IS THE SII PORTION OF THE CROSS-BRIDGE IN GLYCERINATED RABBIT PSOAS FIBERS COMPLIANT IN THE RIGOR STATE?

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ABSTRACT To see whether the SII portion of the cross-bridge in rigor fibers is longitudinally compliant, we chemically cross-linked with dimethyl suberimidate the entire rod portion (including the SII portion) of myosin onto the surface of thick filaments in glycerinated rabbit psoas fibers, and studied the effect of the SII fixation on the stiffness of the rigor fibers. The cross-linking of fiber segments with full filament overlap increased the rigor stiffness by ~ 25%. Almost the same absolute amount of the stiffness increase was also observed in rigor fibers with half- or no filament overlap after the cross-linking, and a similar but somewhat larger increment of stiffness was observed in fiber segments cross-linked in relaxing solution. These results indicate that the stiffness increase is not produced by the fixation of the SII portion onto the thick filament surface, but is caused instead by the cross-linking of some parallel elastic elements in muscle, and therefore indicate that the SII portion of the cross-bridge is hardly longitudinally compliant in rigor fibers.

INTRODUCTION

The cross-bridge in active muscle is compliant (Ford et al., 1981). This compliance appears to play a key role in the force generation mechanisms in muscle (Eisenberg and Hill, 1978). The model of muscle force generation mechanism proposed by Huxley and Simmons (1971) to account for the isometric tension transients contains a series-compliant element in the cross-bridge, and the compliant element is tentatively put in the SII portion of the cross-bridge in the model. As pointed out by Huxley (1974), the structure and the nature of the cross-bridge compliance are not known.

In an attempt to elucidate the nature of the compliant element, we studied the stiffness of glycerinated rabbit psoas fibers in the rigor state. One of the main advantages of using rigor fibers for the stiffness study is that it is easy to study the passive physical nature of elastic elements in muscle.

Rigor fibers are compliant and they can be stretched by ~ 1% of the slack length under an externally applied force corresponding to the maximum isometric tension of active muscles. The magnitude of the stiffness in rigor fibers is close to that in fully activated muscle fibers (Goldman and Simmons, 1977; Güth and Kuhn, 1978; Yamamoto and Herzig, 1978). In the preceding article, we showed that the sarcomere compliance in rigor fibers is mostly the cross-bridge compliance, (Tawada and Kimura, 1984); that in active intact frog fibers, likewise, is mostly the cross-bridge compliance (Ford et al., 1981). Thus, it seems that the physical nature of the compliant element in the cross-bridge in rigor fibers is similar to, or the same as, that in active muscle.

The question we had in this work was whether the SII portion in the cross-bridge comprising the SII portion and the SI heads is longitudinally compliant in rigor fibers. To answer this question, we fixed the SII portion of the cross-bridge on the surface of thick filaments by chemically cross-linking the entire rod portion (including the SII portion) of myosin onto the surface of the filaments (Sutoh and Harrington, 1977; Chiao and Harrington, 1979), and studied the effect of the SII fixation on the stiffness of rigor fibers. We will describe how the SII fixation hardly makes the cross-bridge stiffer and how, therefore, the SII portion in the cross-bridge is scarcely longitudinally compliant in rigor fibers. A preliminary account of the work was reported (Tawada and Kimura, 1983).

MATERIALS AND METHODS

Fiber Preparation

Glycerinated rabbit psoas fibers were prepared as previously described (Tawada and Kimura, 1984). To obtain a preparation of muscle fibers with a long sarcomere, fibers glycerinated in relaxed state (see Tawada and Kimura, 1984) were stretched slowly by a manipulator in a 50% mixture of glycerol and a relaxing solution (150 mM K propionate, 5 mM EGTA, 5 mM phosphate buffer of pH 6.8, 3 mM Mg acetate, 3 mM ATP) at 4° C. It took ~10 h to stretch a bundle of fibers from a sarcomere length at full filament overlap to a sarcomere length with small filament overlap. After the stretching, both ends of the fiber bundles were tied on a thin stick with cotton threads and stored in a 50% mixture of glycerol and a solution (100 mM KCl, 10 mM EGTA, 5 mM $MgCl_2$, 10 mM phosphate buffer of pH 6.8) at −20° C. The sarcomere length was

determined as previously described (Tawada and Kimura, 1984). The fibers were used within 3 mo of preparation.

Protein Preparation

Heavy meromyosin was prepared by chymotryptic digestion of rabbit skeletal myosin by the method of Weeds and Taylor (1975). Myosin was prepared by the method of Perry (1952). Actin was extracted from acetone-dried powder of rabbit muscle and purified by three successive cycles of polymerization and of depolymerization. Tropomyosin was prepared by the method of Baily (1948) with slight modification (Tawada et al., 1975).

Apparatus and Procedure

The apparatus for the stiffness measurements and the laser diffractometry of the sarcomere length changes, and the general procedures for these measurements were the same as previously described (Tawada and Kimura, 1984).

