LOCALIZATION OF THE PARALLEL ELASTIC COMPONENTS IN FROG SKINNED MUSCLE FIBERS STUDIED BY THE DISSOCIATION OF THE A- AND I-BANDS

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ABSTRACT Localization of the parallel elastic components (PECs) in skinned muscle fibers was investigated by analyzing the change of the resting tension, which accompanies the dissociation of the A- and I-bands. The A-band was dissociated from both ends by increasing the concentration of KCl under relaxing conditions (0.09–0.54 M KCl, 4.0 mM MgATP, 1.0 mM Mg^{2+}, 4.0 mM EGTA, pH 6.0–9.0, 20°C). At sarcomere lengths $\geqq 3.5$ μm, the length of the A-band was estimated by comparing the intensity of the first-order optical diffraction line with the results of model calculations. These results were supported by differential-interference microscopy and sodium dodecyl sulfate gel electrophoresis. It was shown that the resting tension decreased nearly in proportion to the residual length of the A-band. At sarcomere lengths $\leqq 4.0$ μm, the resting tension after the dissociation of the A-band was lowered to $<10\%$ of the initial value. On the other hand, at sarcomere lengths $\geqq 5.0$ μm the resting tension after the dissociation of the A-band still showed ~35% of the initial value and did not change even after the I-band was dissociated by a solution containing KI. From these results, we propose that most of the PECs contributing to resting tension bind almost uniformly to the A-band and there are also PECs connecting Z-lines.

INTRODUCTION

A striated muscle has an ordered structure. This ordered structure is necessary for repetitious and effective contraction in a striated muscle. Optical and electron microscopic observations have suggested that certain fibrous or network structures, other than thick and thin filaments and Z- and M-lines, maintain the ordered structure (6, 12, 23, 31). On the basis of the tension-length relation of a relaxed skinned fiber, Natori showed that a parallel elastic component (PEC) exists in a skinned fiber (18). Maruyama et al. suggested that the PEC that still remained after the extraction of myofilaments plays a part in maintaining the ordered structure of a striated muscle (13, 14). Recently, by using an indirect immunofluorescence method Maruyama et al. and Wang et al. showed that connectin (titin) (13, 30), one of the PECs, is located in A-I junction areas (16, 30).

Electron microscopy and immunofluorescence would be useful methods to investigate the localization of the PECs, but they cannot clarify the physiological role of the PECs. Here we attempt to clarify the localization and possible physiological roles of the PECs in a skinned fiber mainly by measuring the decrease in the resting tension, which accompanies the dissociation of the A- and I-bands. It was recently shown that the A-bands in myofibrils in the presence of pyrophosphate dissociate from both ends (8) and the length of the A-band in a muscle fiber could be estimated by an optical diffraction method (9). By using this method, it was possible to investigate the relationship between the resting tension and the residual length of the A-band, which accompanies its dissociation. As a result, we propose two classes of PECs; one connecting thick filaments with a Z-line and the other connecting Z-lines. A preliminary report of the present study has been presented at the 21st Annual Meeting of the Biophysical Society of Japan (4).

MATERIALS AND METHODS

Fiber Preparation

A mechanically skinned single muscle fiber (18) was prepared from semitendinosus muscle of frog (*Rana catesbeiana*) in a relaxing solution (90 mM KCl, 5.2 mM $MgCl_2$, 4.3 mM Na_2ATP, 4 mM EGTA, 10 mM PIPES, pH 7.0; see Table I). A segment of a skinned fiber, ~5 mm long, was mounted horizontally in a chamber (3 × 5 × 20 mm) between a tension transducer (UL2-240; Shinkoh Inc., Tokyo, Japan) and a micromanipulator MM-33; Narishige; Scientific Inst. Lab., Tokyo, Japan). The solution was changed by pouring a new solution in at one end of the chamber and sucking the old solution out at the other end.

Optical Diffraction

An apparatus was essentially the same as that in Umazume and Fujime (26) except that a He-Ne laser, (GLG 5700; NEC Co., Inc. Tokyo, Japan) (25 mW output intensity, ~1 mm beam diameter on a fiber), was

TABLE I
SOLVENT COMPOSITIONS AT THE IONIC STRENGTH OF 0.15 M

pH	$MgCl_2$	Na_2ATP	KCl
	mM	*mM*	*mM*
6.0	5.0	4.6	93
7.0	5.2	4.3	90
7.5	5.6	4.3	85
8.0	6.8	4.3	89
8.5	7.6	4.3	81
9.0	7.2	4.3	77

Concentrations of EGTA and pH buffers were 4.0 and 10 mM, respectively. 2-(*N*-morpholino) ethanesulfonic acid monohydrate (MES) (pH 6.0) or PIPES (pH 7.0, 7.5) or *N*-2-hydroxyethylpiperazine-*N'*-3-propane-sulfonic acid (EPPS) (pH 8.0, 8.5, 9.0) was used as a pH buffer. The pH values were adjusted by KOH. Concentrations of Mg^{2+} and MgATP in solutions were kept at 1.0 and 4.0 mM, respectively. When changing the ionic strength, the KCl concentration was changed.

used. A laser beam was aligned to illuminate a skinned fiber normally. The sarcomere length (L) of a skinned fiber was calculated from the separation between the two first-order diffraction lines. The intensity of the first-order diffraction line (I_1) was measured by a photomultiplier tube (1P28; Hamamatsu TV, Hamamatsu, Japan).

Experimental Procedures on Dissociation of the A- and I-Bands

A skinned fiber was stretched to an appropriate sarcomere length in the relaxing solution. Then, it was immersed for 15 min in a relaxing solution containing 0.5% Brij-58 and subsequently for ~30 min in the relaxing solution again. When the A-band was dissociated at pH 6.0 or 8.0, a skinned fiber was further immersed for ~10 min in a preincubating solution of the ionic strength of 0.30 (pH 6.0) or 0.10 M (pH 8.0), respectively (see Table I). The dissociation of the A-band was initiated by exchanging the relaxing or the preincubating solution with that of higher ionic strength (the ionic strength of 0.35, 0.25, and 0.15 M at pH 6.0, 7.0 and 8.0, respectively, see Table I). Then, the A-band was dissociated further by the stepwise increase of the KCl concentration and the pH value, which kept both the pH value and the ionic strength constant. The final dissociation of the A-band was performed by exchanging the solution of high ionic strength at various pH values with the solution of 0.54 M KCl at pH 7.0. The ionic strength was adjusted by changing the KCl concentration such as shown in Table I. Finally the I-band was dissociated by exchanging the KCl solution with a solution containing KI instead of KCl. All solutions contained 1 mM Mg^{2+} and 4 mM MgATP. All procedures were performed at 20°C.

