Extensibility of the Myofilaments in Vertebrate Skeletal Muscle as Revealed by Stretching Rigor Muscle Fibers

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ABSTRACT The extensibility of the myofilaments in vertebrate skeletal muscle was studied by stretching glycerinated rabbit psoas muscle fibers in rigor state and examining the resulting extension of sarcomere structures under an electron microscope. Although stretches applied to rigor fibers produced a successive yielding of the weakest sarcomeres, the length of the remaining intact sarcomeres in many myofibrils was fairly uniform, being definitely longer than the sarcomeres in the control, nonstretched part of rigor fibers. The stretch-induced increase in sarcomere length was found to be taken up by the extension of the H zone and the I band, whereas the amount of overlap between the thick and thin filaments did not change appreciably with stretches of 10–20%. The thick filament extension in the H zone was localized in the bare regions, whereas the thin filament extension in the I band appeared to take place uniformly along the filament length. No marked increase in the Z-line width was observed even with stretches of 20–30%. These results clearly demonstrate the extensibility of the thick and thin filaments. The possible contribution of the myofilament compliance to the series elastic component (SEC) in vertebrate skeletal muscle fibers is discussed on the basis of the electron microscopic data and the force-extension curve of the SEC in rigor fibers.

INTRODUCTION

In the sliding-filament model of contraction in striated muscle (Huxley, 1957), it has been assumed that both the thick and thin filaments are effectively rigid, so that the forces exerted by the cross-bridges within a half-sarcomere sum additively. This assumption is supported by the X-ray diffraction studies (Huxley and Brown, 1967) that showed that there is no distinct change in filament lengths during isometric contraction in vertebrate skeletal muscle.

The mechanical behavior of active muscle can be explained by postulating an elastic component in series with the contractile component (Hill, 1938). In vertebrate skeletal muscle, the extension of the series elastic element (SEC)

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J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/83/04/0531/16 $1.00 531
Volume 81 April 1983 531–546

under the maximum isometric force (P_o) is ~1% of the slack length (L_o) (Jewell and Wilkie, 1958), i.e., ~100 Å per half-sarcomere. Huxley and Simmons (1971*a*, 1973) found that the force-extension curves of the SEC in vertebrate skeletal muscle fibers were scaled down in proportion to the isometric force immediately before quick length changes. This has been taken as evidence that the SEC is not separate from the contractile component, but largely resides in the cross-bridges.

On the other hand, it has been pointed out that the dependence of the force-extension curves of the SEC on the magnitude of isometric force can also be accounted for by assuming a passive SEC having an exponential force-extension relation (T. L. Hill, 1975; Meiss and Sonnenblick, 1974; Podolsky and Teichholz, 1970). It seems possible that an elastic extension of the myofilaments, which is within the limit of accuracy of the X-ray diffraction measurements, takes place during isometric force generation in each sarcomere to contribute significantly to the SEC together with the elastic extension of the cross-bridges (White and Thorson, 1973). As a matter of fact, recent experiments of Ford et al. (1981) could not exclude the contribution to the SEC of myofilament compliance in tetanized muscle fibers.

The present work was undertaken to explore the possibility of myofilament extensibility in vertebrate skeletal muscle by stretching muscle fibers in rigor state and examining the resulting myofilament extension under an electron microscope. Since all the cross-bridges are permanently bound to the thin filaments in rigor state (H. E. Huxley, 1968; Reedy et al., 1965), rigor muscle fibers were stretched in the hope that the resulting increase in sarcomere length may be taken up by the extension of the myofilaments per se, but not by the sliding between them, so that their extension could be visualized under an electron microscope. It will be shown that both the thick and thin filaments are highly extensible, which suggests a significant contribution of their elastic extension to the SEC in vertebrate skeletal muscle fibers. Preliminary accounts of this work have already appeared (Sugi, 1979; Sugi and Suzuki, 1980).

METHODS

Preparation

Strips of rabbit psoas muscle (2–4 mm in width and 3–4 cm in length) were tied to glass rods, left to stand for 12–14 h in a solution containing 50% glycerol, 2 mM ethyleneglycol *bis*(β-aminoethylether)-*N*,*N*-tetraacetate (EGTA), and 10 mM histidine (pH 6.8) at 0°C, and then transferred to a fresh solution of the same composition for storage at −20°C until use (2–4 mo). Small bundles consisting of three to five muscle fibers (20–50 μm diam) were excised from the glycerinated muscle in a solution containing 20% glycerol, 2 mM EGTA, and 10 mM histidine (pH 6.8) under a dissecting microscope. The glycerinated fiber bundle preparation made in this way (~1 cm at slack length, L_o) was mounted horizontally in a glass experimental chamber (3 ml) filled with a relaxing solution containing 65 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 5 mM ATP, and 10 mM histidine (pH 6.8). Both ends of the preparation were glued with Aron alpha A adhesive (Sankyo Inc., Tokyo) between two stainless-steel rods (1 mm diam with tapered tips), one being connected to a force transducer

(U-gage, Shinkoh Tsushin Kogyo Inc., Tokyo), while the other was fixed in position. The force transducer was a strain gauge that had a compliance of 1 μm/g and a resonance frequency of 150 Hz. The sarcomere length was measured by optical diffraction with He-Ne laser light at several points along the length of the preparation and was proved to be fairly uniform in relaxed state. In most cases, the initial sarcomere length was adjusted to 2.10–2.30 μm with a micromanipulator carrying the strain gauge, the movement of which was sensed by a differential transformer (Trans-Tek Inc., Ellington, CT). The length and force changes were simultaneously recorded on an ink-writing oscillograph (Recticorder, Nihon Koden Kogyo Inc., Tokyo).

