PASSIVE INTERACTION BETWEEN SLIDING FILAMENTS IN THE OSMOTICALLY COMPRESSED SKINNED MUSCLE FIBERS OF THE FROG

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ABSTRACT Shortening and lengthening velocities, instantaneous stiffness, and tension transients after stretch were measured in compressed muscle fibers from the frog in the presence or absence of polyvinylpyrrolidone (PVP K30) or Dextran T70. Both shortening and lengthening velocities clearly decreased with the concentration of polymer. In the presence of polymer, "passive" stiffness was observed in relaxing solution depending on fiber diameter, and stiffness increased further by activation. This increase by activation above "passive" stiffness was nearly constant in the wide range of polymer concentrations. These active and "passive" stiffnesses were found to be dependent on sarcomere length. The stiffness of a compressed rigor fiber was indicated to be composed of constant rigor stiffness and a variable "passive" one. The tension transient after stretch in a compressed active or rigor fiber was also indicated to be composed of two kinds of transients. The above results suggest that (*a*) there exist two kinds of interactions in parallel in a compressed active or rigor fiber: one active or rigor and another "passive" between sliding filaments, and (*b*) the decrease in shortening velocity in a compressed fiber may be brought about by this "passive" interaction.

INTRODUCTION

It has long been known that a resting intact skeletal muscle has a special kind of elasticity which is due to a component lying between the two sets of filaments. Hill (1968) demonstrated that a resting muscle of frog possessed an elasticity ("short-range elastic component" [SREC]) and a resting tension ("filamentary resting tension" [FRT]), which were shown to rise to high values when the external solution was made hypertonic. It was suggested that these properties had an active rather than a passive origin (Hill, 1970).

On the other hand, Berman and Maughan (1982) measured the axial elastic modulus as a function of relative fiber width in relaxed skinned muscle fibers, and they found that the elastic modulus increased steeply as the fiber width was reduced. Their interpretation of this phenomenon was that the increase in the elastic modulus with compression might not reflect an active interaction but a rigor-like one between crossbridges and thin filaments.

The present experiment was undertaken to elucidate the properties of the interaction between thick and thin filaments in compressed relaxed, activated, and rigor skinned fibers. It is demonstrated that in a compressed activated or rigor fiber, there exist two kinds of interactions, one active or rigor and another "passive."

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METHODS

Preparation and Solutions

Skinned fibers were prepared from semitendinosus, or adductor magnus muscles of the frog *Rana catesbeiana* in relaxing solution (Natori, 1954). A segment of a single-skinned fiber, 2–4-mm long, was connected to the hooks from the tension transducer and from the arm of servo-motor by silk thread.

A fiber was immersed in one of 18 baths machined into a brass block, the surface of which was covered completely with silicone rubber. The block was recessed in a mount that allowed it to be lowered, moved sideways, and raised once a new bath was correctly positioned. Each bath had a glass floor through which a laser beam for the measurement of sarcomere length or illumination for microscopical observation passed.

The standard relaxing solution contained (mM) 5 ATP, 6 $MgCl_2$, 5 EGTA, 135 KCl, 10 imidazole (pH 6.8), 10 creatine phosphate, and 100 U/ml creatine kinase. Activating solution contained (mM) 5 ATP, 6 $MgCl_2$, 15 EGTA, 14 $CaCl_2$, 110 KCl, 10 imidazole (pH 6.8), 10 creatine phosphate, and 100 U/ml creatine kinase. Rigor solution contained (mM) 10 EGTA, 10 imidazole (pH 6.8), and KCl was increased so that total ionic strength might be equal to that in relaxing and activation solutions, and in some experiments 10 mM $CaCl_2$, was added to rigor solution. Dextran T70 (70,400 mol wt) or PVP K30 (40,000 mol wt) was added to the solution to increase the osmotic pressure in all the experiments except for the one to check the effects of viscosity, in which Dextran T500 (461,000 mol wt) was also used. The concentration of polymer referred to in the text denotes percentage (weight of polymer/volume of solution). All experiments were performed at 5°–10°C.

Measurements of Velocity, Stiffness, and Fiber Diameter

Servo-motor (model 303; Cambridge Technology, Inc., Cambridge, MA) was used for the measurement of isotonic shortening and lengthening velocity. The experiments for the measurement of unloaded shortening were performed using the so-called "slack test." For this purpose, a quick release of varying magnitude was carried out to allow a fiber to shorten between ~2.3- and 2.0-μm sarcomere lengths, and the slope of the length step vs. slack time was taken as the velocity of unloaded shortening (Edman, 1979).

Tension was measured by a tension transducer (model AE801; Aksjeselskapet, Horten, Norway), the resonant frequency of which was improved to 15 kHz in air. Increase in muscle length of 0.5% l_0 (l_0; muscle length) with a rise time of 0.7 ms was applied by a servo-motor (model G-100PD; General Scanning, Cambridge, MA), and length and tension changes during the stretch were displayed horizontally and vertically, respectively, on a digital oscilloscope (model 4094; Nicolet Instrument Corp., Madison, WI) or a storage oscilloscope (model 5113; Tektronics, Beaverton, OR). Stiffness was measured by the slope of a length–tension diagram during stretching (Güth et al., 1981).