Sodium Dodecyl Sulfate (SDS) Gel Electrophoresis

A bundle of 10 skinned fibers was rinsed with 50 μl of an extracting solution of various ionic strengths for 5 or 10 min at 20°C. SDS slab gel electrophoresis was performed by the Laemmli method with slight modification (10). To each rinsing solution and muscle residue were added 5 μl of 10% SDS and 10% 2-mercaptoethanol, and each solution was incubated for 5 min at 100°C. The 15 and 5% acrylamide gels were used for a separation gel and a stacking gel, respectively.

Differential-Interference Micrograph

Both ends of a skinned fiber were laid on two pieces of double stick tape that had been stuck to a glass slide. The fiber axis was situated in the direction of a shear between two light beams divided by the Nomarski prism. The micrograph of the fiber was taken with a differential-interference microscope (Zeiss Ultraphoto III; Carl Zeiss, Inc., Thornwood, NY) on a Kodak Tri-X film (Eastman Kodak Co., Rochester, NY). The length of the A-band in a skinned fiber on negative films was measured with a comparator (V-12. Nikon Inc., Tokyo, Japan).

RESULTS

Changes of the Resting Tension and the Intensity of the First-order Diffraction Line I_1 during Dissociation of the A- and I-Bands

We measured the changes of the resting tension and I_1 induced by the dissociation of the A- and I-bands at various conditions. When the fiber was stretched in a relaxing solution, an instantaneous increase of the resting tension was followed by an exponentially decreasing phase with a relaxation time of ~10 min. At ~45 min after stretching, the resting tension reached almost a steady level. Then the dissociation of the A-band was initiated by increasing the KCl concentration. The resting tension decreased on the stepwise increase of the KCl concentration as shown by the solid line in Fig. 1. The resting tension did not decrease any more after the A-band were almost completely dissociated by 0.54 M KCl solution. On further dissociation of the I-band by exchanging KCl with KI, the resting tension hardly decreased (Fig. 1). No change of the resting tension was observed by exchanging 0.60 M KI with the relaxing solution. After the resting tension decreased at a high KCl concentration, no change of the resting tension was observed by re-incubating the fiber in a low KCl solution. When pH value was increased stepwise from 7.0 to 9.0 at the ionic strength of 0.25 M, the resting tension also decreased. The resting tension did not change by reducing pH value again from 9.0 to 7.0.

Fig. 1 also shows the change of the first-order intensity, I_1. With the stepwise increase of the KCl concentration, I_1 decreased to zero and then increased. That is, the intensity reached nearly zero in 0.24 M KCl and ~1.7 times the original value in 0.54 M KCl. After that, I_1 decreased again on further dissociation of the I-band by exchanging KCl with KI (Fig. 1). It did not change any more by exchanging 0.60 M KI with the relaxing solution again. The changes of I_1 in various ionic strengths at L = 3.5, 4.0, 5.0, and 6.0 μm were shown in Fig. 2. At these sarcomere lengths, I_1 first decreased and then increased with increasing the ionic strength.

At $L < 3.5$ μm, I_1 decreased and then increased with increasing ionic strength, but decreased again at KCl concentrations of 0.34 M and over at pH 7.0. Contrary to the case of a rabbit glycerinated psoas muscle (9), the disorder of the sarcomere repetitious structure was observed under a differential-interference microscope and the width of diffraction line increased at KCl concentrations of 0.34 M and over. For these reasons, all the following analyses of I_1 were performed at $L \geq 3.5$ μm.

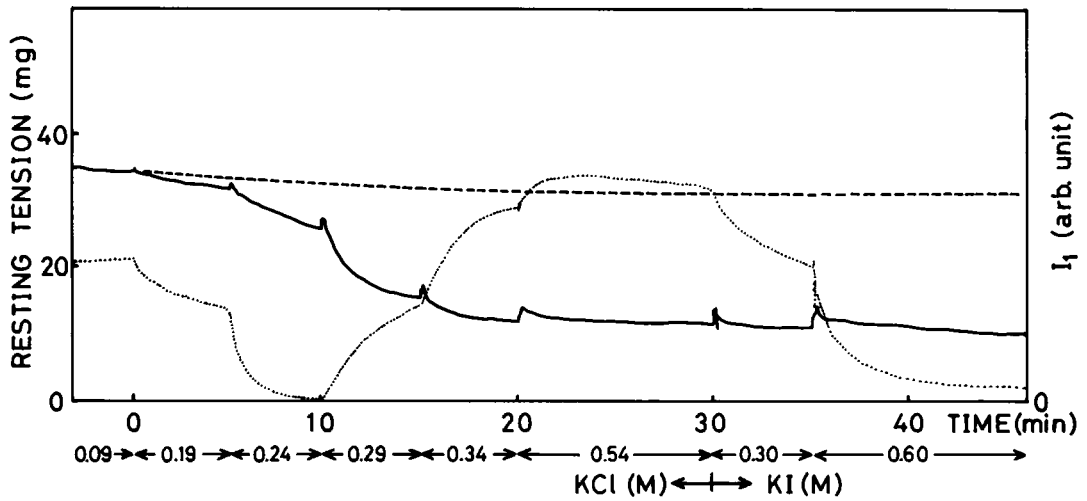

FIGURE 1 Time course of change of the resting tension and I_1 during dissociation of the A- and I-bands. (—), the resting tension. (· · ·), I_1 in an arbitrary unit. (---), the resting tension at 0.09 M KCl (ionic strength of 0.15 M), which was drawn by exponentially extrapolating the observed values at various times after stretching the fiber. The concentration of KCl or KI in the relaxing solution was increased stepwise at an interval of 5 or 10 min as shown on the abscissa. L = 5.0 μm and pH 7.0.