All experiments were performed at room temperature (18–20°C).

Electron Microscopy of Rigor Fibers

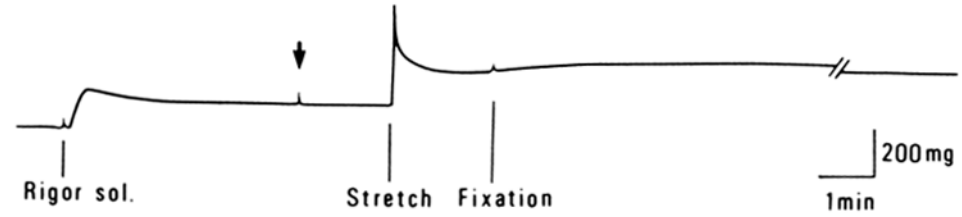
The preparation was put into rigor state by applying a rigor solution containing 65 mM KCl, 5 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM histidine (pH 6.8) unless otherwise stated. When the isometric force, which was almost as large as the maximum Ca-activated isometric force (2–3 kg/cm^2; Fig. 1A), was fully developed, the middle of the preparation was further glued to another stainless-steel rod which was also fixed in position. The fibers showed no appreciable tension redevelopment after a quick release applied at >3 min after the isometric force development, which indicates the establishment of rigor force. Then, one half of the preparation between the strain gauge and the middle rod was stretched by 10–30% with velocities of <1 mm/s, while the length of the other half remained unchanged to serve as control. The tension in the stretched half continued to rise during stretch and started to decay at the end of stretch to reach a new steady level higher than the initial isometric force (Fig. 1A). The force developed by a given amount of stretch showed a wide range of variation. At the end of 10% stretches, for instance, the force attained was 5.3 ± 1.8 P_o (n = 8). The large variation of stretch-induced force may be due to the rupture of myofibrils (Fig. 1B), the degree of which differed from preparation to preparation.

As soon as the final steady isometric force was attained after stretch, the whole preparation was fixed by replacing the rigor solution with a 2.5% glutaraldehyde solution (pH 7.2 by 0.1 M cacodylate buffer, containing 2 mM $CaCl_2$). The preparation was further postfixed in 2% OsO_4 (unbuffered), dehydrated with a graded series of ethanol, and embedded in Epon 812. Ultrathin sections (thickness ~700 Å) were cut on a Porter-Blum MT-2 ultramicrotome (DuPont Instruments-Sorvall, DuPont Co., Wilmington, DE) with a glass knife and double-stained with uranyl acetate and lead citrate. Care was taken to cut sections with a knife edge parallel to the long axis of the fibers, so that there was no compression of sections in the longitudinal direction during the cutting stroke. The section were examined and photographed with a Hitachi HU-12AS electron microscope (Hitachi Ltd., Tokyo), and magnifications were calibrated with a diffraction grid. The lengths of sarcomeres and sarcomere structures were measured on electron micrographs of longitudinally sectioned fibers. The Student's t distribution was used to determine the significance of the difference between the stretched and nonstretched sarcomeres.

Physiological Experiments

The force-extension relations of the SEC in rigor and Ca-activated muscle fibers were examined by applying quick length changes (complete within 0.5–1 ms) and recording the resulting force changes (see Fig. 6); it was assumed that the force changes coincident with the quick length changes were due to the response of the SEC (Jewell

and Wilkie, 1958). For this purpose, the preparation (L_o, ~0.5 cm) was mounted horizontally between the extended shaft of a servo-motor (GP-100; General Scanning Inc., Watertown, MA) and another force transducer (AE 802; Aksjeselskapet Mikro-Elektronikk, Horten, Norway) having a compliance of 2 μm/g and a resonance frequency of 5 kHz. The servo-motor contained a built-in differential transformer



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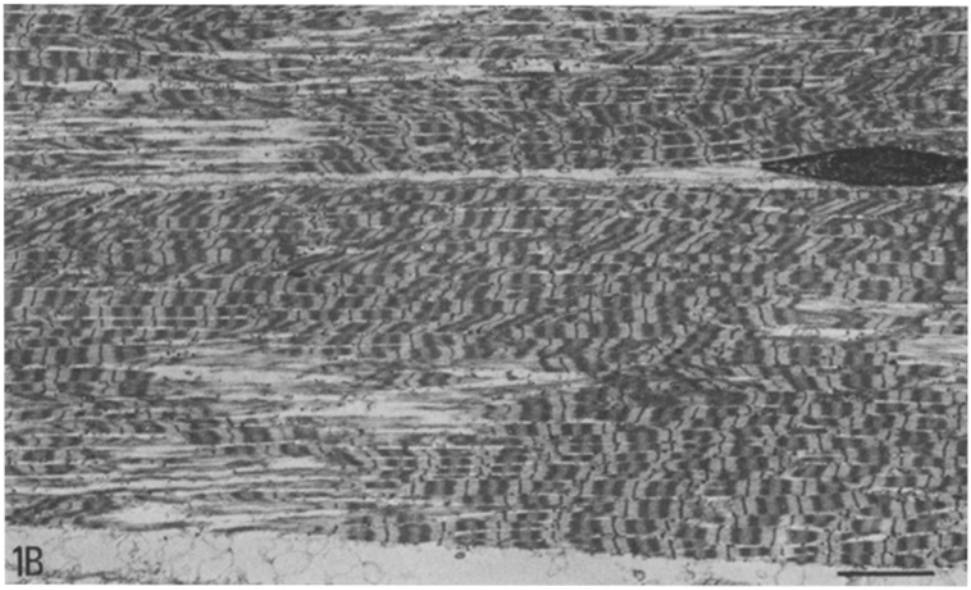