The change in diameter of a fiber by polymer was recorded under a microscope (model SR; Zeiss, Oberkochen, West Germany) by a video system (model HSV-200; Nac Inc., Tokyo, Japan) before and after the change of solution; then the diameter of a fiber was measured by a scaler installed in the video system. Special care was taken so that a fiber might not be twisted when it was set in the bath. Sarcomere length was measured either directly by light microscopy (Diaphoto-TMD; Nikon, Tokyo, Japan) or by a helium neon laser (beam diam 1 mm) (model GLG 5350; Nihon Electronics Co., Tokyo, Japan) in the middle part of a fiber. In most of the experiments, the sarcomere length was adjusted to 2.0–2.3 μm in relaxing solution.

RESULTS

Time Course of Change in Fiber Diameter

When relaxing solution without polymer was replaced with one containing polymer, the diameter began to decrease rapidly until it reached a final value within 2 or 3 min; it did not change during immersion in polymer solution for more than 1 h (Fig. 1 *A*). However, bathing in a solution containing polymer for extended periods of time was avoided in order that the diffusion of the smaller polymer molecule into a fiber might be minimized. When the bathing solution was replaced with relaxing solution without polymer again, a fiber swelled very rapidly at first, then the diameter was restored gradually.

When a fiber was activated in polymer solution, a fiber was first equilibrated for more than 12 min in relaxing solution and for 3 min in relaxing solution with polymer, and then activated by activating solution with the same concentration of polymer. Diameter change on activation was measured (Fig. 1, *B* and *C*). On activation without polymer, the diameter increased by ~5% at the time of tension rise, and this was followed by a small reduction up to 5%; on relaxation it was restored quickly. (Fig. 1 *B*). When a fiber was activated in a moderate concentration of polymer, a transient increase in diameter took place, followed by the recovery as seen in Fig. 1 *C*. When effects

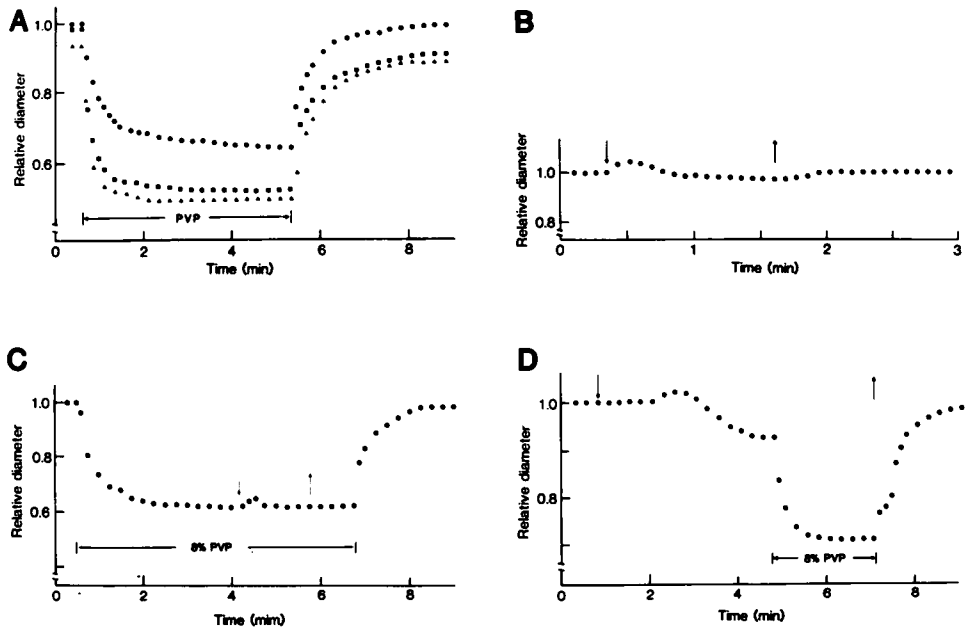

FIGURE 1 The relative diameter change of a skinned fiber with time. (*A*) The diameter change of a relaxed fiber in 8% (*circles*), 16% (*squares*), and 24% (*triangles*) of PVP. The period of the application of PVP is shown in the figure. (*B*) The diameter change of a fiber by activation without polymer. The moments of the beginning and the end of the application of activating solution are indicated by arrows oriented upward and downward, respectively. (*C*) The diameter change of a fiber activated in 8% PVP. The period of the application of PVP is shown in the figure, and the moments of the beginning and the end of the application of activating solution are indicated by arrows oriented downward and upward, respectively. (*D*) The diameter change of a rigor fiber in 8% PVP. The duration of the application of PVP is shown in the figure, and the moments of the beginning and the end of the application of rigor solution are indicated by arrows oriented downward and upward, respectively.

of osmotic compression on rigor were observed, a fiber was first made to be rigor and then compressed. A fiber shrank remarkably after a transient swelling when ATP and Mg ions were removed, and this rigor fiber shrank further in the presence of polymer as illustrated in Fig. 1 *D*.

Tension Development in Compressed Fibers

The effects of compression on tension development were examined in fully activated fibers (pCa 4.5) (Fig. 2). Active tension increased by the addition of small amounts of PVP (4%) or dextran (4–6%). The average relative tension in 4% PVP and 6% dextran compared with that in polymer-free solution was 1.21 ± 0.13 (n = 21) and 1.19 ± 0.26 (n = 12), respectively. The further increase in polymer concentration suppressed the tension development. The rapid transient tension rise as shown in Fig 2 *D* and *E* was induced by changing the solution from an activating one with high polymer to a relaxing one without polymer. This phenomenon might be brought about by the recovery of the diameter sooner that that of the Ca^{++} concentration inside a fiber. After the activation was repeated eight times in various concentrations of polymer, a fiber developed ~90% of the tension in the first activation, and the activation in a strong compression damaged a fiber less than in a weak compression.

