ms of the release.

As pointed out by Schoenberg (1980), a 2% change in osmotic pressure of an externally applied solution (about 4.6 mOsm) is needed to cause a 1% lattice compression in an intact fiber. This is much larger than that of a solution producing the same spacing change in a relaxed skinned fiber (about 0.2 mOsm, Matsubara et al., 1984) because the intact fiber spacing change causes concentration of all cell constituents, whereas compression of the lattice of the skinned fiber concentrates only the lattice and any counter ions associated with it, which in total may exert a considerably smaller expansive force. For this reason, if radial forces acting in the intact fiber change the apparent lattice volume without change in total fiber volume, they will be much smaller than those needed to change total fiber volume by the same amount. Because of this difference between intact and skinned preparations, we are unable to perform the same sort of calibration of radial forces by variation of osmotic pressure that has been possible for skinned fiber experiments (Matsubara et al., 1984; Brenner and Yu, 1991; Xu et al., 1993). The lattice spacing of a skinned fiber is generally thought to be determined by the interaction of electrostatic, mechanical, osmotic, and entropic mixing forces (Maughan and Godt, 1980; Matsubara et al., 1984). The electrostatic force is considered to be repulsive and arises from osmotic effects caused by a Donnan-like distribution of ions caused by fixed charges on the filaments (Flory, 1953; Elliott, 1973), the osmotic force (resulting from the tonicity of the bathing medium) and the mechanical force (resulting from cross-bridges and mechanical linkages within the lattice) are considered attractive. In addition, there should be an entropic mixing force contributed by the filaments themselves which is expansive. It seems likely that this expansive force is of the same magnitude as axial tension, based on the osmotic pressure needed to recompress the lattice to its spacing in the intact cell (Brenner and Yu, 1991). In the analysis of Maughan and Godt (1980), it is principally the equilibrium between this force and the elastic restraining forces on the lattice that determines the lattice spacing of an uncompressed skinned fiber. In the intact cell, this entropic force is partially balanced by an additional osmotic constraining force provided by the cell membrane. In this scheme, the change in the balance of forces accompanying activation would be caused by the formation of cross-bridges and the contribution of their (compressive) radial force. If the dependence of lattice spacing on radial forces found in relaxed skinned frog muscle by Matsubara et al. (1984) is similar in the intact fiber lattice, then the compression of the intact fiber lattice by 0.771 nm during the redevelopment of isometric tetanic tension would require a radial force of about 74 pN per thick filament, assuming that activation produces only an increase in compressive radial forces within the lattice. For the reasons discussed earlier in the methods section, these values are likely to underestimate somewhat the true lattice compression.

Our findings show that upon the onset of activation of the intact fiber in standard tonicity saline, there is an increase in lattice spacing that is largely attributable to a small amount of shortening against series compliance. When corrected for sarcomere length changes as a constant volume system, or performed under length clamp control, this initial expansion is reduced and sometimes completely abolished. However, we could not exclude the possibility that there may be an increase in expansive forces early in a tetanus. If so, this would lead to an underestimate of the radial force generated by the cross-bridges in skinned fiber experiments, where the difference in osmotic radial forces required to maintain the same lattice spacing in activated and relaxed fibers would not simply equal the radial force contribution of the cross-bridges, but the difference between compressive cross-bridge force and increased expansive force at that spacing. Our experiments with BDM lead us to the conclusion that the expansion of the lattice at the rise of tetanic tension is not caused by the appearance of an expansive force associated with calcium activation. The early expansion may be the result of a difference in behavior between the length of fiber exposed to the laser beam (0.25 mm) and the much longer length of fiber sampled by the x-rays (4 mm). An alternative explanation might be found in the failure of the fitted line in Fig. 1 to pass through the origin. If the fiber undergoes significant shortening before the development of the compressive cross-bridge force, its lattice spacing may be governed by the relationship in Fig. 1, which would predict a small increase in apparent lattice volume and, therefore, the expansion would be diminished but not eliminated in a plot of lattice volume. In preparations in which shortening upon activation was prevented or reduced (i.e., under length clamp control), the expansion would be less evident and might disappear completely. The findings of other groups (Huxley, 1953; Rome, 1968; Millman el al., 1981) suggest that the intact fiber maintains a constant volume system over a much greater range of sarcomere lengths than that we examined. Nevertheless, our static changes in spacing with sarcomere length agree well with the dynamic increase in apparent lattice volume that we observe in response to the stretch of a relaxed fiber. Furthermore, one group (Haselgrove and Huxley, 1973) has reported a dependence of lattice spacing on sarcomere length even further removed from a constant volume system than that reported here. However, we cannot exclude the possibility that the apparent lattice expansion upon activation may be associated with the partially synchronized entry into the AM·ADP·P state that predominates upon activation of a relaxed fiber. The subsequent compression would then indicate the change to a subsequent state that predominates in the asynchronous cross-bridge cycling during an isometric tetanus, and is associated with a compressive force between filaments. If a repulsive force associated with the AM·ADP·P state were small compared with the compressive force of other (later) states in the cross-bridge cycle, it would only be evident when cross-bridge activity was synchronized, which must occur to some extent during the rise of tetanic tension. The possibility that different cross-bridge states might have different equilibrium spacings (and, therefore, potentially different directions of radial forces at some lattice spacings) has already been proposed (Xu et al., 1993).