Estimation of the Residual Length of the A-Band

We estimated the residual length of the A-band by comparing I_1 at various conditions with the result of model calculation. Model 1 in Ishiwata et al. (9) was used for the analyses. The assumption in this model that the A-band dissociates from both ends, which keeps the refractive index (i.e., the local concentrations of proteins) of the A-band constant, was confirmed by us, as described later. We set the relative refractive index of the A-band to 1, that of the Z-line to 1.5, the length of the I-band to 2 μm, and the width of a Z-line to 0.1 μm, as in Ishiwata et al. (9). We defined the refractive index of an I-band as n_I, whose value will be determined experimentally. Then, we use the theoretical expression for the first-order diffraction line,

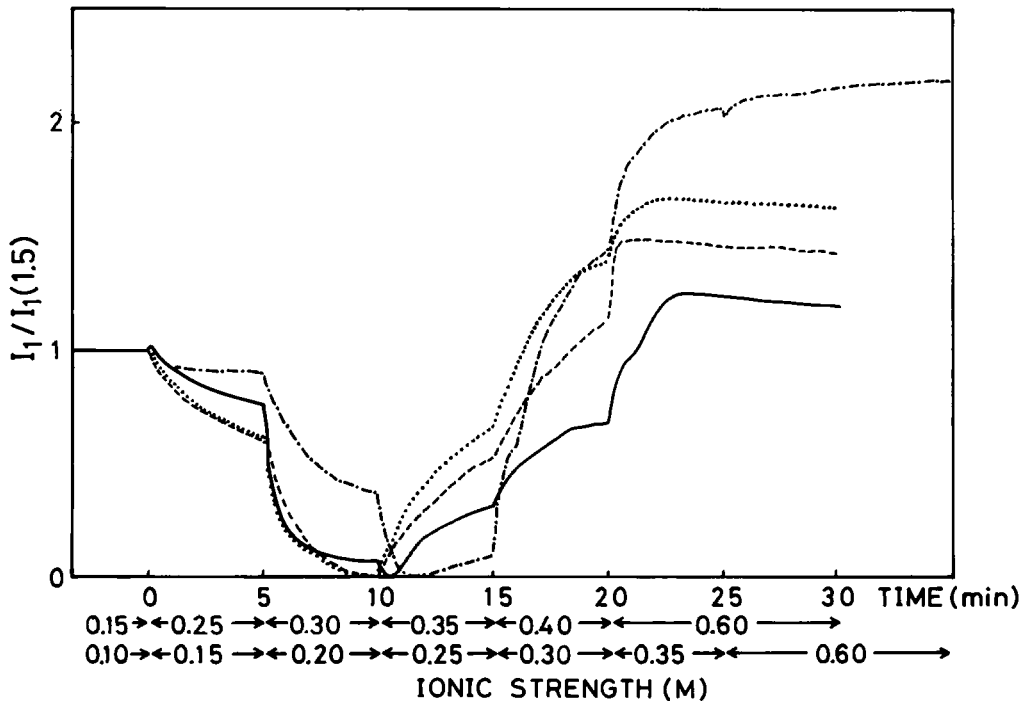

FIGURE 2 Examples of the change of I_1 at L = 3.5 μm (—), 4.0 μm (---), 5.0 μm (· · ·), and 6.0 μm (– · –). The ordinate shows $I_1/I_1(1.5)$. The ionic strength was increased stepwise and sequentially at an interval of 5 min. At $L \leq$ 5.0 μm, the ionic strength was increased from 0.15 to 0.60 M at pH 7.0. At L = 6.0 μm, the ionic strength was changed from 0.10 to 0.35 M at pH 8.0 and finally 0.60 M at pH 7.0.

TABLE II
INTENSITY RATIO ($I_1[0]/I_1[1.5]$) AND CALCULATED VALUE OF REFRACTIVE INDEX OF I-BAND (n_I) AT VARIOUS SARCOMERE LENGTHS

Sarcomere length	$I_1(0)/I_1(1.5)$	n_I
μm		
3.5*	1.4 ± 0.2	0.40 ± 0.02
4.0*	1.7 ± 0.2	0.40 ± 0.02
5.0‡	1.9 ± 0.4	0.39 ± 0.02
6.0*	2.5 ± 0.3	0.41 ± 0.02

Mean ± SD.
*Three fibers measured.
‡Nine fibers measured.

I_1^*, at a given length of the A-band L_A, in an arbitrary unit as follows (1, 9) $I_1^*(L_A) = \Psi \times [\sin(L_A\pi/L) - n_I \sin(2\pi/L) - 1.5 \sin(0.1\pi/L)]^2$, where L is the sarcomere length in microns and Ψ includes all factors other than the unit cell structure factor $[\sin(L_A\pi/L) - n_I \sin(2\pi/L) - 1.5 \sin(0.1\pi/L)]^2$. We obtained experimentally the ratio of the diffraction intensity, before the dissociation of the A-band, $I_1(1.5)$, to that value calculated after the almost complete dissociation with 0.54 M KCl at pH 7.0, $I_1(0)$. Ishiwata et al. (9) suggest that the factor Ψ was nearly constant throughout the dissociation process of the A-band. If this were true for the present case, the n_I could be determined from the relation: $I_1^*(0)/I_1^*(1.5) = I_1(0)/I_1(1.5)$.

Table II summarizes the experimental ratio $I_1(0)/I_1(1.5)$ and the estimated value of n_I for various sarcomere lengths. Note that the estimated values of n_I are very close to 0.40 in spite of large differences in the ratio $I_1(0)/I_1(1.5)$ for different sarcomere lengths. This again suggests that Ψ was nearly constant throughout the dissociation of the A-band. In what follows, the average value of $n_I = 0.40$ was used for analyzing the experimental intensity at any sarcomere length and any solvent condition we studied. This value is similar to the values obtained by Huxley and Hanson (7), $n_I = 0.50$, and Huxley (5), $n_I = 0.35$. Fig. 3 shows $I_1^*(L_A)$ in a wide range of L. The change of $I_1^*(L_A)/I_1^*(1.5)$ was similar to that of experimentally observed $I_1/I_1(1.5)$. The length of the A-band at the given condition was estimated by the relation: $I_1^*(L_A)/I_1^*(1.5) = I_1/I_1(1.5)$. Plausibility of the estimated L_A will be confirmed by microscopy measurements given later.