Cross-Linking of Glycerinated Rabbit Psoas Fibers

Glycerinated rabbit psoas fibers were cross-linked with dimethyl suberimidate (DMS) at 4°C, according to the method of Sutoh and Harrington (1977). For the cross-linking of rigor fibers, we used rigor solution containing DMS, i.e., 5.5 mM DMS, 80 mM KCl, 40 mM imidazole. For the cross-linking of relaxed fibers, we used relaxing solution containing DMS, i.e., 5.5 mM DMS, 5 mM Na_2ATP, 1 mM $MgCl_2$, 5 mM EGTA, 80 mM KCl, 40 mM imidazole. The pH of these solutions was 7.35 at 4°C.

The cross-linking of the SI and rod segments of myosin in muscle fibers was followed by the methods similar to those described by Chiao and Harrington (1979). Small (< 1 mm diam) bundles of muscle fiber were suspended in a cross-linking solution and cross-linked at 4°C. The cross-linking reaction was terminated by washing the fiber bundles three times with a solution (80 mM KCl, 40 mM imidazole of pH 7.0), and the fiber bundles were homogenized in a blender (Nippon Seiki Co., Ltd., Tokyo, Japan) in 80 mM KCl, 40 mM imidazole of pH 7.0. The homogenized fiber bundles (≃3 mg/ml) at various stages of cross-linking was digested by chymotrypsin. The proteolysis was started by adding 0.1 vol of 0.1 M EDTA and 0.1 vol of 3 mg/ml chymotrypsin to the homogenate and carried out for 15 min at 4°C. The digestion was terminated by adding phenylmethylsulphonyl fluoride (final concentration of ≃6 mM). After the termination of the digestion, the samples were applied to sodium dodesyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) The kinetics of cross-linking the SI and rod segments in myosin was determined by densitometry of the SI and rod bands on the gels.

To see whether the proteolytic digestion by chymotrypsin of the SI/rod hinge of myosin in muscle fibers was unaltered by cross-linking reaction, we used dimethyl 3, 3′– dithiobispropionimidate, a cross-linker containing a S–S bond cleavable by reduction (DTBP), instead of DMS for cross-linking muscle fibers, as had been used by Sutoh and Harrington (1977) to check the same question with synthetic-myosin thick filaments.

In the stiffness measurement study, a segment of single fiber, with one end of the segment tied to a force transducer and with the other end tied to the tip of a servomotor arm, was cross-linked in a cross-linking solution at 4°C. The cross-linking reaction was terminated by rinsing the fiber segment with rigor solution or relaxing solution and the stiffness was determined.

When the time-course of the stiffness change during the cross-linking reaction was followed, the stiffness was measured in the cross-linking solution. The pH of the cross-linking solutions is different from that of rigor or relaxing solution without DMS. However, the pH difference (0.35 pH unit) is not crucial for the stiffness measurements because the rigor stiffness is not sensitive to pH in this range (Tawada and Kimura, 1984). Free DMS has no direct effect on the stiffness, because the stiffness of rigor fibers was the same in rigor solutions with and without DMS.

Cross-Linking of Heavy Meromyosin-Actin-Tropomyosin Complexes

To study whether DMS cross-links heavy meromyosin (HMM) to thin filaments, and to study the binding capacity of HMM to thin filaments before and after DMS cross-linking, a solution of HMM-actin-tropomyosin complexes was mixed with DMS (final concentration, 3 mM). The concentrations of HMM, actin, and tropomyosin were 1, 0.45, and 0.1 mg/ml, respectively. Other conditions were the same as those for fiber cross-linking. The DMS cross-linking reaction was terminated by adding one volume of 0.2 M maleic acid (pH 6.0). The final pH was ~6.1. At this pH, DMS does not cross-link HMM-actin complexes, myosin filaments, or two subunits of rod in myosin at all (Kimura, unpublished observation). The extent of cross-linking of HMM-actin-tropomyosin complexes was examined with SDS-PAGE. The effect of the DMS cross-linking reaction on the binding capacity of HMM to thin filaments was studied by examining with SDS-PAGE the content of HMM in the supernatant and pellet fractions after centrifugation of the mixture solution to spin down F-actin (with tropomyosin and HMM bound).

Reagents

DMS was purchased from Nakarai Chemical Co. (Kyoto, Japan) and α-chymotrypsin was purchased from Millipore Corp., Bedford, MA

Other Solutions

Rigor Solution. 80 mM KCl, 40 mM imidazole (pH 7.0 at 0°C), 4 mM EDTA.

Relaxing Solution. 80 mM KCl, 40 mM imidazole (pH 7.0 at 0°C), 5 mM Na_2ATP, 2 mM $MgCl_2$, 5 mM EGTA.