FIGURE 1. Stretch-induced force development and structural changes in glycerinated rabbit psoas muscle fibers in rigor state. (A) Typical force record during the procedure used to study the myofilament extensibility. The preparation was first put into rigor state, and when steady rigor force was developed, the middle of the preparation was glued to a stainless-steel rod (arrow) to divide the fibers into two parts. Then, one half of the preparation was stretched by 10% and fixed with glutaraldehyde for electron microscopic observation. (B) Low-magnification electron micrograph of rigor fibers in longitudinal section showing rupture of myofibrils after a 30% stretch. Note that there are still many intact sarcomeres with fairly uniform length. Bar, 5 μm.

that sensed the length changes of the preparation. The maximum Ca-activated contraction was produced by applying a contraction solution, which was prepared by adding $CaCl_2$ to the relaxing solution to result in pCa 5.3, taking the apparent stability constant of Ca-EGTA complex to be 1.95×10^6/M (Portzehl et al., 1964; Tanaka et al., 1979).

The force-extension curve of the SEC was obtained by plotting the force immediately after a quick change in fiber length against the corresponding amount of length change (Fig. 6). The force and the length change were expressed relative to P_o and L_o, respectively.

RESULTS

Stretch-induced Increase in Sarcomere Length

The sarcomere length in the control nonstretched part of rigor muscle fibers was always fairly uniform everywhere, whereas it was variable in the stretched part because of local damage to the myofibrils as reported by previous authors (Herlihy et al., 1972; Mulvany, 1975). At the damaged region, the sarcomeres were overextended or ruptured to result in a disappearance of normal sarcomere structures (Fig. 1B). The proportion of the damaged sarcomeres in the stretched half of the preparation increased with an increasing amount of stretch. In spite of the local sarcomere damage, however, many myofibrils were still observed in which the length of remaining intact sarcomeres was fairly uniform and definitely longer than that in the nonstretched half of the preparation. Fig. 2 shows examples of longitudinal sections of the myofibrils in the control nonstretched part (A) and the stretched part (B) of rigor muscle fibers. It can be seen that the sarcomere length, i.e., the distance between adjacent Z lines, is increased as a result of stretch. The extent of increase in sarcomere length in the stretched part was roughly proportional to the amount of stretch (see Figs. 3 and 4 and Tables I and II).

These results indicate that although stretches applied to rigor muscle fibers produce a successive yielding of the weakest sarcomeres (Mulvany, 1975), the remaining intact sarcomeres can be stretched fairly uniformly. This may be taken to imply that, in many myofibrils, the force could still be transmitted across the damaged sarcomeres until they were completely ruptured; it may be that during the course of rise in force during a stretch a fairly uniform extension of intact sarcomeres takes place as well as the overextension and the rupture of weakest sarcomeres, and the decay of tension after the end of stretch (Fig. 1A) results from a still-continuing rupture of the myofibrils. If the rupture of the myofibrils takes place at the early part of stretch, the length of intact sarcomeres within the same myofibrils may not increase appreciably compared with the control part, since the force is no longer transmitted across the ruptured sarcomeres. As a matter of fact, in some myofibrils in the stretched part, the intact sarcomere length was not significantly different from that in the nonstretched part. In the comparison of sarcomere length between the nonstretched and the stretched parts, such sarcomeres were not taken into consideration.

Stretch-induced Extension of Sarcomere Structures

To determine by what sarcomere structures the stretch-induced sarcomere lengthening was taken up, comparisons were made between the sarcomeres in the control and the stretched halves of the fibers. Typical examples of electron micrographs showing the changes in sarcomere structures after two different

amounts of stretch are shown in Figs. 3 and 4. It will be seen that, in both cases, the increase in sarcomere length as a result of stretch is taken up by the extension of both the A and I bands. The A-band extension was found to be confined to its middle region, i.e., the H zone, where the thick and thin filaments do not overlap. On the other hand, the amount of overlap between the thick and thin filaments did not change markedly in spite of the marked increase in sarcomere length.