Time Course of the Length Change of the A-Band

Fig. 4 shows the time course of the length change of the A-band at various conditions. Rapid dissociation of the A-band was observed just after the stepwise increase of the ionic strength (Fig. 4 *a*) or the pH value (Fig. 4 *b*). The lower the pH, the higher the critical ionic strength for the dissociation of the A-band was. Note that the dissociation velocity of the A-band was hardly dependent on L (filled symbols in Fig. 4 *a*).

SDS Gel Electrophoresis

We analyzed the proteins dissociated from skinned fibers by SDS gel electrophoresis. A bundle of 10 skinned fibers

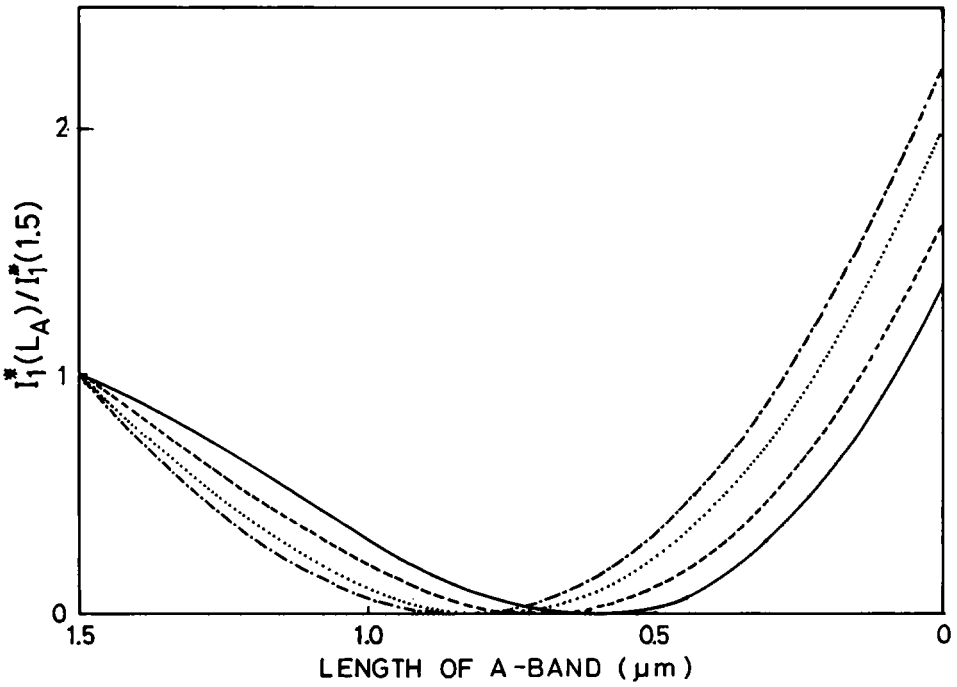

FIGURE 3 The intensity change $I_1^*(L_A)/I_1^*(1.5)$ based on equation in the text for $n_I = 0.40$ (see Table II). $L = 3.5$ μm (—), 4.0 μm (---), 5.0 μm (· · ·), and 6.0 μm (– · –).