RESULTS

Cross-Linking of Glycerinated Rabbit Psoas Fibers

The main strategy used in the study was (*a*) to fix the SII portion of the cross-bridge onto the surface of the thick filaments in rigor or relaxed fibers by chemical cross-linking, and (*b*) to compare the rigor stiffness values before and after the SII fixation. IF the SII portion is the only compliant element in the cross-bridge, the stiffness after SII fixation will increase at least five times, as is estimated in the Appendix.

It should be pointed out that it is not absolutely necessary to cross-link the SII portion of the thick filament surface for SII fixation, but the SII portion can be fixed by cross-linking both the light meromyosin (LMM) and SI portions on the filament surface, because the SII portion is between these two portions.

Harrington and his colleagues (cf., Sutoh and Harrington, 1977; Chiao and Harrington, 1979; Ueno and Harrington, 1981) showed that DMS cross-links initially the LMM portion, next the SII portion, and finally the SI portion of myosin on the thick filament surface. Therefore,

the LMM portion has been cross-linked when the rod portion is cross-linked, since the rod portion comprises the LMM and SII portions. Thus, to follow the SII fixation we monitored the chemical cross-linking of the rod and SI portions. Following the methods developed by Harrington and colleagues, we monitored the chemical cross-linking by measuring the amounts of un-cross-linked SI segment and un-cross-linked rod segment of myosin with SDS-PAGE after digesting cross-linked muscle fibers with chymotrypsin.

The proteolytic digestion of the SI/rod hinge of myosin with chymotrypsin is unaltered by the cross-linking reaction, as shown with synthetic myosin filaments by Sutoh and Harrington (1977). We also carried out a similar check with glycerinated rabbit psoas fibers in rigor, using DTBP, and found that the proteolytic digestion of the hinge was unaltered by the cross-linking reaction in the fibers as well (data not shown).

Fig. 1 shows the time-courses of the cross-linking with DMS of thick filaments in glycerinated rabbit psoas fibers at full filament overlap in rigor and relaxing solutions, and also of that in fibers at small (25%) filament-overlap in rigor. The three time courses of the cross-linking were nearly the same. The intensity of the rod band on the gel disappeared and that of the SI band decreased by 80%, within 5 h. This means that the SII portion of more than 80% of the cross-bridges is fixed on the surface of thick filaments within 5 h.

Ueno and Harrington (1981) showed that DMS does not cross-link SI to thin filaments and that DMS cross-linking of myofibrils does not affect the binding of SI to thin filaments, using SI-thin filament complexes in myofibrils. We carried out similar experiments using HMM-actin-tropomyosin complexes and obtained the same results (data not shown). It appears from these results that the SI portion of the cross-bridges is not cross-linked to and is not induced to detach from, thin filaments by DMS cross-linking of rigor fibers.

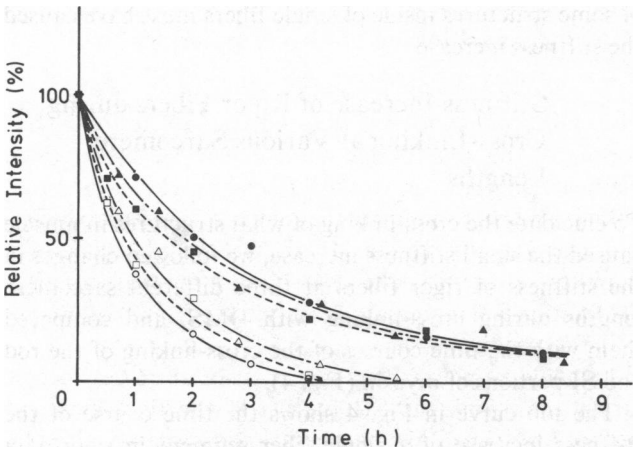

FIGURE 1 Time course of cross-linking rod and SI segments of myosin in glycerinated muscle fibers in rigor and relaxing solutions. The relative intensities of the rod band (○, □, △) and the SI band (●, ■, ▲) were determined by densitometry on SDS gels of cross-linked fibers after chymotryptic digestion. Cross-linking was carried out in rigor (○, ●; △, ▲) and in relaxing (□, ■) solutions (pH 7.35) with 5.5 mM DMS at 4 C. (○, ●; □, ■) 100% filament overlap (sarcomere length = 2.3 ± 0.1 μm [SD, n = 13]). (△, ▲) 25% filament overlap (sarcomere length = 3.4 ± 0.3 μm [SD, n = 10]).

Recently, Labbé et al. (1982) reported that small fraction of SI in acto-SI complexes is cross-linked to actin filaments by DMS at pH 8.3 and at 20°C. The difference of their result from ours and Ueno and Harrington's result may be due to differences in cross-linking conditions (pH 7.35 at 4°C in our case, and pH 7.0 at 20°C in Ueno and Harrington's case).

The fact that the time-course of the SI cross-linking relative to that of the rod cross-linking in glycerinated rabbit psoas fibers is virtually identical to that observed with synthetic filaments (Chiao and Harrington, 1979) also supports that the SI portion is not cross-linked to structures other than to the surface of the thick filaments in the fibers.