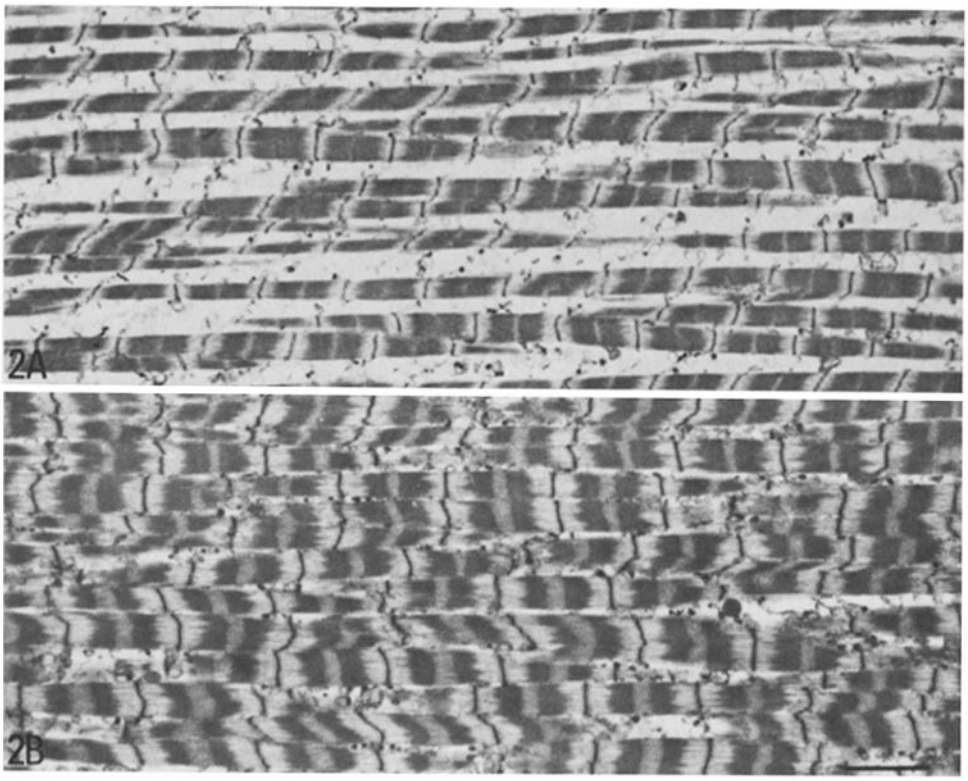
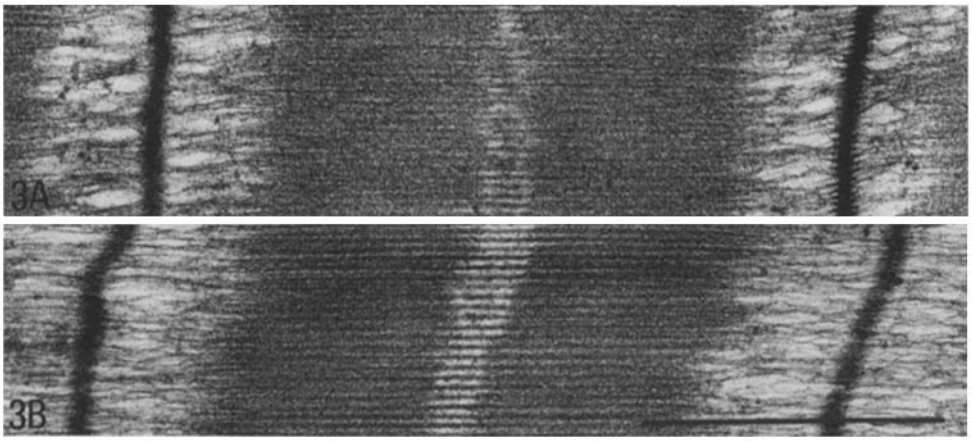


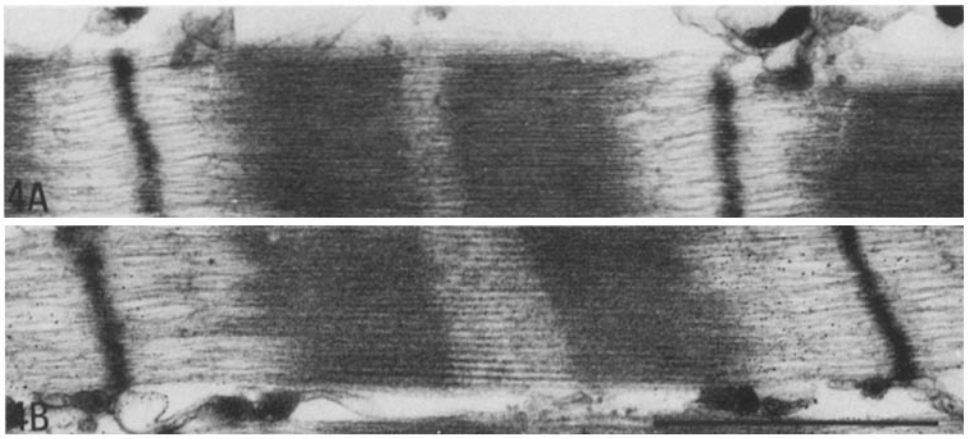
FIGURE 2. Longitudinal sections of myofibrils in the control nonstretched part (A) and the stretched part (B) of the fiber bundle preparation in rigor state. The amount of stretch applied was 30%. Note that the length of sarcomeres is fairly uniform in both cases, being definitely longer in B than in A. Bar, 2 μm.