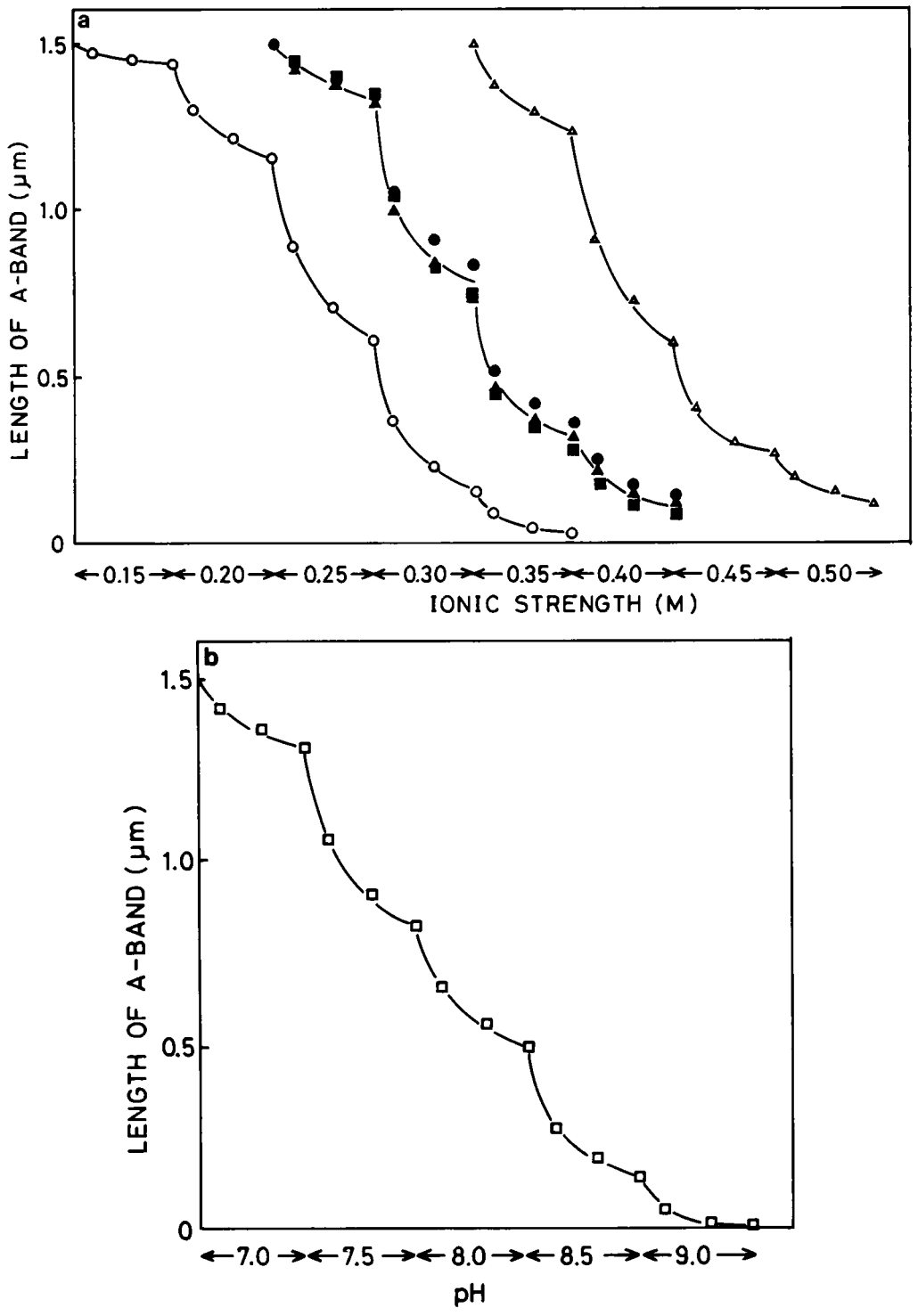

FIGURE 4 Time course of the length change of the A-band during the stepwise increase of the ionic strength and pH value. For the estimation of the residual length of the A-band, see text. (*a*) L = 3.5 μm (●), 4.0 μm (■), and 5.0 μm (▲) at pH 7.0; L = 5.0 μm (△) at pH 6.0; and L = 6.0 μm (○) at pH 8.0. (*b*) L = 5.0 μm (□) at ionic strength of 0.25 M. Data points were obtained by averaging data measured for three fibers at each condition. The ionic strength and the pH value were increased stepwise at an interval of 5 min as shown on the abscissa.

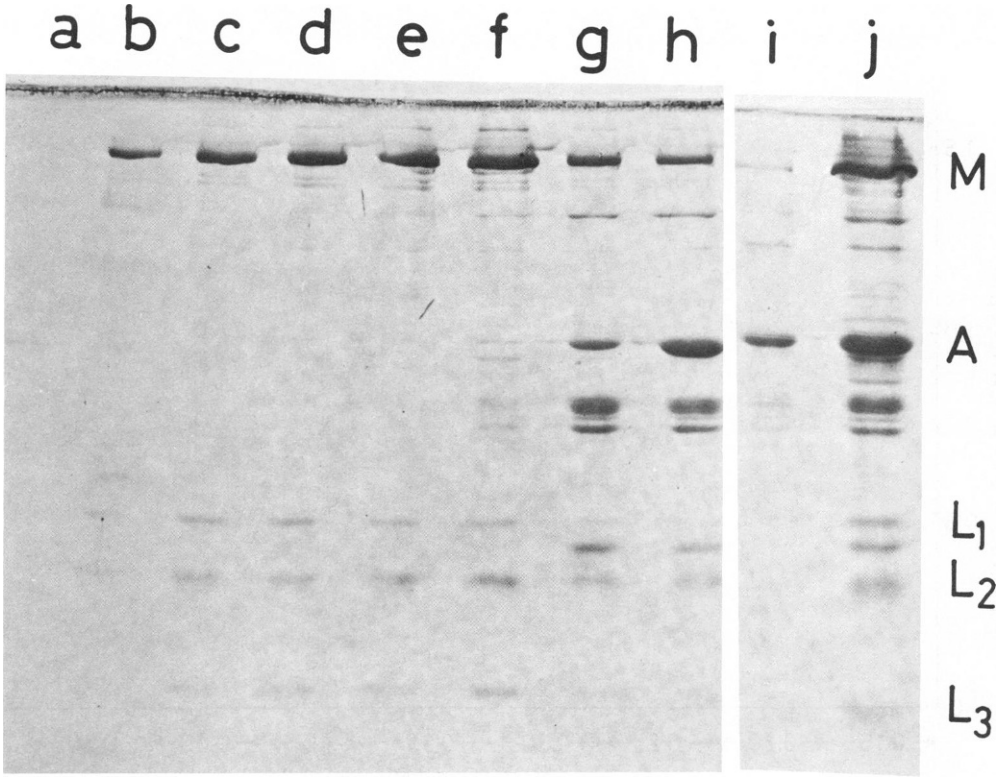

FIGURE 5 SDS gel electrophoresis demonstrating the release of myosin or actin from skinned fibers. *a–h*, extraction for 5 or 10 min at 20°C, successively changing the concentration of KCl or KI by the same procedure as that shown in Fig. 1. (*a*) 0.09 M, (*b*) 0.19 M, (*c*) 0.24 M, (*d*) 0.29 M, (*e*) 0.34 M, (*f*) 0.54 M KCl, (*g*) 0.30 M, and (*h*) 0.60 M KI. (*i*) Residue obtained after *h*. (*j*) Untreated skinned fibers. *M*, *A*, L_1, L_2, and L_3 denote myosin heavy chain, actin, myosin light chains 1, 2, and 3, respectively.