Force–Velocity Relation and Velocity of No Load Shortening in Compressed Fibers

The force–velocity relationship in compressed fibers was obtained by isotonic shortening and lengthening, and the maximum velocity of shortening was calculated from Hill's equation fitted to the data points (Hill, 1938).

The records of isotonic shortening in the presence of 0% (*A*), 4% (*B*), 8% (*C*), and 12% (*D*) PVP are illustrated in Fig. 3, in which the relative load is all adjusted to 0.6. It is obviously seen that the shortening velocity decreases as the concentration of PVP increases. The records of isotonic lengthening under the same relative load (1.24 P_0) in the presence of 0% (*A*), 4% (*B*), 8% (*C*), 12% (*D*) PVP are illustrated in Fig. 4, in which it can be seen that the velocity of isotonic lengthening is slower with the increase of the concentration of PVP. Force–velocity data thus obtained in four different concentrations of PVP are shown in Fig. 5. The velocities of shortening and lengthening were measured 70 and 900 ms after the application of load step, respectively, and the data were from 18 different fibers. It should be noted here that the length vs. time traces within 50 ms after the release are curved, indicating that the shortening velocity decreases during the early phase after release. Therefore, if initial velocity just after release are plotted, the effects of dextran may be less pronounced (see Fig. 3 *C*). To know the maximum velocity of shortening, all data were first plotted on the scale for the linearized form of Hill's equation, and a mean line was determined by the least squares method. The solid line below P_0 in Fig. 5 is Hill's equation thus obtained. The maximum velocities of shortening Fig. 5 were 1.68, 1.23, 0.78, and 0.03–0.06 in 0, 4, 8, and 12% PVP, respectively. The velocities of isotonic lengthening in 0 and 4% PVP were not significantly different but were reduced remarkably in 8 and 12% PVP. With the current set up, no clear length transients were observed in normal and compressed fibers, as shown by Sugi and Tsuchiya (1981*a*, *b*)

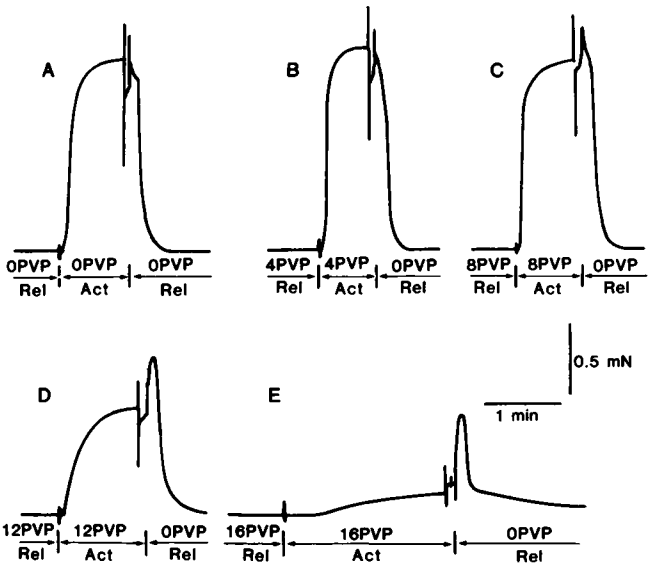

FIGURE 2 The tension development in 0%, (*A*), 4% (*B*), 8% (*C*), 12% (*D*), and 16% PVP (*E*). The periods of the applications of PVP and relaxing (Rel) and activating (Act) solutions are shown in the figure.

The velocities of no load shortening measured by the "slack test" were 1.88 ± 0.22, 1.38 ± 0.06, 0.55 ± 0.06, 0.03 ± 0.02 l_o/s (n = 4) in 0, 4, 8, and 12% PVP, respectively, which were not largely different from those obtained from force–velocity data.

Stiffness Changes in Relaxed, Activated, and Rigor Compressed Fibers

Measurements of longitudinal instantaneous stiffness, active tension, and relative diameter of a fiber in relaxed and activated states were carried out using one fiber, and the results in Fig. 6 were the average of data from five fibers. In relaxing solution, stiffness was detected above 8% dextran, and it increased linearly with the concentration of dextran. Active stiffness in 4% dextran was higher by 10–20% than that in dextran-free solution, and it increased as the concentration of dextran increased until 20–24%, above which it decreased. If the slopes of active and resting stiffness are compared between the range of 4–20% dextran, it should be noted that two lines are considerably parallel. Interpretation of this phenomenon will be discussed later together with the results in rigor fibers. Active tension was constant in the range of 4–16% dextran, and it decreased with further compression as shown by Gulati