Quantitative studies on the stretch-induced changes in sarcomere structures were made by measuring their average lengths within single sarcomeres in each myofibril on the electron micrographs of longitudinal sections of the myofibrils in the control and the stretched parts. Examples of the results obtained are given in Tables I and II. As shown in Table I, there was indeed no significant change in the amount on overlap between the filaments when rigor fibers were stretched by ~10%, although the lengths of the H zone and the I band were definitely increased. Similar results were obtained on five

other preparations stretched by 10–20%, the initial sarcomere length ranging from 2.10 to 2.30 μm.

With extreme stretches of ~30%, the results were somewhat variable; in two preparations examined, one (initial sarcomere length 2.16 μm) showed no significant change in the amount of overlap between the filaments, while the





FIGURES 3 and 4. Longitudinal sections of myofibrils showing stretch-induced extension of sarcomere structures with two different degrees of stretch applied to rigor fibers. In each figure, A and B show the sarcomeres in the control and the stretched part, respectively. The amount of stretch was 10% in Fig. 3, and 30% in Fig. 4. Note that the increase in sarcomere length is taken up by the extension of the H zone and the I band, while the amount of overlap between the thick and thin filaments appears not to change appreciably. Bar, 1 μm.

other (initial sarcomere length 2.39 μm) exhibited a significant decrease in the amount of overlap, which indicates that a relative sliding between the filaments took place to a certain extent in addition to the marked elongation of the H zone and the I band (Table II).

These results demonstrate that both the thick and thin filaments are actually extensible, and that the relative sliding between the filaments is not readily produced when rigor muscle fibers with the initial sarcomere length of 2.10–2.30 μm are stretched by <30%, because of the presence of rigor linkages between the filaments, as was expected at the beginning of the present study.

TABLE I

STRETCH-INDUCED EXTENSION OF SARCOMERE STRUCTURES IN GLYCERINATED RABBIT PSOAS MUSCLE FIBERS IN RIGOR STATE

Length of sarcomere structure	Nonstretched part (Mean ± SD) $n = 100$	Stretched part* (Mean ± SD) $n = 100$	Level of significance
Whole sarcomere‡	2.30±0.04	2.58±0.05	<0.001
A band	1.52±0.05	1.62±0.05	<0.001
H zone	0.11±0.03	0.17±0.01	<0.001
Half-overlap zone	0.70±0.03	0.73±0.03	>0.3
Half-I band	0.39±0.03	0.48±0.02	<0.001

* The amount of stretch applied to the preparation was ~10%.

‡ Sarcomere length was measured as the distance between adjacent Z lines.

STRETCH-INDUCED EXTENSION OF SARCOMERE STRUCTURES IN GLYCERINATED RABBIT PSOAS MUSCLE FIBERS IN RIGOR STATE

Length of sarcomere structures	Nonstretched part (Mean ± SD) $n = 100$	Stretched part* (Mean ± SD) $n = 100$	Level of significance
Whole sarcomere	2.39±0.07	2.88±0.06	<0.001
A band	1.57±0.06	1.62±0.05	<0.001
H zone	0.11±0.01	0.54±0.06	<0.001
Half-overlap zone	0.72±0.03	0.55±0.06	<0.001
Half-I band	0.41±0.02	0.62±0.03	<0.001

* The amount of stretch applied was ~30%.

NATURE OF THE H-ZONE ELONGATION The marked extension of the H zone, accompanied by no significant decrease in the amount of overlap between the filaments (Figs. 3 and 4, Table I), was always observed with initial sarcomere lengths of 2.10–2.30 μm, including the range where all the

cross-bridges along the thick filaments could form rigor linkages with the thin filaments (e.g., Gordon et al., 1966). This suggests that the marked H-zone extension by stretch is localized in the bare region of each thick filament, i.e., the middle region lacking the cross-bridges.

In one preparation, serial transverse sections (thickness ~750 Å) were cut through the extended H zones. The simple hexagonal array consisting only of the thick filaments with no projections of the cross-bridges was always observed throughout the extended H zone, which is consistent with the view that the stretch-induced H-zone extension is due to the elongation of the bare regions of the thick filaments.

The nature of the H-zone elongation was further studied by putting the preparation into rigor state with a Ca-free rigor solution containing 1 mM EGTA instead of 1 mM $CaCl_2$. Although the resulting rigor force was <50% of the maximum Ca-activated isometric force, the dense M line at the center of the H zone was well preserved in the control nonstretched part of the preparation as well as a narrow zone of lower density on either side of the M line (Fig. 5A); the width of this whole central zone was ~1,500 Å, in agreement with the previous report (H. E. Huxley, 1972). The M-line structures at the center of the H zone were found, however, to disappear completely with stretches of >10%. With stretches of <10% on the other hand, some sarcomeres were observed to still exhibit the M-line structures, which were appreciably elongated compared with the control part (Fig. 5B). Since the M-line structures are believed to represent the bare region of the thick filaments (H. E. Huxley, 1972), these results also indicate that the stretch-induced H-zone extension may be due to the elongation of the bare regions of the thick filaments.