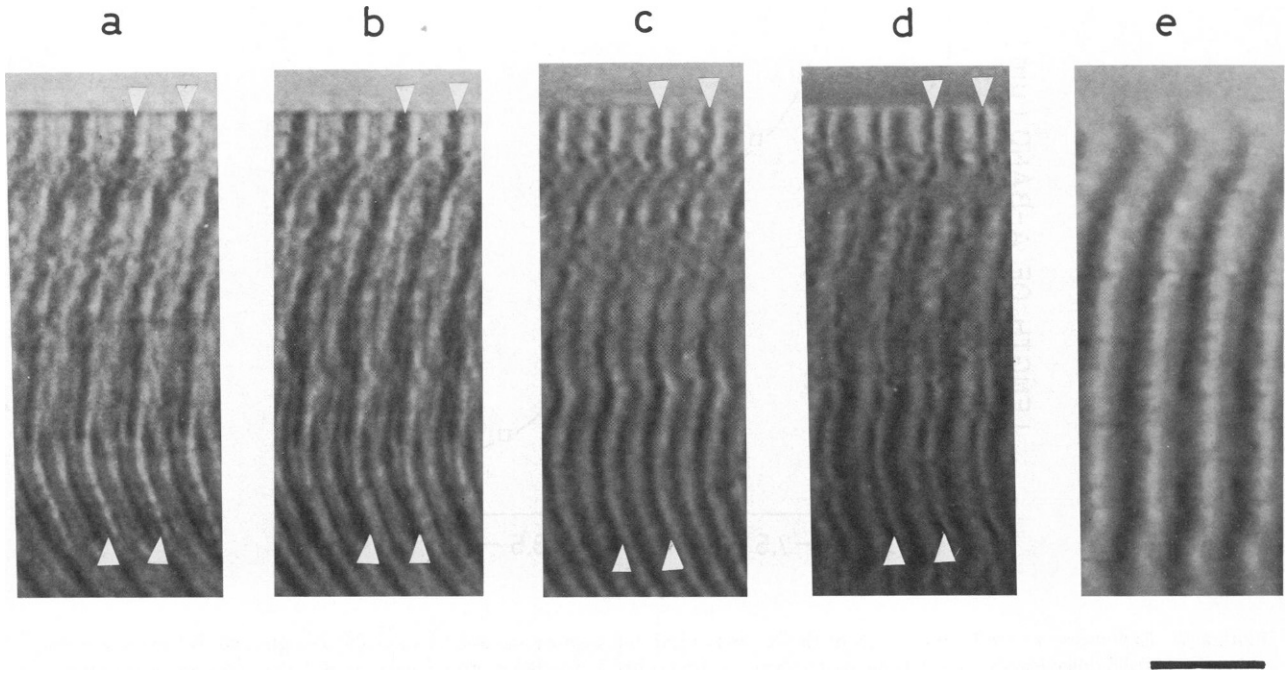

FIGURE 6 Dissociation of the A-band in a skinned fiber under a differential-interference microscope. A fiber was placed on a glass slide. Other conditions for dissociation of the A-band were the same as those in Fig. 1. (*a*) Skinned fiber in the relaxing solution. Micrographs were taken at 3 min after the stepwise increase of KCl concentration of 0.19 M (*b*), 0.24 M (*c*), 0.29 M KCl (*d*) and at 10 min after the treatment with 0.54 M KCl (*e*). Arrows show the location of the center of A-bands. L = 4.9 μm. Diameter of a fiber was ~100 μm. Scale bar, 10 μm.

TABLE III

THE LENGTH OF THE A-BAND ESTIMATED BY DIFFERENTIAL-INTERFERENCE MICROSCOPY AND OPTICAL DIFFRACTION

Ionic strength	OD	DIC	
		surface	core
M	*μm*	*μm*	
0.15	1.5	1.5 ± 0.1	1.6 ± 0.2
0.25	1.35 ± 0.04	1.4 ± 0.1	1.4 ± 0.2
0.30	0.84 ± 0.08	0.9 ± 0.2	0.8 ± 0.2
0.35	0.37 ± 0.02	0.5 ± 0.1	0.5 ± 0.1

Mean ± SD for measurements on five A-bands in both the surface and core regions of skinned fiber in Fig. 7 and three fibers at 3 min after the stepwise increase of KCl concentration in Fig. 4 (L = 5.0 μm, pH 7.0, ▲).

was mounted in the chamber of a Plexiglas block drilled with a series of rectangular wells. Each chamber contained 50 μl of the solutions used in Fig. 1. The bundle was successively incubated in each solution for 5 or 10 min according to the experimental procedure illustrated in Fig. 1. Then each solution and the bundle were analyzed. We confirmed that myosin was selectively released from fibers in solutions in which the KCl concentration was ≧0.19 M (Fig. 5). Bands of myosin in columns *g* and *h* of Fig. 5 did not result from undissociated A-bands but rather from myosin not yet diffused. This will be confirmed below by microscopy. Release of actin was hardly observed in the KCl solutions but observed in KI solutions (Fig. 5).

Differential-Interference Microscopic Observation

Under a differential-interference microscope, we observe an optically thin section in a bulk object without strong disturbance from regions above and below the section. This microscope enabled us to observe the simultaneous dissociation of the A-bands at surface and core regions of a skinned fiber when ionic strength is increased; we also attempted to verify that the length of the A-bands was properly estimated from the diffraction intensity I_1. The A-bands dissociated from both ends in nearly the same way with almost equal velocity both at surface and core regions of a skinned fiber (Fig. 6). The A-bands were dissociated almost completely with 0.54 M KCl. The register of fibrils and an ordered structure of sarcomere hardly changed by dissociation of the A-bands. The length of the A-bands at the surface and core regions of a skinned fiber was nearly the same as that estimated from I_1 (Table III), although the results were questionable when the ionic strength was 0.34 M because of the resolution of the microscope.

Correlation between the Resting Tension and the Length of the A-Band

We examined the correlation between the resting tension and the length of the A-band in a skinned fiber at various conditions. The absolute value of the total tension of a fiber depends on such factors as the fiber thickness, the sarcomere length, the length of the A-band, etc. Therefore, we defined the relative resting tension by setting the extrapolated resting tension at 1 (e.g., that shown by the dashed line in Fig. 1), and the resting tension after the treatment of the fiber with 0.54 M KCl at pH 7.0 for 10 min at zero. Figs. 7 *a* and *b* show that the relative resting tension changes nearly in proportion to the residual length of the A-band and independently of L and of the solvent conditions for the dissociation of the A-band.

Relation between the Sarcomere Length (L) and the Resting Tension

We investigated the relation between L (2.3–6.0 μm) and a resting tension before and after the dissociation of the A-bands or the A- and I-bands in a skinned fiber. As shown in Fig. 8, the resting tension was largely decreased by the dissociation of the A-bands; it was nearly zero at $L \leqq 3.0$ μm, <10% of the initial value at L = 3.5 to 4.0 μm and ~35% of the initial value at $L \geqq 5.0$ μm. The resting tension

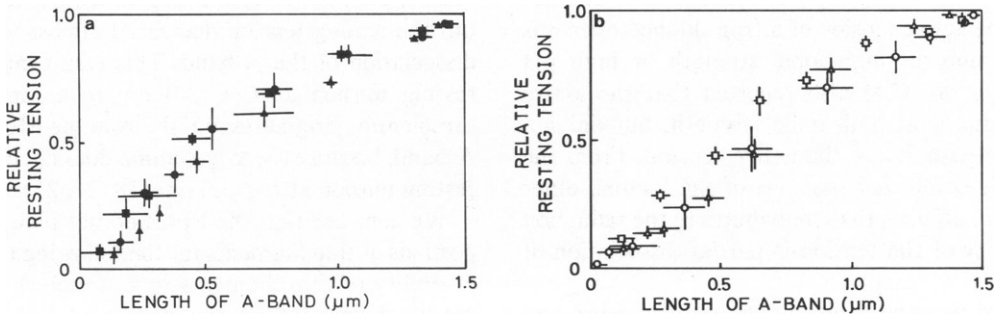

FIGURE 7 Correlation between the resting tension and the residual length of the A-band at various conditions. Experimental procedures were the same as those in Fig. 4. The resting tension and I_1 were measured at 1 and 5 min after the stepwise increase of ionic strength or pH value. See text for normalization of the resting tension. (*a*) L = 3.5 μm (●), 4.0 μm (■), and 5.0 μm (▲) at pH 7.0. (*b*) L = 5.0 μm (△) at pH 6.0; L = 6.0 μm (○) at pH 8.0; L = 5.0 μm (□) at ionic strength of 0.25 M. Symbols and bars indicate the mean and the SD of each parameter, respectively, for three fibers.

was not significantly decreased further by dissociation of the I-bands with 0.60 M KI at every sarcomere length.