Fibers cross-linked with DMS in relaxing solution for 10 h at pH 7.35 were able to be stretched from a sarcomere length with full filament overlap to >4.3 μm in relaxing solution. This means that the SI portion is not cross-linked to thin filaments in relaxed fibers and no cross-bridge is formed by DMS cross-linking of relaxed fibers.

Stiffness Change of Rigor Fibers After Cross-Linking

To study the effect of the SII fixation on the rigor stiffness, we cross-linked segments of single fiber in rigor with DMS extensively (for 8 h), and compared the stiffness values of the fiber segments before and after the cross-linking. The length of the cross-linker is ~1 nm and short enough for the purpose of our experiments, because muscle fibers are stretched by ~10 nm per half-sarcomere for the stiffness measurement.

The stiffness was determined by measuring the tension increment at one end of a fiber segment while quickly stretching the other end of the segment (Tawada and Kimura, 1984). Fig. 2 shows examples of oscilloscope traces of the tension change produced by quickly stretching a segment of single fiber with full filament overlap in rigor, before and after the cross-linking. A larger response in the tension to a given stretch was observed after the cross-linking. The initial tension increment was plotted against the extent of the quick stretching (Fig. 3). The stress-strain relations before and after the cross-linking are linear. The slope of each linear line gives the stiffness. The rigor stiffness of the fiber segment at a sarcomere length with full filament overlap increased by 25% after the extensive cross-linking. One immediate conclusion from this fact is that the contribution of the SII portion to the cross-bridge compliance, even if the SII portion is compliant, is relatively small in rigor fibers (see the Appendix).

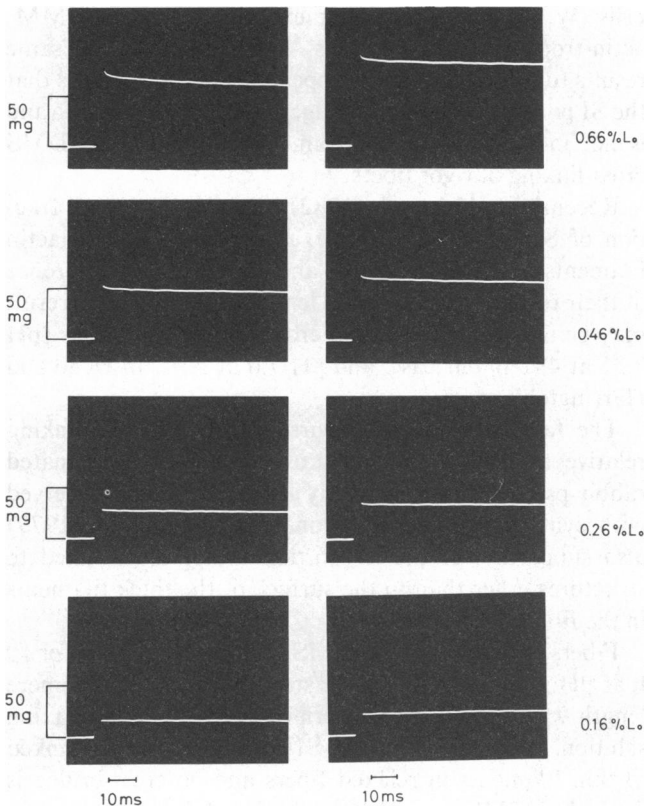

FIGURE 2 Tension changes due to stretch of a single fiber segment before and after cross-linking in rigor. After taking the records (*left*) in rigor solution at 10° C, the fiber was cross-linked in rigor solution with DMS at pH 7.35 at 4° C for 8 h. The fiber was transferred into another new DMS solutions at 3 and 6 h after the start of the cross-linking reaction. After the cross-linking, the fiber segment was rinsed with rigor solution without DMS and the records shown (*right*) were taken in the rigor solution at 10 C. Muscle segment length = 0.32 cm. Sarcomere length = 2.3 μm.

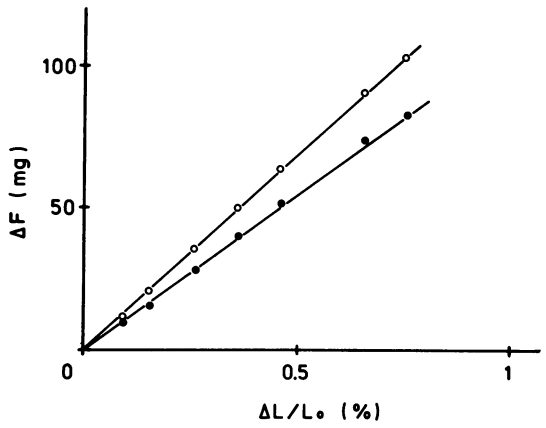

FIGURE 3 Stress-strain relations of a single fiber segment before and after cross-linking in rigor. (●) Before cross-linking. (○) After cross-linking for 8 h with DMS at 4° C. Muscle segment length = 0.32 cm. Sarcomere length = 2.3 μm. The stiffness was measured in rigor solution at 10° C.