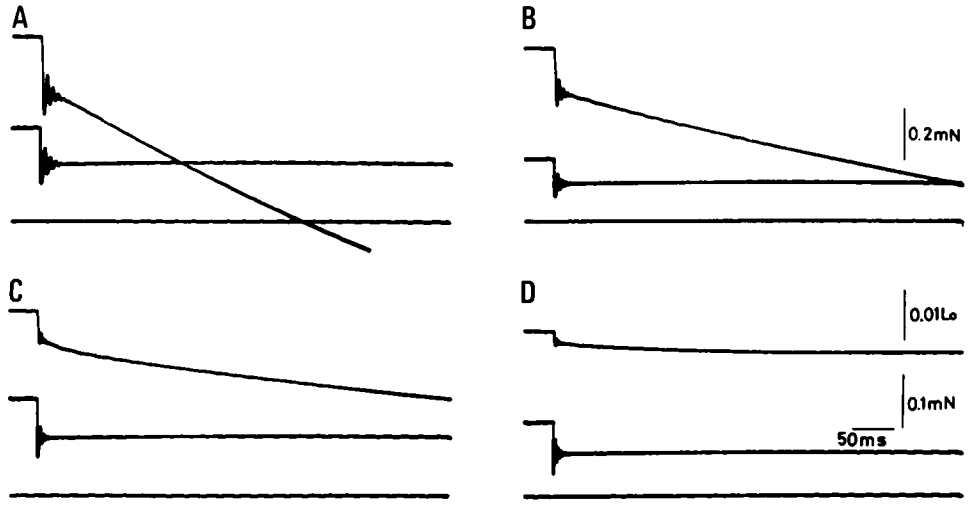

FIGURE 3 Records of isotonic shortening in 0% (*A*), 4% (*B*), 8% (*C*), 12% (*D*), PVP. Upper, middle, and lower traces in each panel are length, tension, and zero level of tension records, respectively. All relative loads during shortening are adjusted to 0.6. The tension in *A*, *C*, and *D* was recorded at the same sensitivity.

and Babu (1985). Fiber diameters in activating and relaxing solutions were not different above 8% dextran, whereas a fiber shrank slightly by activation in 0 and 4%.

The effects of osmotic pressure on stiffness and diameter in rigor and relaxed states were examined in one fiber, and the results in Fig. 7 are the average of data from five fibers. Stiffness of a rigor fiber, unexpectedly, increased obviously as the concentration of dextran increased. This phenomenon appears to be similar to that shown in Fig. 6, but the two curves of stiffness are not parallel. In addition, diameter in rigor was larger than that in rest above 8% of dextran, as pointed out by several authors (Maughan and Godt, 1981; Matsubara et al., 1984; Umazume and Kasuga, 1984).

From the above results, it is natural to conceive that there may be a close relation between the parallelism of two stiffness curves and small differences in fiber diameter in Fig. 6, and it is assumed that the "passive" stiffness does not depend on the existence of Ca and ATP but solely depends on fiber diameter. On the basis of the above assumption, the "passive" stiffness in rigor fibers was re-estimated in Fig. 7.

An example of the re-estimation is illustrated in Fig. 7 by arrows for 16% dextran. Thus, first the diameter of a rigor fiber in 16% dextran is found to be the same as that of a relaxed fiber in ~9.5% of dextran (first horizontal arrow), the stiffness of which is read (vertical arrow), and this value is taken to be the "passive" stiffness of a rigor fiber in 16% dextran (second horizontal arrow). The diameter of a rigor compressed fiber is larger than that of a relaxed compressed one in the same concentration of dextran; therefore, the re-estimated "passive" stiffness is smaller than that before the re-estimation. It can be seen that the stiffness curve of a rigor fiber is nearly parallel with the curve of the re-estimated "passive" stiffness, suggesting the possibility that two kinds of stiffness may exist even in a rigor compressed fiber.

The fundamental step to know the properties of the "passive" stiffness is to carry out the measurement of the stiffness change as the sarcomere length varies. The results in an activated and a relaxed fiber are illustrated in Fig. 8 *A*. The stiffness of a relaxed fiber without polymer above 3.0 μm was not negligible in comparison with that of a rigor or an activated fiber; therefore, it was subtracted

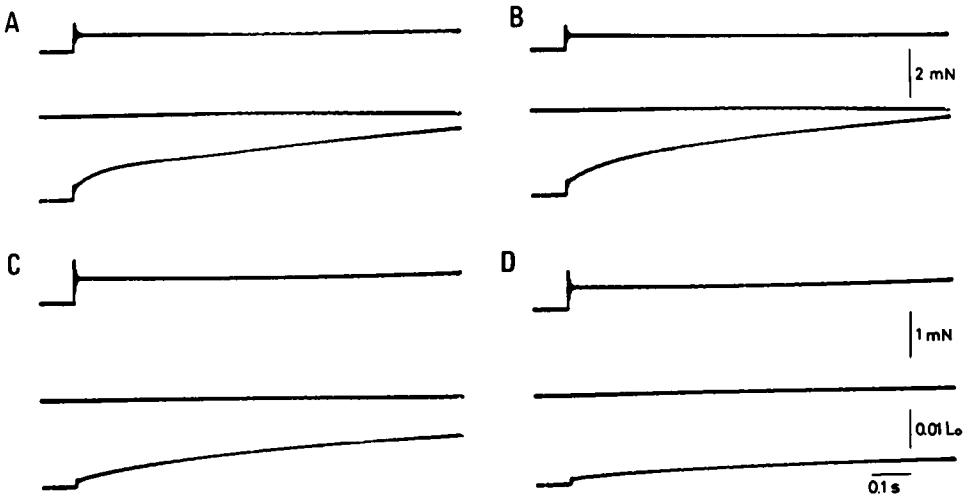

FIGURE 4 Records of isotonic lengthening in 0% (*A*), 4% (*B*), 8% (*C*), and 12% (*D*) PVP. Upper, middle, and lower traces in each panel are tension, zero level of tension, and length records, respectively. All relative loads during lengthening are adjusted to 1.24. The tension in *C* and *D* was recorded in twice as high sensitivity as in *A* and *B*.