DISCUSSION

We studied extremely stretched skinned fibers using the following strategies.

(*a*) To avoid contributions to the resting tension from possible rigor complexes in the resting fibers, sarcomere lengths ≥ 3.5 μm were chosen. Due to these long sarcomere lengths, the resting tension was much larger than that at physiological lengths, making measurements much easier. In addition, we could use MgATP instead of Mg-pyrophosphate in experiments over a very wide range of solvent conditions.

(*b*) In our method of estimating the residual length of the A-band by optical diffraction, i.e., by use of the relation $I_{\uparrow}^{*}(L_A)/I_{\uparrow}^{*}(1.5) = I_1/I_1(1.5)$, it was necessary to change only one parameter value, L, in order to verify the plausibility of our results (Table II and closed symbols in Fig. 4 *a*). Although experimental conditions in both the sarcomere length and the solvent composition were far from physiological, and the estimation of the residual length of the A-band by optical diffraction might not be very accurate, the present results are worth noting as far as the items discussed below are concerned.

Dissociation of the A-Band

For analyzing the dissociation process of the A-band, the optical diffraction method has been employed in frog skinned fibers (28, 32) and rabbit glycerinated psoas fibers (3, 9). In the present study, we showed that the A-band dissociated rapidly just after the stepwise increase of the ionic strength or the pH value. This suggests that the dissociation velocity of the A-band increases when the ionic strength or the pH value is increased. Also, we showed that the dissociation velocity of the A-band was hardly dependent on L. These results are consistent with those of Ishiwata et al. (9) and Higuchi and Ishiwata (3) except that the A-band in a frog skinned fiber dissociates at a lower ionic strength than that in a rabbit glycerinated muscle fiber. Gordon et al. (2) and Robertson et al. (22) reported that the active tension of a frog skinned fiber was reduced irreversibly at high ionic strength or high pH value. Thames et al. (24) also reported that the active tension was reduced at high ionic strength, but did not examine the reversibility of the active tension. From the present study, it is inferred that one of the factors, other than acto-myosin affinity, that contributes to the reduction and irreversibility of the tension is partial dissociation of the A-band.

We confirmed that the length of the A-band estimated from I_1 was consistent with differential-interference microscopic observations. Also, the phase contrast microscopic observations confirmed that the A-band in a myofibril, prepared by homogenizing frog semitendinosus muscle, dissociated from both ends, keeping the refractive index of the A-band constant in the presence of MgATP at high ionic strength (see Fig. 2 in reference 17). Moreover, electron micrographs of longitudinal sections of frog skinned fibers showed that thick filaments dissociate from both ends without changing their diameter and number (Higuchi, H., T. Yoshioka and Y. Umazume, unpublished results). These results suggest that there is no essential difference between rabbit and frog muscles in the dissociation of thick filaments at high ionic strength, and that the A-band in frog skinned fibers dissociates according to the model 1 of Ishiwata et al. (9). Unfortunately, the present results on the dissociation of the A-band in a frog skinned fiber cannot be compared with those of in vitro studies because there have been few studies on physicochemical properties of synthetic thick filaments of frog myosin.

Elastic Components

Some recent papers reported that the resting tension or the resting stiffness decreased on the dissociation of the A-band in frog skinned fibers (11, 13, 28) and rabbit glycerinated psoas muscle fibers (3). But nothing has been reported on the relation between the resting tension and the residual length of the A-band. We showed that the relative resting tension in a skinned fiber decreased nearly in proportion to the residual length of the A-band. This proportional relation was obtained at several sarcomere lengths and different conditions for the dissociation of the A-band (Fig. 7). It would be accidental if disassembly of the parallel elastic components (PECs) occurred in the same conditions as those of dissociation of the A-band. Therefore, it is quite possible to interpret that the PECs bind almost uniformly to the A-band and that the dissociation of the A-band results in disassembly of these PECs.

The relationship between the resting tension and L in a skinned fiber (Fig. 8, ■) is consistent with that obtained by Umazume (25). The resting tension at $L = 5.0$ μm in Fig. 8 was about one-fifth of the active tension at $L = 2.2$ μm, ionic strength of 0.15 M, pCa = 5.4, and 10°C (data not shown). After the dissociation of the A-band, the resting tension greatly decreased (Fig. 8, ▲). Especially at $L \leq 3.0$ μm the resting tension decreased almost to zero after the dissociation of the A-band. This result indicates that the resting tension at $L \leq 3.0$ μm in an intact fiber with sarcolemma originates mainly from the PECs bound to the A-band, because the sarcolemma does not contribute to the resting tension at $L \lesssim 3.0$ μm (18, 21, 25).