There is a small contribution of the end compliance in the stiffness measurements of rigor fibers (Tawada and Kimura, 1984). When the length of a fiber segment is 0.3 cm, for example, ~80% of a length change given at one end of the fiber segment in rigor goes to the sarcomere at the middle of the segment. This means that the true stiffness of the fiber segment in rigor is 1/0.8 (=1.25) times larger than the observed value (see Appendix A of Tawada and Kimura. 1984). Thus, if the contribution of the end compliance disappeared during the cross-linking reaction, the rigor stiffness of a fiber segment after the cross-linking would be 25% larger than the original value obtained before the cross-linking as actually found.

To compare the contribution of the end compliance in the stiffness measurements of rigor fibers before and after the cross-linking with DMS, we measured, by laser diffractometry of the changes in sarcomere length, what percentage of a length change given at one end of a fiber segment in rigor goes to the sarcomere at the middle of the fiber segment (see Tawada and Kimura, 1984). About 80% of a length change given at one end of a fiber segment (0.31 cm long) went to the sarcomere after as well as before the cross-linking. Therefore, the small stiffness increase observed with rigor fibers after the cross-linking is not due to the disappearance of the contribution of the end compliance.

Single glycerinated rabbit psoas fibers still retain the permeable outer membrane. To see whether the cross-linking of the ghost membrane caused the small stiffness increase, we removed the outer membrane of a single fiber segment by the hand-skinning technique (Natori, 1954), and cross-linked the fiber segment with DMS. An increase of 25% of the rigor stiffness was again observed with the hand-skinned fiber segment at a full overlap sarcomere length after extensive cross-linking. Thus, the cross-linking of some structures inside of single fibers must have caused the stiffness increase.

Stiffness Increase of Rigor Fibers during Cross-Linking at Various Sarcomere Lengths

To elucidate the cross-linking of what structures in muscle caused the small stiffness increase, we followed changes in the stiffness of rigor fibers at three different sarcomere lengths during cross-linking with DMS, and compared them with the time courses of the cross-linking of the rod and SI portions of myosin (Fig. 4).

The top curve in Fig. 4 shows the time course of the stiffness increase of a single fiber segment in rigor at a sarcomere length with full filament overlap during the cross-linking with DMS. The stiffness increase leveled off in 4 h after the start of the reaction and ~25% increment of the rigor stiffness was observed.

A similar experiment was carried out at the sarcomere length with 50% filament overlap. Initially, the sarcomere length of a single fiber segment was set to a value with full filament overlap in relaxing solution. After transferring the

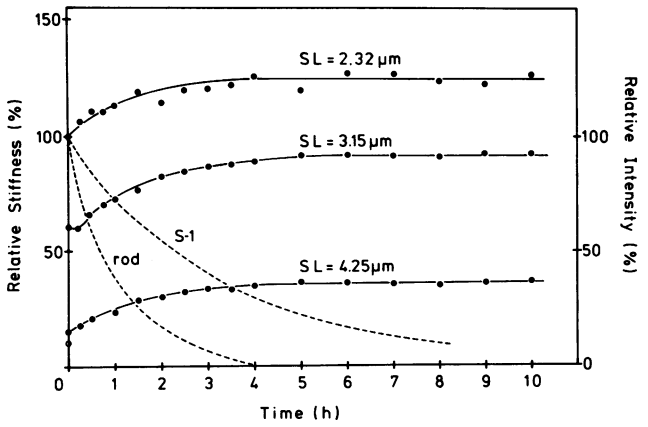

FIGURE 4 Time course of stiffness increase of single fiber segments at three different sarcomere lengths in rigor during cross-linking. *Top* curve: muscle segment length = 0.31 cm; sarcomere length = 2.32 μm. *Middle* curve: muscle segment length = 0.41 cm; sarcomere length = 3.15 μm. *Bottom* curve: muscle segment length = 0.47 cm; sarcomere length = 4.25 μm. (O) Resting stiffness at the sarcomere length of 3.15 and 4.25 μm. The cross-linking with DMS was carried out at 4° C and the stiffness was measured in the cross-linking solution. In each experiment, the cross-linking solution was replaced with new ones at 3 and 8 h after the start of the cross-linking reaction. Time-courses of cross-linking rod and SI segments in glycerinated fibers in rigor are shown by broken line (replotted from Fig. 1).

fiber segment to the rigor solution, the rigor stiffness at the full overlap sarcomere was determined, and the fiber was transferred back to the relaxing solution. In the relaxing solution, the sarcomere length of the fiber was set to a value with 50% filament overlap by manually stretching the fiber segment, and the resting stiffness was determined. After the transfer of the fiber segment to the rigor solution, the rigor stiffness of the segment at the new sarcomere length was determined. Then, the fiber segment was immersed in another rigor solution containing DMS and the change in rigor stiffness during the cross-linking reaction was followed. The resting stiffness and the rigor stiffness of the fiber segment at the intermediate overlap were normalized with the rigor stiffness of the segment at the full overlap. The middle curve in Fig. 4 shows the time course of the change in the rigor stiffness at the intermediate filament overlap. The rigor stiffness increased from 60 to 90%, leveling off in 4 h after the start of the cross-linking reaction.