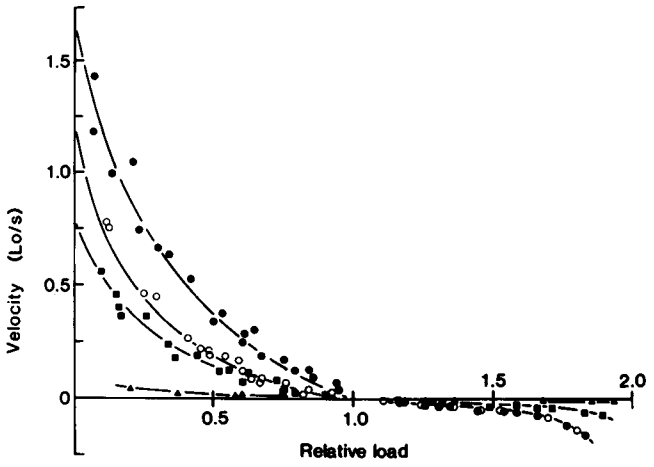

FIGURE 5 Force–velocity relation in 0% (*solid circles*), 4% (*open circles*), 8% (*solid squares*), 12% (*solid triangles*) PVP.

from total stiffness. Not only the stiffness of an active fiber in the absence of dextran but also the stiffness of a relaxed and an activated fiber in the presence of dextran were greatly reduced with the increase of the sarcomere length. In a rigor fiber (Fig. 8 *B*) also, irrespective of presence or absence of dextran, the sharp decrease of the stiffness was observed as the sarcomere length increased. At a sarcomere length of 3.8 μm, there is significant stiffness in compressed, relaxed, activated, and rigor fibers. This may not imply that there is a component of "passive" stiffness that is caused by interactions other than those between thick and thin filaments, because at 3.8 μm of sarcomere length, the stiffness of a compressed relaxed fiber increased clearly by activation as shown in Fig. 8 *A*, suggesting that overlap of both filaments remained in a compressed fiber at 3.8 μm. Goldman and Simmons (1986) also reached the similar conclusion by the experiments at varied filament overlap that polymer increased the stiffness of the sarcomeres.[1]

Tension Transients after Stretch in a Compressed Fiber

Tension transients after stretch of 0.5% l_0 in relaxed, activated, and rigor states were recorded in the absence or presence of dextran (Fig. 9), and they were vertically scaled so that the peak tensions could be equal to compare the rate of tension decay (Fig. 10). Fig. 9 demonstrates that the tension responses by stretch became higher with the concentration of dextran in relaxed, activated, and rigor states. It should be noted that the time course of tension transient of relaxed compressed fiber was not similar to that of an activated or a rigor fiber. It is evident from Fig. 10 that the characteristic feature of the tension transient of a relaxed compressed fiber is fast recovery phase and that active and rigor tension transients in 24% dextran have faster recovery phase than those in 0%.

Influence of Viscosity and Low Molecular Sugar

The viscosity of PVP or dextran solution was higher than that without these polymers, accordingly, the effects of the viscosity of the polymer on stiffness and the tension transients should be checked. A segment of a fiber before mechanical skinning was connected to the tension transducer and to the servo-motor in the similar way as in a skinned fiber, and the tension responses after stretch were measured in relaxing solution and in rigor solution with various concentration of Dextran T70 or T500 up to 28%, and of PVP up to 24%. In all relaxing solutions with polymer examined, the tension response to quick stretch was negligible. The tension responses of rigor fibers in the presence and absence of polymer were compared, and no difference was detected between them.

It is conceivable that some of the low molecular weight fraction of polymer penetrated into fiber lattice, and some direct effects of polymer on intracellular structure increased axial stiffness and affected tension responses after stretch, although skinned fibers were immersed in polymer solution at most for only 4 min to avoid the penetration of polymer. To examine the above possibility, skinned fibers were immersed in the solution with Dextran T10, which was expected to penetrate into a skinned fiber. However, it was observed that a skinned fiber shrank in the solution, suggesting that Dextran T10 was not appropriate to the above purpose, though this fact is contrary to the observation of Berman and Maughan (1982).

When a skinned fiber was immersed in relaxing solution containing 16% sucrose, the diameter of a fiber reduced

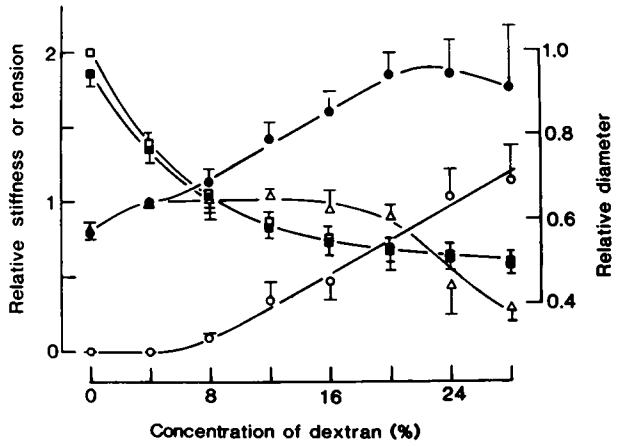

FIGURE 6 Effects of dextran concentration on relative stiffness, active tension, and relative diameter. *Solid circle*, relative stiffness in activating solution; *open circles*, relative stiffness in relaxing solution; *hollow triangles*, active tension; *open squares*, relative diameter in relaxing solution; *solid squares*, relative diameter in activating solution. Data are means and standard deviations for five different fibers.