When activated fibers are allowed to shorten, the loss of axial tension causes a rapid increase in lattice spacing that resembles the recoil of a strained elasticity when the distorting force is suddenly removed. When the discharge of axial force was smaller because of a slower shortening velocity, the recoil of the radial elasticity was also reduced. As can be seen in Fig. 6, apparent lattice volume also increases as a result of the discharge of axial force, but by a smaller relative amount. This cannot be explained by the deviation from true constant volume behavior seen in Figs. 1 and 2 because the effect of this would be to cause a reduction in apparent lattice volume rather than an increase. Instead, we believe that this is because some of the lattice spacing change accompanying the loss of axial tension results from the shortening of the fiber, which behaves more like the relaxed preparation, with quasi-constant volume properties. Nevertheless, during this period of low axial force it is known that in our preparation substantial numbers of cross-bridges are still attached (Cecchi et al., 1991), so it would appear that attachment per se is not responsible for the decreased lattice spacing during activation, and the radial stiffness of the activated fiber at zero axial force seems equivalent to that of the relaxed preparation inasmuch as the lattice spacing at a given sarcomere length corresponds with that of the relaxed fiber. What clearly emerges from these results is that both in the response of lattice spacing to rapid unloading of axial tension by shortening, and in the similarity of the time course of the loss of radial compression and that of axial force relaxation at the end of a tetanus, there is a clear correlation between the behavior of axial and radial forces.

Under hypertonic conditions (1.4× standard osmolarity), activation of the fiber causes virtually no change in lattice spacing, and isotonic shortening causes an increase in lattice spacing largely accounted for by constant volume behavior of the relaxed preparation. The reduction of radial forces arising from cross-bridge formation in the compressed lattice is supported by the virtual absence of a recompression upon tension redevelopment. In hypotonic conditions (0.8× standard osmolarity) the reverse is true; much larger changes in lattice spacing are observed than in the standard Ringer osmolarity, which cannot be accounted for by constant volume constraints. Although a change in tonicity also affects axial force, the sensitivity of lattice spacing changes to axial force levels seen in Figs. 5 and 7 suggests that such a pronounced effect of tonicity on lattice spacing changes could not arise from alteration of the peak tetanic force levels. So despite the apparently strong correlation between axial and radial forces seen in response to changes in axial force, changes in tonicity can virtually abolish or greatly increase radial force with relatively little effect on axial tension. Such behavior is consistent with the predicted alterations in the disposition of the S2 component of the cross-bridge, whose angle of contact with the myosin filament would increase as lattice spacing increased, leading to a variation in the magnitude of the radial

component of cross-bridge force exerted on the lattice. If, as seems plausible, the angle between S2 and the myosin filament axis were small, then effect on the radial force of lattice spacing changes would be much more pronounced than than on axial force, yet mechanical interventions that reduced axial force would have a similar effect on radial force. Furthermore, in such an arrangement the ends of the S2 element would be required to act as hinges; otherwise, no radial force could result from exertion of axial tension. However, the change in radial force seems very pronounced compared with the expected change in S2 orientation, given a length of 40 nm for S2. This should lead to a change in S2 angle at the myosin filament of at most 5° for an attached cross-bridge going from 1.4× tonicity to 0.8×, which would be accounted for much better by a larger change in angle with lattice spacing at the S2 junction. This could be achieved either by having the free portion of S2 much shorter than its full length (Bagni et al., 1990) or by having S2 attach to myosin in such a way that the projection of S2 into a plane containing actin and myosin filaments was much shorter than its true length. Indeed, such an angled attachment of S1 seems a requirement of the cross-bridge model, because most recent descriptions of the dimension and attachment of the S1 head show its length to be 16–19 nm (Garrigos et al., 1992; Rayment et al., 1993), whereas the space between the surface of the actin and myosin filaments is only 12 nm at slack length. It should be noted that use of NaCl to vary saline tonicity and, hence, lattice spacing causes a sarcoplasmic ionic strength change that may also affect the magnitude of the radial force. However, it is apparent that in skinned fiber experiments (Brenner and Yu, 1991), where lattice spacing was changed at constant ionic strength, the lattice compression upon activation showed a similar dependence on lattice spacing to that reported here in intact cells.

Thus, the change in spacing accompanying the release of an activated fiber has two components, one arising from the quasi-constant volume relationship of the relaxed preparation, the other from the loss of axial force. It is of interest that the static equilibrium spacing of Brenner and Yu (1991) for activated fibers (that spacing at which activation causes neither compression nor expansion of the lattice) corresponds to 39 nm between adjacent myosin filaments, close to our spacing in 1.4× hypoosmotic saline (38.36 nm) at which we also observe a marked reduction of lattice compression component associated with the loss of axial force. A similar value for the equilibrium spacing of nearest neighbor thick filaments (38.10 nm) is quoted by Matsubara et al. (1984) for the comparison of the rigor and relaxed states. This suggests that the radial forces detected in static measurements on the skinned fiber preparation have the same origin as dynamic forces we observe in living cells. However, skinned fibers under rigor conditions failed to show any change in lattice compression when axial force was varied (Matsubara et al., 1985), unlike the close correlation between axial and radial forces we report here. Also, the static radial stiffness of rigor fibers is also much higher than in fully calcium-activated preparations (Brenner and Yu, 1991), although a similar number of attached cross-bridges are thought to be present (Goldman and Simmons, 1977). These results could be accommodated within the double-hinged cross-bridge model if it were assumed that under rigor conditions the S1-S2 joint became locked, as a result fixing the angle between the S2 moiety and the axis of the myosin filament and, therefore, the lattice spacing. In this situation, the cross-bridge would become a rigid structure, and axial force changes would no longer alter lattice spacing. However, because this hinged region is at a considerable distance from the nucleotide binding site on the S1 moiety, such a mechanism would require interaction at a surprisingly long distance along the myosin head.

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