The bottom curve in Fig. 4 shows the time course of the stiffness change of another segment in rigor at a non-overlap sarcomere length during the cross-linking reaction. Here again, the stiffness increased with time, leveling off in 4 h after the start of the cross-linking reaction. The stiffness increment was ~20%. The stiffness increase at the three different overlaps leveled off by the time when the rod of myosin was almost completely cross-linked but well before the completion of the cross-linking of the SI portion.

We carried out similar experiments with another 12 fiber segments at three different filament overlaps. The stiffness increase was characterized by measuring the stiffness increment and the half-time of the increment which is the time for the stiffness increment to reach the half-maximum. Table I summarizes the results. The half-times of the stiffness increment at these three different filament overlaps were nearly identical. The stiffness increments were also similar.

TABLE I
STIFFNESS INCREMENT AND HALF-TIME OF STIFFNESS INCREMENT IN DMS CROSS-LINKING OF SINGLE FIBERS IN RIGOR AT THREE DIFFERENT FILAMENT OVERLAPS AT pH 7.35 AND 4°C

Filament overlap	Δ Stiffness	$t_{1/2}$
%	%	*h*
100	24 ± 2	1.1 ± 0.2
50	23 ± 4	1.3 ± 0.2
0	18 ± 4	1.3 ± 0.1

Muscle segment length = 0.30 ± 0.02, 0.42 ± 0.02, and 0.48 ± 0.03 cm for 100%, 50%, and 0% filament overlap, respectively. Sarcomere length = 2.31 ± 0.05, 3.12 ± 0.08, and 4.20 ± 0.11 μm for 100%, 50%, and 0% filament overlap, respectively. Values are means ± SD (n = 5).

If the small stiffness increase observed during the cross-linking was due to the fixation of the SII (or a part of the SII) portion onto the surface of the thick filaments, the stiffness increment would be smaller with the decrease of the filament overlap. However, this was not the case within the experimental errors (Table I). Thus, most (if not all) of the small stiffness increase is likely produced by cross-linking of some other structures in muscle.

Since the time course of the stiffness increase at the non-overlap sarcomere was similar to that at the full overlap and intermediate overlap sarcomeres, the stiffness increase may be due to cross-linking with DMS of some parallel elastic components in muscle such as connectin (Maruyama et al., 1977).

Stiffness Increase of Relaxed Fibers during Cross-Linking Reaction

If the small stiffness increase observed with rigor fibers during the cross-linking reaction was due to the cross-linking of some parallel elastic components in muscle, one would also observe a similar stiffness increase with relaxed fibers. This was the case as shown in Table II. The stiffness of segments of relaxed single fiber with full filament overlap increased from 0 to 40% after the cross-linking for 3.5 h with DMS.

When the cross-linked fiber segments were transferred to rigor solution, the fibers went into rigor and the stiffness increased. The rigor stiffness was 22% larger than that of the fibers in rigor before the cross-linking (Table II). This value is close to the stiffness increment observed with fibers cross-linked in rigor solution (see Table I).

TABLE II
STIFFNESS CHANGES OF SINGLE FIBER SEGMENTS AFTER CROSS-LINKING IN RELAXING SOLUTION WITH DMS FOR 3.5 *H*

	Solution	Stiffness
Before cross-linking	Rigor	100
	Relaxing	0
		mean ± SD (n = 2)
After cross-linking	Relaxing	40 ± 1
	Rigor	122 ± 9
	Relaxing	40 ± 1

Muscle segment length = 0.35, 0.34 cm; sarcomere length = 2.35 μm. Cross-linking condition: 5.5 mM DMS, 80 mM KCl, 40 mM imidazole (pH 7.35), 5 mM Na_2ATP, 1 mM $MgCl_2$, 5 mM EGTA, 4°C. The stiffness of the cross-linked fiber segments was first measured in relaxing solution, then in rigor solution, and again in relaxing solution, without DMS.

DISCUSSION

This study showed that the stiffness of glycerinated rabbit psoas fibers in rigor, when cross-linked with DMS, increased by ~25% by the time the rod of myosin was almost completely, but the SI portion of the cross-bridge was not yet completely, cross-linked onto the surface of the thick filaments. After that, there was no more increase in stiffness although the cross-linking of the SI proceeded further until its completion. A similar absolute value for the rigor stiffness increment was observed at the full overlap, intermediate overlap sarcomeres, and even at non-overlap sarcomere length. A similar but somewhat higher value for the stiffness increment was also observed after cross-linking of relaxed fibers with DMS.