[1]After the submission of this paper, the paper by Goldman and Simmons (1986) appeared in *J. Physiol. (Lond.)* and the discussion about their work was added during the revision of this paper.

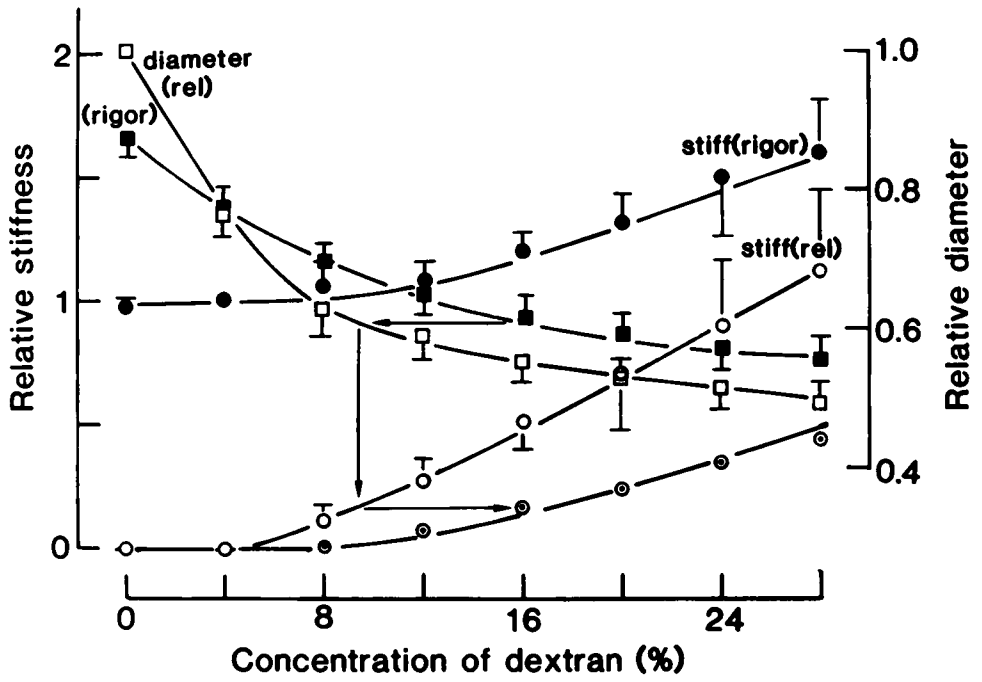

FIGURE 7 Effects of dextran concentration on relative stiffness and relative diameter. *Solid circles*, relative stiffness in rigor solution; *open circles*, relative stiffness in relaxing solution; *open squares*, relative diameter in relaxing solution; *solid squares*, relative diameter in rigor solution; *double circles*, re-estimated "passive" stiffness. The arrows indicate the process of the re-estimation of the "passive" stiffness. Data are means and standard deviations for five different fibers. See text.

slightly and then recovered fully in 5 min. No stiffness and tension transient after stretch were detected in this fiber. A fiber in relaxing solution containing 16% sucrose and 16% Dextran T70 demonstrated a little lower stiffness and tension response after stretch than in the solution containing only 16% Dextran T70. A fiber that was first soaked in sucrose and then made to rigor showed a little less stiffness and tension responses than a fiber that was first made to rigor and then soaked in sucrose. These results provided the evidence that the stiffness and the tension response after stretch was not brought about by the sugar of low molecular weight penetrated into a fiber. On the contrary, it might inhibit appreciably the interaction between crossbridges and thin filaments.

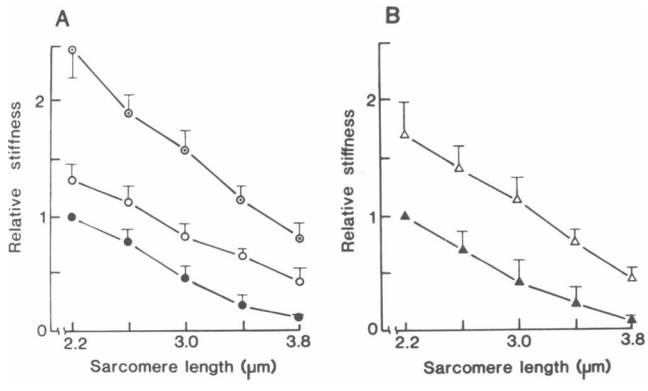

FIGURE 8 The relation between relative stiffness and sarcomere length. (*A*) *open circles*, in relaxing solution containing 16% PVP; *solid circles*, in activating solution without PVP; *double circles*, in activating solution containing 16% PVP. (*B*) *Solid triangles*, in rigor solution in 0% PVP; *open triangles*, in rigor solution containing 16% PVP. Data are means and standard deviations for five different fibers.