NATURE OF THE I-BAND EXTENSION The marked extension of the I band (Figs. 3–5, Table I) was due almost entirely to the extension of the thin filaments out of the zone of overlap between the filaments, since the width of the Z line did not increase markedly even with stretches of 20–30%. This indicates that the contribution of the elongation of Z-line structures to the I-band extension is negligibly small compared with that of the elongation of the thin filaments.

REVERSIBILITY OF THE STRUCTURAL CHANGES IN STRETCHED SARCOMERE When the preparation was stretched by 20–30%, released again to its initial length, and then returned to the relaxing solution, the isometric force in response to the subsequent application of the rigor or activating solution was reduced nearly to zero. Under the electron microscope, the remaining intact sarcomeres in such preparations also exhibited an appearance similar to that in Fig. 4B, which indicates that the extension of sarcomere structures was plastic and irreversible.

If, on the other hand, the preparation was stretched by 5–8% and then returned to the initial length, the reduction in the amount of the subsequent isometric force development (20–30%) was not so marked, and the remaining intact sarcomeres in such preparations were not clearly distinguishable in appearance from those in unstretched preparations.

These results suggest that, with stretches of <5–8%, the extension of sarcomere structures may be elastic and reversible, and the force-generating mechanism in the remaining intact sarcomeres may not be markedly impaired, though much more accurate studies are needed concerning the reversible elastic filament extension.

Force-Extension Curves

Fig. 6 shows typical force-extension curves of the SEC in glycerinated rabbit psoas muscle fibers, which were obtained by applying quick length changes to the fibers in rigor and Ca-activated states. The magnitude of force change for

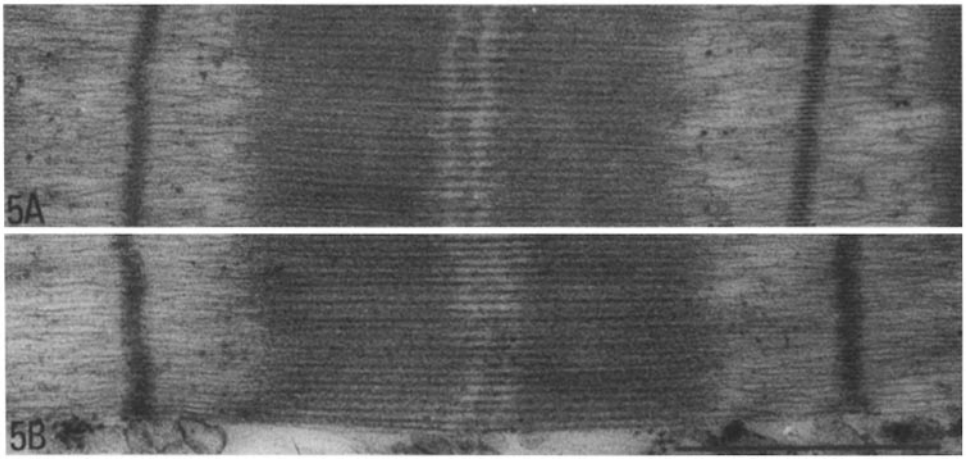


FIGURE 5. Longitudinal sections of myofibrils showing stretch-induced elongation of the M-line structures. The fibers were put into rigor state with a Ca-free rigor solution in which the M-line structures were well preserved. A and B show the sarcomeres in the control and the stretched part, respectively. The amount of stretch was 8%. Note appreciable elongation of the M line and the two adjacent zones of low density by stretch. Bar, 1 μm.

a given amount of length change was greater in rigor than in Ca-activated contraction. For stretches up to 1% and releases of <0.3%, the data points fell around a straight line in both rigor and Ca-activated states, so that the initial slope was the same for both quick stretches and quick releases. Similar results were obtained on 10 other preparations examined. The minimum amount of quick release required to drop P_o to zero was ~1% of L_o in rigor fibers, and ~1.5% of L_o in Ca-activated fibers. These values are analogous to those obtained by other investigators in intact, skinned, or glycerinated vertebrate skeletal muscle fibers (Goldman and Simmons, 1977; Güth and Kuhn, 1978; Heinl et al., 1974; Huxley and Simmons, 1971*a*; Yamamoto and Herzig, 1978).

DISCUSSION

Extensibility of the Myofilaments

The present experiments have clearly demonstrated a marked extensibility of the myofilaments; when glycerinated rabbit psoas muscle fibers in rigor state were stretched, the resulting increase in sarcomere length was found to be taken up by the extension of the H zone and the I band (Figs. 3 and 4). The