These facts indicate that most of the stiffness increase observed after the DMS cross-linking of rigor fibers is attributable not to the fixation of the SII (or a part of the SII) portion onto the thick filament surface but rather to cross-linking of some parallel elastic elements. In other words, the SII fixation increases almost negligibly the rigor stiffness apart from the contribution of the stiffened parallel elastic elements. As is evident from the estimation given in the Appendix, this means that the SII fixation hardly makes the cross-bridge stiffer.

It is conceivable that the SII portion of the cross-bridge is longitudinally compliant by nature but the SII portion of the cross-bridge in rigor fibers can not be stretched because it is already tightly held down on the surface of the thick filaments in the fibers before the cross-linking. If so, fixation of the SII portion on the surface by chemical cross-linking will not have any effect on the rigor stiffness. However, this is not the case as reasoned below.

The SII portion of the cross-bridge in rigor fibers is detached from the thick filament surface at alkaline pH, e.g., pH 8.5, but moves in close contact with the surface when the pH is lowered to neutral pH, e.g., pH 7.0 (Ueno and Harrington, 1981). Nontheless, the stiffness of rigor fibers at pH 7.0 is the same as that at pH 8.5 (Tawada and Kimura, 1983*b*). Thus, the rigor stiffness is the same no matter when the SII portion is detached from, or held down on, the thick filament surface. This means that the SII portion of the cross-bridge in rigor fibers is not stretchable at all, or stretchable with the same extent of easiness, no matter when it is detached from, or held down on, the thick filament surface. Thus, the above possibility raised is not the case. The present chemical cross-linking study shows that the SII portion is hardly stretchable in rigor fibers.

That the SII portion of the cross-bridge in rigor fibers is not stretchable simply means that the passive physical nature of the SII portion is not longitudinally compliant. Thus, our result with rigor fibers does not rule out any other possible characteristics of the SII portion which may appear in active muscle such as the melting in the SII portion assumed to occur during the cross-bridge cycle in the muscle force generation model proposed by Harrington (1971, 1979).

There are several reports that the SII fragment of the myosin molecule is flexible. From the temperature-dependence study of the helix-coil transition of the SII fragment and the viscosity of the SII fragment, Tsong et al. (1979) reported that the SII particle is significantly flexible at physiological temperature. Highsmith et al. (1977) also reported the flexibility of the SII fragment based on the pulse fluorimetric study. However, these flexibilities do not necessarily mean that the SII fragment is longitudinally compliant, and therefore these results are not inconsistent with our results. Recently, Hvidt et al. (1982) described that the SII portion of the rod can not be longitudinally compliant, deriving from viscoelastic measurements of myosin rod solution.

Implications

In the preceding article, we showed that most of the sarcomere compliance in rigor fibers is assigned best to the cross-bridge compliance (Tawada and Kimura, 1984). The present study showed that the SII portion is unlikely to be compliant in rigor fibers. These two results lead to an implication that the SI head itself is compliant or the angle of the SI binding to thin filaments is not rigid in rigor fibers, because the cross-bridge comprises the SII portion and the SI heads.

On the other hand, x-ray (Naylor and Podolsky, 1981) and paramagnetic-resonance (Cooke, 1981) studies of rigor fibers have shown that the SI (or a domain of the SI) head bound to thin filaments does not rock in rigor fibers when the fibers are stretched. Inasmuch as the SI head has a "pear-like" shape with a thinner proximal end (Elliot and Offer, 1978), however, the x-ray study may not have detected such structural changes as those occurring in the thin proximal domain of the SI head in rigor fibers. If so, a possible explanation for the cross-bridge compliance in rigor fibers may lie in the assumption that there is such a length change or such an angle change in the proximal domain of the SI head as to cause compliance in rigor

fibers. The domain must be between the point in the SI of cross-linking to the thick filament and the position (the cysteine, designated SH_1, on the SI heavy chain) of the spin label used in the study by Cooke (1981), if we assume that the spin label measures the appropriate SI angle with respect to the thin filament. Recent work has shown that the location of the SH_1 is close to actin-binding sites on the SI heavy chain rather than to the C terminus of the polypeptide chain, i.e., the SI/SII joint (Gallagher and Elzinga, 1980; Sutoh, 1981, 1982, 1983). Therefore, the domain could be large enough for causing the compliance.

Conclusion

The passive physical nature of the SII portion in the cross-bridge is hardly longitudinally compliant in rigor fibers.