Re-composition of Tension Response after Stretch in a Compressed Fiber

The results from the measurement of stiffness as depicted in Figs. 6 and 7 suggest the existence of the "passive" stiffness or linkages parallel to normal active or rigor linkages, and this interpretation is further supported qualitatively by the results of the tension responses after stretch as shown in Figs. 9 and 10. From the results of stiffness (Fig. 6), it may be written that

$$S_{\text{act comp}} = S_{\text{act}} + S_{\text{rel comp}},$$

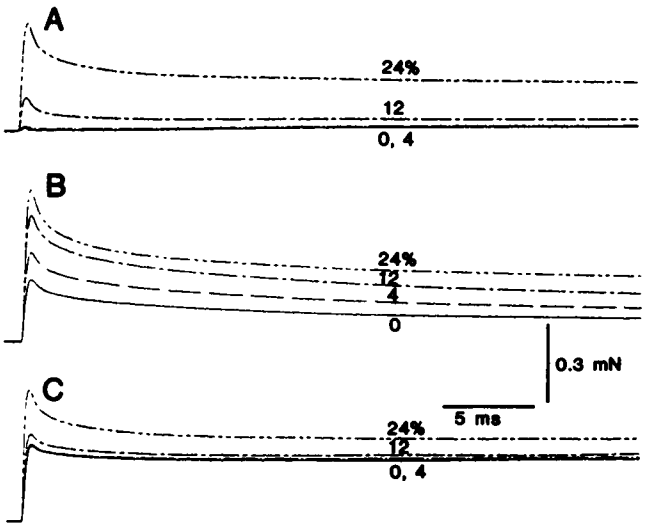

FIGURE 9 The tension records after 0.5% stretch in relaxing solution (*A*), in activating solution (*B*), and in rigor solution (*C*) containing 0, 4, 12, and 24%, dextran. The figures denote the concentration of dextran in percentage.

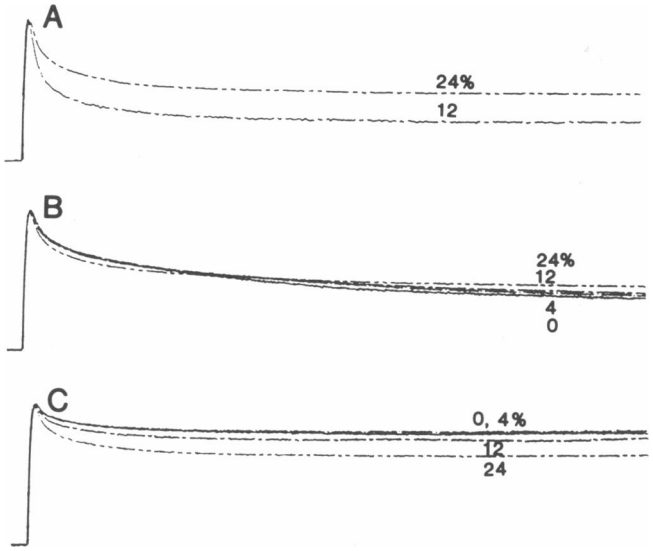

FIGURE 10 Vertical scaling of the tension records after stretch shown in Fig. 9 so that peak tensions could be equal. (*A*) In relaxing solution; (*B*) in activating solution; (*C*) in rigor solution. The figures denote the concentration of dextran in percentage.

in a certain range of polymer concentrations, where $S_{\text{act comp}}$ denotes the stiffness of a compressed fiber in activating solution by a given concentration of polymer; S_{act} denotes the stiffness of an activated fiber in 4% dextran or PVP, in which the diameter of a skinned fiber is nearly the same as that of an intact fiber; $S_{\text{rel comp}}$ denotes the "passive" stiffness of relaxed compressed fiber in the same concentration of polymer as that in $S_{\text{act comp}}$. If the tension responses after stretch are linearly related to the stiffness, it is written that

$$T_{\text{act comp}} = T_{\text{act}} + T_{\text{rel comp}},$$

where T is the tension response after stretch; the subscripts have the same meaning as for S. It is possible to see whether the above assumption is correct or not by comparing $T_{\text{act comp}}$ calculated by the above equation from observed T_{act} and observed $T_{\text{rel comp}}$ with the observed $T_{\text{act comp}}$. Examples of the comparison of two tension responses are illustrated in Fig. 11, *A* and *B*, where vertical scaling to equalize the tension peaks is not carried out and considerably good coincidence can be seen. Better fit was obtained in the lower concentration of polymer, and the difference was more marked with the increase of the concentration of polymer.

Analogously, the re-composition of the tension response in a compressed rigor fiber was carried out (Fig. 11, *C* and *D*). The stiffness of a rigor compressed fiber was assumed to be the sum of the rigor stiffness and the re-estimated "passive" stiffness. Better fits were seen in rigor fiber in all concentrations of polymer than in an activated fiber.

DISCUSSION

An increase in stiffness or occurrence of elastic component in osmotically compressed muscle has been reported in intact whole muscles (Howarth, 1958; Hill, 1968, 1970), in intact isolated fibers (Lännergren, 1971), and in skinned fibers (Berman and Maughan, 1982; Umazume and Kasuga, 1984; Gulati and Babu, 1985; Kawai and Schulman, 1985; Goldman and Simmons, 1986).