We consider that the PECs do not bind to the free end portions of thin filaments for the following reasons. (*a*) The flexibility of thin filaments in a stretched skinned fiber is hardly dependent on the degree of stretching and the dissociation of the A-band (32), and (*b*) an electron micrograph shows that the free ends of thin filaments in an extremely stretched skinned fiber are not straight (19). Therefore, we conclude that one end of each PEC binds to

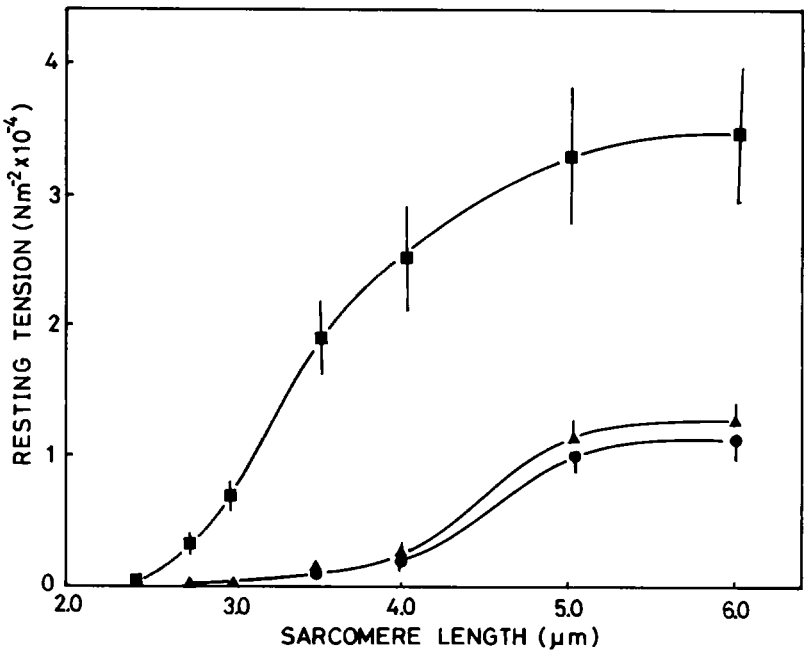

FIGURE 8 The relation between L and the resting tension before and after the dissociation of the A- and I-bands in a relaxing condition. A different fiber was used at each sarcomere length. ■ were obtained at ~ 45 min after stretching a skinned fiber. ▲ and ● were obtained after a skinned fiber was treated with 0.54 M KCl and further with 0.60 M KI, respectively, for 10 min at pH 7.0. The ordinate represents the resting tension per unit area. The cross-sectional area of a fiber was determined as follows. The fiber in a relaxing solution was rotated around the long axis and the largest and smallest widths at a middle portion of the fiber were measured at L = 2.5 μm under a microscope. The measurement was performed in each fiber before each experiment. The cross-sectional area was calculated from the two diameters assuming that the cross-sectional shape of the fiber was elliptic and independent of the sarcomere length and the treatment of the fiber. Symbols and bars show the mean and SD for three fibers at each sarcomere length.

the A-band (the thick filaments) and the other end binds directly or indirectly to a Z-line and/or some part of a thin filament near the Z-line. On the other hand, an indirect immunofluorescence microscopy showed that connectin (titin) was located at the A-I junction and the whole A-band, except the bare zone in stretched myofibrils of frog muscle (17). (But since the I-band was faintly fluorescent, it is difficult to determine whether or not connectin was located at the I-band [17]). Therefore, one constituent of the PECs bound to the A-band may be connectin, which might bind to Z-line directly or to a Z-line indirectly via another PEC at the I-band region.

As shown in Fig. 8, the resting tension hardly changed on the dissociation of the I-band. This result qualitatively agrees with the result of Maruyama et al. (13). The remaining resting tension after the dissociation of the A- and I-bands will be ascribed to the PECs that connect Z-lines and do not dissolve either at high concentrations of KCl or KI. One component of these PECs would be connectin (titin) (12, 13, 30). These PECs would be slack at $L \leqq 4.0$ μm, because the resting tension diminished after the dissociation of the A- and I-bands as shown in Fig. 8.

It has been suggested that an elasticity of the skinned fiber described by Natori (18), an S-filament proposed by Huxley and Hanson (6), a gap filament observed by Sjöstrand (23) and connectin found by Maruyama et al. (12, 13) play a part in maintaining the ordered structure of a striated muscle. The elasticity of the skinned fiber would be ascribed to the elasticity of the PECs in the present study. Some parts of PECs would correspond to an S-filament, a gap filament, and connectin. Therefore, we consider that the PECs bound to the A-band described here are important in maintaining the ordered structure in relaxing and contracting states of striated muscle at $L \leqq$ 3.5 μm. At a physiological sarcomere length of ~2.5 μm the resting tension is small. But during contraction the random movements of the A-bands along the fiber axis by ~0.2 μm are expected (1, 20, 27). In this case, the PECs

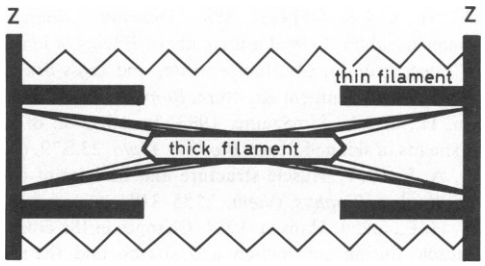

FIGURE 9 A possible model of localization of the parallel elastic components (PECs) in a sarcomere. Thin lines show the PECs. Thin zig-zag lines represents slack PECs. Z, Z-line. L = 3.5 μm. This figure was drawn very symbolically. For details, see text.

would be stretched locally and stabilize the sarcomere repetitious structure during contraction.

Finally, we propose a possible model on the localization of the PECs in a sarcomere on the basis of the present results (symbolically drawn in Fig. 9). From the present study alone, it is difficult to conclude that one end of PECs (thin lines) is distributed uniformly on each thick filament as Fig. 9 shows. The almost linear relationship between the resting tension and the residual A-band length, however, strongly suggests that one end of PECs distributes uniformly over an A-band in a myofibril and the other end links to a Z-line directly or indirectly via other PECs. In the latter case, it is possible that a small number of PECs bind randomly to each thick filament. In this sense, the figure was drawn diagrammatically and symbolically. In either case, the PECs would be entities connecting a thick filament with a nearby Z-line and a Z-line with the nearest Z-line. Some of the PECs connecting Z-lines may correspond to connecting and core filaments proposed by Magid et al. (11) and/or longitudinal intermediate filaments observed by Wang and Ramirez-Mitchell (31).

Maruyama et al. suggested that the elastic components form three-dimensional networks in a sarcomere (15). It is probable that the PECs bound to thick filaments and/or Z-lines also bind to the adjacent elastic components, which are running in the radial direction and are also contributing to the radial elasticity of muscle. Our next step will be to clarify the localization of the radial elastic components and its relation to the radial stiffness of a skinned fiber (29).

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