APPENDIX

Estimation of the Rigor-Stiffness Increment Due to the Fixation of the SII Portion in the Cross-Bridge onto the Surface of the Thick Filaments

The sarcomere compliance of muscle fibers in rigor (C_s) may be written as

$$C_s = C_c + C_o, \tag{A1}$$

where C_c is the cross-bridge compliance and C_o is the compliance of structures other than the cross-bridges in the sarcomere, such as the compliance of Z-membrane or thin filaments (see Appendix A of Ford et al., 1981 and Appendix B of Tawada and Kimura, 1984). As is apparent from Eq. A1, the sarcomere compliance defined here does not include the compliance due to parallel elastic elements in muscle. Their contribution will be discussed later. The sarcomere compliance is a function of the overlap between thin and thick filaments. In this Appendix, we are considering muscle fibers with full filament overlap.

Since the cross-bridge consists of the SI heads and the SII portion connected in series, the cross-bridge compliance may be given by

$$C_c = C_1 + C_2, \tag{A2}$$

where C_1 is the compliance of the SI heads and C_2 is the compliance of the SII portion. C_1 includes the compliance due to the bending or stretches or rocking of the SI head bound to thin filaments.

In the preceding article, (Tawada and Kimura, 1984), we estimated that the cross-bridge compliance comprises >80% of the sarcomere compliance. Thus, we assume

$$C_c/(C_c + C_o) = 0.8.$$

This gives

$$C_o = C_c/4 = (C_1 + C_2)/4. \tag{A3}$$

When the SII portion is fixed onto the surface of the thick filaments by the chemical cross-linking, $C_2 = 0$. In the following, we assume that the compliance of the SI heads is the same before and after the cross-linking reaction, and consider two extreme cases concerning the possible effects of cross-linking sarcomere structures responsible for C_o.

Case I. It is assumed that the cross-linking reaction has no effect on C_o.

The ratio of the sarcomere compliance after the SII fixation to that before the SII fixation is given by

$$\begin{aligned}\text{compliance ratio} &= (C_1 + C_o)/(C_1 + C_2 + C_o) \\ &= (5 - 4\gamma)/5,\end{aligned} \tag{A4}$$

where Eqs. A1, A2 and A3 were used and $\gamma = C_2/(C_1 + C_2)$ $(0 \le \gamma \le 1)$.

If the SII portion is compliant and the SI heads are not compliant in the cross-bridge before the SII fixation, then $\gamma = 1$. In this case, Eq. A4 shows that the sarcomere compliance after the SII fixation will be 1/5 of that before the SII fixation. In other words, the rigor stiffness will be five times greater after the SII fixation.

If the contribution of the SII portion to the cross-bridge compliance before the SII fixation is 25%, $\gamma = 0.25$. Then, Eq. A4 gives 4/5 for the compliance ratio. This means that the rigor stiffness will be 1.25 times greater after the SII fixation.

If the SI heads are compliant and the SII portion is not compliant before the SII fixation, $\gamma = 0$ and therefore the compliance or stiffness does not change after the SII fixation, as seen from Eq. A4.

Case II. It is assumed that the cross-linking reaction makes the sarcomere structures that are responsible for C_o very stiff.

Here we put $C_o = 0$ after the SII fixation and we have

$$\begin{aligned}\text{compliance ratio} &= C_1/(C_1 + C_2 + C_o) \\ &= 4\,(1 - \gamma)/5.\end{aligned} \tag{A5}$$

If the contribution of the SII portion to the sarcomere compliance is 100% before the SII fixation, $\gamma = 1$. Then, Eq. A5 shows that the sarcomere compliance will be zero after the SII fixation. Thus, the rigor stiffness will be infinitely large after the SII fixation.

If the contribution of the SII to the cross-bridge compliance is 25% before the SII fixation, $\gamma = 0.25$, and therefore the compliance ratio will be 0.6. Thus, the rigor stiffness will be 1.67 times greater after the SII fixation.

If the contribution of the SII to the cross-bridge compliance is zero before the SII fixation, $\gamma = 0$, and therefore the compliance ratio will be 4/5. Thus, the rigor stiffness will be 1.25 times greater after the cross-linking reaction for the SII fixation.

As a more general treatment, we should consider possible effects of cross-linking on C_1 as well. However, the results of calculation will be the same insofar as a 100% contribution of the SII portion to the cross-bridge compliance before the SII fixation is assumed.

Parallel elastic elements in rigor fibers do not contribute to the rigor stiffness at full filament overlap (Tawada and Kimura, 1984). If the parallel elastic elements are, however, assumed to become stiffer as a result of their crosslinking and to contribute to the rigor stiffness after the cross-linking, it is easy to show that the rigor stiffness will be more than five times greater after cross-linking the parallel elastic elements and the SII portion insofar as a 100% contribution of the SII to the cross-bridge compliance before the SII fixation is assumed.

In this Appendix, we neglected the contribution of the end compliance to the rigor stiffness, because the extent of the end-compliance contribution to the rigor stiffness is almost the same before and after the cross-linking of muscle fibers, as shown in Results.

To summarize, the rigor stiffness will increase at least five times after the SII fixation, if the SII portion is the only compliant element in the cross-bridge before the SII fixation.

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