It is indicated in this experiment that two kinds of stiffness exist in parallel in a compressed fiber, one being from active or rigor linkages and another from the "passive" linkages between thick and thin filaments. This conclusion is supported by the following three reasons. First, the two curves of the stiffness–polymer concentration relationship in Figs. 6 and 7 are parallel in a wide range of concentrations, suggesting that coexistence of the constant active or rigor stiffness and the increasing "passive" stiffness with polymer concentration. Second, the time courses of tension transients after stretch of relaxed compressed fibers were quite different from those of rigor or active uncompressed fibers (Figs. 9 and 10), and in addition, the tension response after stretch in a compressed active or rigor fiber could be shown to be about the sum of the two responses (Fig. 11). Third, the stiffness of relaxed, activated, or rigor fiber in polymer solution was clearly reduced as the sarcomere length increased. Goldman and Simmons (1986) also obtained the result that the increase in active stiffness in PVP was comparable to the increase of the stiffness in relaxed fibers caused by PVP.

Howarth (1958) indicated that the considerable tension developed when an intact, unstimulated shrunken muscle was stretched might be brought about by greatly increased viscosity. In the present experiment, the effects of the

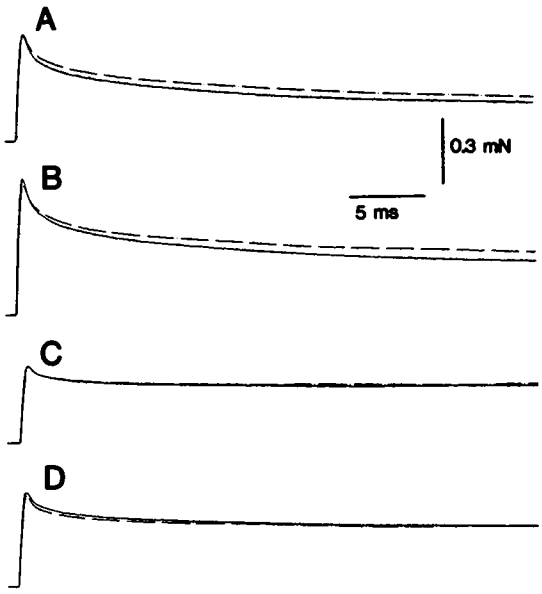

FIGURE 11 Comparison between re-composed tension responses (*solid lines*) and real records (*broken lines*). (*A*) In activating solutions containing 12% dextran; (*B*) in activating solutions containing 18% dextran; (*C*) in rigor solution containing 12% dextran; (*D*) in rigor solution containing 18% dextran. No adjustment of peak tension by vertical scaling was made in all records. Note that good fitting can be seen in the responses in rigor (C, D).

viscosity inside and outside a fiber were allowed for and it was proved that the increased viscosity of the surrounding fluid brought about a negligible effect on mechanical responses, and that the sugar of low molecular weight which penetrated into a fiber, if any, did not induce stiffness, even though it might inhibit the active or rigor interaction between thick and thin filaments. Endo et al. (1979) reported the inhibitory effects of viscosity on tension development. Further evidence that the mechanical responses were not produced by viscosity were also provided by the observation in a compressed fiber that the force level after stretch was maintained (Figs. 9 and 10) and that the tension continued to rise during stretch of constant velocity when stiffness was measured.

Another property of the "passive" stiffness disclosed in the present experiment is that this linkage could exist in relaxed, activated, and rigor states, regardless of the presence or absence of Ca^{++} or ATP. Berman and Maughan (1982) found that the responses after quick stretch in various ionic strengths were similar at the same degree of compression, namely ionic strength per se had no significant effect on the response. If all of the above results are taken into consideration, it is natural to conclude that this linkage between thick and thin filaments is not active but passive.

Matsubara et al. (1984) measured the lateral filament spacing of skinned fibers. According to their results, the 1,0 lattice spacing of a relaxed fiber at the sarcomere length of 2.3 μm in the presence of 0, 4, and 7% PVP were 41.0, 35.6, and 30.8 nm, respectively. Assuming that the diameters of a thick and a thin filament are 15 and 8 nm, respectively, the surface-to-surface distances between both filaments in each concentration of PVP are 15.8, 12.2, and 9 nm, respectively. Allowing for these distances and the size of S1 (15–19-nm long) (Elliott and Offer, 1978), it is reasonable to postulate that in a compressed fiber, passive interaction is constituted between filaments.

In the present experiment, it is demonstrated that the decrease of the shortening velocity and the active force, which was preceded by the transient increase, was brought about in weakly or moderately compressed fibers, and the decrease of active stiffness was observed in strongly compressed fibers. The order of susceptibility of the mechanical parameters to the inhibiting effects of shrinkage can be written as below.

Decreasing in shortening velocity / Decrease in active force / Decrease in active stiffness.

Maughan and Godt (1979) found that the diameter increased by 28% by skinning, and 4% PVP was necessary to recover the diameter to that before skinning. As shown in Fig. 5, the velocity without polymer was faster than in 4% PVP, and if the reason for this decrease in shortening velocity is supposed to be the "passive" interaction between thick and thin filaments, small "passive" force may work even in the fiber of normal diameter. In an intact single fiber, the shortening in hypotonicity was shown to be faster by 12% than that in normal tonicity (Edman and Hwang, 1977).

If the weak passive interaction operates at the normal lattice spacing, it may have relation to other phenomena. Hill (1968) found that SREC and FRT existed in an intact resting muscle and that both were increased by making the external solution hypertonic. Comparing the properties of the SREC and those of the "passive" stiffness in the present paper, it may be able to say that the SREC and the "passive" stiffness resulted from the same source and accordingly that the SREC and the FRT may be the reflection of "passive" interaction between thick and thin filaments.

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