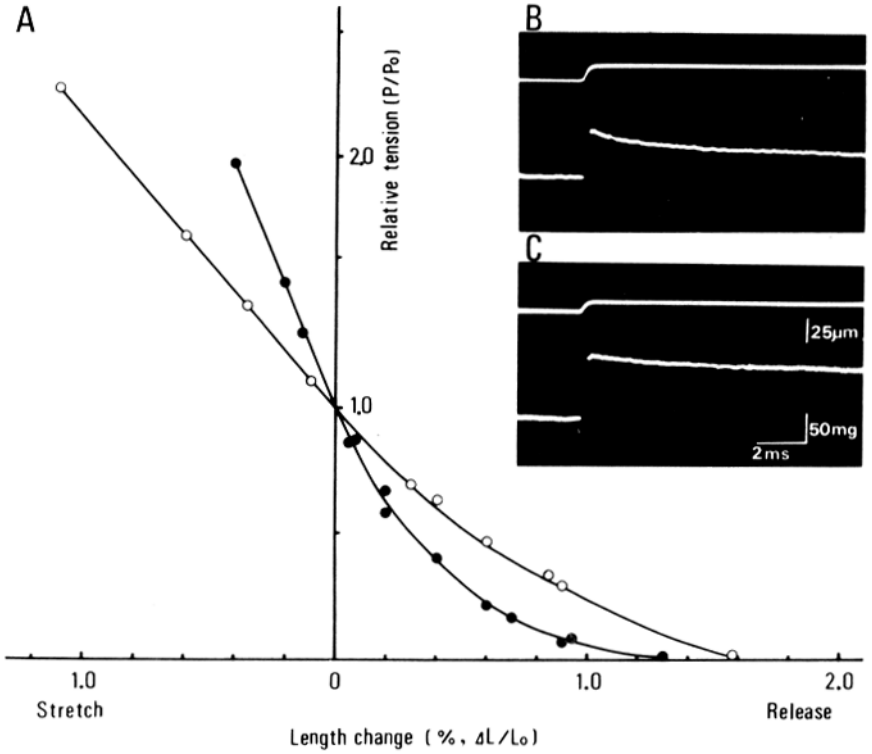


FIGURE 6. (A) Force-extension curve of the SEC in glycerinated rabbit psoas muscle fibers in rigor (filled circles) and in Ca-activated state (open circles). In this preparation, the isometric force was almost the same in both rigor and Ca-activated contraction. The force (P) immediately after a quick length change is plotted against the amount of length change (ΔL). P and ΔL are expressed relative to P_o and L_o, respectively. (B and C) Examples of length and force records when a quick stretch was applied to the preparation in Ca-activated state (B) and in rigor state (C).

extension of sarcomere structures appeared to be elastic and reversible if the amount of stretch was not too large. When rigor fibers with nearly maximum amount of overlap between the filaments were stretched by <20%, the amount of overlap between the filaments did not change significantly (Table I). This result, together with the stretch-induced extension of the M-line structures

(Fig. 5), may be taken to indicate not only the presence of tight rigor linkages to resist sliding between the filaments, but also the inextensibility of the thick filaments except for the bare region. The rigidity of the thick filaments except for the bare region may provide a mechanical condition in which the cross-bridge forces sum additively within a half-sarcomere, as has been supposed in the Huxley model (A. F. Huxley, 1957). On the other hand, the extension of the I band may indicate that the thin filaments can be extensible anywhere along their entire length.

The rate of extension of the bare region in the thick filaments was much more marked than that of the thin filaments in the I band; with extreme stretches of 30% (Table II), the I-band length increased by ~50%, while the length of the bare region was estimated to increase about threefold, even when the decrease in the amount of overlap between the filaments was taken into consideration. It seems possible that the rod portions of myosin molecules which form the thick filament (H. E. Huxley, 1963) stagger at the center of the thick filament as a result of stretch to produce a localized extension at the bare region. In this connection, it is of interest that in horseshoe crab striated muscle fibers, the marked length changes of the thick filaments are not accompanied by the change in the 14.5-nm period, which is believed to represent the period of myosin or paramyosin molecules forming the thick filament (Dewey et al., 1979); this also suggests that the thick filament length changes are due to the staggering of myosin molecules.

Concerning the extension of the thin filaments in the I band, on the other hand, there was no indication that their elongation was localized in some definite regions. It would be of interest to examine whether the helical pitch of actin monomers in the thin filaments (Hanson and Lowy, 1963) increases as a result of stretching rigor muscle fibers. It is also possible, especially in the case of extreme I-band extension with 30% stretches, that the thin filaments are not actually extended, but broken at many points and supported by some structures such as the gap filaments extending along the whole sarcomere.

In contrast with the present results, it has been reported that when mouse skeletal muscles in rigor state are stretched, the sliding between the thick and thin filaments takes place (Hegarty, 1972; Herlihy et al., 1972). It seems possible, however, that the muscles were not fully in rigor state, since the muscles still contained a fairly high concentration of ATP (0.7 μmol/g). On the other hand, Mulvany (1975) studied the effect of stretch on frog skeletal muscles in rigor state produced by iodoacetic acid poisoning and reported that stretch produced a successive yielding of the weakest sarcomeres while the lengths of the remaining intact sarcomeres remained unchanged. In his experiments, the measurement of sarcomere lengths was made by examining the histological sections under a light microscope or by laser diffraction, and there was a large dispersion in the length of sarcomeres as compared with the present study. It seems possible, therefore, that the extension of the filaments was obscured by the large dispersion in the sarcomere length, which might result, at least in part, from a nonuniformity in the development of rigor state among the component muscle fibers. The possibility should also be kept in

mind, however, that the response of the sarcomeres to stretch might vary according to the type of rigor induced.

Possible Contribution of the Myofilament Elasticity to the Series Elastic Component

Although the present electron microscopic work has revealed the detailed nature of the myofilament extensibility, it remains to be determined whether or not the reversible elastic extension and recoil of the myofilaments take place during contraction to contribute significantly to the SEC in muscle fibers. In rabbit psoas muscle fibers, the extension of the SEC at P_o was ~1.5% of L_o in Ca-activated state, and ~1% of L_o in rigor state (Fig. 6). In the contraction model of Huxley and Simmons (1971*b*), the SEC (or the instantaneous elasticity) largely originates from an elastic extension of the cross-bridges because of their head rotation during Ca-activated contraction. In rigor state, on the other hand, the cross-bridges are generally believed to be fixed on the thin filaments, so that their heads can no longer rotate (Heinl et al., 1974; Reedy et al., 1965). This view has been supported by the absence of the quick force recovery after a quick release in rigor fibers (Heinl et al., 1974) and by a lack of significant changes in the equatorial X-ray diffraction pattern (Podolsky et al., 1981) and in the angular distribution of the paramagnetic probes (Cooke, 1981) when rigor fibers are stretched. On this basis, the steeper force-extension relation of the SEC in rigor fibers than that in Ca-activated fibers (Fig. 6) (Goldman and Simmons, 1977; Heinl et al., 1974; Yamamoto and Herzig, 1978) may be due to the absence of the cross-bridge head rotation in response to quick length changes. This implies that the force-extension relation of the SEC in rigor fibers might largely originate from a reversible, elastic extension of sarcomere structures other than the cross-bridges.

The possible contribution of the myofilament elasticity on the instantaneous elasticity has recently been studied by Ford et al. (1981) by examining the force-extension relations at various amounts of overlap between the filaments, which suggests that ~80% of the instantaneous elasticity is attributable to the cross-bridge elasticity at full overlap between the filaments. In this connection, the present results indicate that the remaining 20% of the instantaneous elasticity may result from the elasticity of the thick and thin filaments but not from the Z line, since it has been shown in the present study that the Z line is very rigid. It should be mentioned that although the degree of contribution to the SEC of the thin filaments in the I band (or nonoverlap zone) can be estimated by changing the amount of overlap between the filaments, the degree of contribution of the thick filaments to the SEC cannot be readily determined, since the thick filament elasticity is believed to be confined to the bare regions (Fig. 5, Table I), the length of which cannot be varied experimentally.

It would be of interest to estimate the values of stiffness in rabbit psoas muscle fibers from the present results. In vertebrate skeletal muscle, the thick filaments are packed hexagonally ~450 Å apart from each other (Elliott et al., 1963; H. E. Huxley, 1953). If the entire cross-section of the fiber is assumed to be occupied by the myofilaments, the number of the thick filaments per

unit cross-sectional area is $\sim 6 \times 10^8/mm^2$. Since the thin filaments are located in trigonal positions between the thick filaments, their number per unit cross-sectional area is $\sim 1.2 \times 10^9/mm^2$. Since the maximum isometric force P_o at L_o in both Ca-activated and rigor states was ~ 2.5 kg/cm^2 or 0.25 N/mm^2 in the present study, the force exerted by the cross-bridges is ~420 pN/thick filament and ~210 pN/thin filament. If it is assumed that the force-extension curve for rigor fibers (Fig. 6) largely reflects the Hookean myofilament elasticity, the slope of the curve indicates an ~26-fold greater force on the myofilaments in intact myofibrils when rigor fibers are stretched by 10%.

On this basis, the values of stiffness of the myofilaments can be tentatively estimated from the experimental data such as shown in Table I. For example, the bare region of the thick filament is extended by ~0.06 μm when the sarcomeres are stretched by ~10%. Since the bare region is ~0.2 μm in length (Gordon et al., 1966), the extension of the bare region is ~30% when the force in the thick filament is increased ~26-fold, giving a stiffness times unit length of the bare region of $\sim 3.6 \times 10^4$ pN. Meanwhile, the thin filament is extended at the nonoverlap zone by ~23%, and the same argument leads to a thin filament stiffness times unit length of $\sim 2.4 \times 10^4$ pN, a value not much different from that estimated by White and Thorson (1973) (1.8×10^4 pN), assuming that the myofilaments are extended by ~0.5% under the maximum isometric force P_o, an amount which is below the limit of accuracy of X-ray diffraction measurements.

The total contribution of the myofilament elasticity to the SEC in glycerinated rabbit psoas muscle fibers is determined by the degree of extension of the bare region of the thick filament and the thin filament in the I band with P_o. This can be simply estimated by drawing a line tangent to the force-extension curve at P_o and measuring its point of intersection with the horizontal axis (Fig. 6), being ~0.4% of L_o. On the other hand, Ford et al. (1981) are of the opinion that the total contribution of the myofilament elasticity to the instantaneous elasticity in tetanized fibers is <0.1% of L_o, the thin filament stiffness times unit length being on the order of 3×10^5 pN, a value ~10 times stiffer than the value estimated in the present study. Much more experimental work is needed on the physical properties of the myofilaments responsible for muscle contraction.

We wish to thank Prof. H. Hiramoto of the Tokyo Institute of Technology for suggesting the use of rigor muscles for studying the myofilament extensibility, and to Misses S. Gomi and N. Hirao for technical assistance.

Received for publication 21 January 1982 and in revised form 3 September 1982.

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