DISASSEMBLY KINETICS OF THICK FILAMENTS IN RABBIT SKELETAL MUSCLE FIBERS

Effects of Ionic Strength, Ca^{2+} Concentration, pH, Temperature, and Cross-Bridges on the Stability of Thick Filament Structure

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ABSTRACT The kinetics of dissociation from both ends of thick filaments in a muscle fiber was investigated by an optical diffraction method. The dissociation velocity of thick filaments at a sarcomere length of 2.75 μm increased with increasing the KCl concentration (from 60 mM to 0.5 M), increasing the pH value (from 6.2 to 8.0) or decreasing the temperature (from 25 to 5°C) in the presence of 10 mM pyrophosphate and 5 mM $MgCl_2$. Micromolar concentrations of Ca^{2+} suppressed the dissociation velocity markedly at shorter sarcomere lengths. The dissociation velocity, v, decreased as thick filaments became shorter, and $v = -db/dt = v_0 \exp(\alpha b)$, where b is the length of the thick filament at time t and v_0 and α are constants. The v_0 value was largely dependent on the KCl concentration but the α value was not. The stiffness of a muscle fiber decreased nearly in proportion to the decrease of overlap between thick and thin filaments induced by the dissociation of thick filaments. This indicates that cross-bridges are uniformly distributed and contribute independently to the stiffness of a muscle fiber during the dissociation of thick filaments.

INTRODUCTION

As described in the preceding paper (Ishiwata et al., 1985), the length of myofilaments in a striated muscle fiber can be estimated by an optical diffraction method (Fujime, 1975, 1984; Fujime and Yoshino, 1978). It is now possible to study the relationship between the stiffness or active tension of a muscle fiber and the length of thick filaments in the disassembly and assembly process of the filaments. In this work, we investigated the disassembly process of thick filaments in a glycerinated skeletal muscle fiber after stepwise increasing KCl concentration in the presence of 10 mM pyrophosphate (PPi)[1] and 5 mM $MgCl_2$ at various pH values, temperatures, Ca^{2+} concentrations and sarcomere lengths. The results were compared with those obtained in vitro in an equilibrium state (Kaminer and Bell, 1966; Josephs and Harrington, 1966, 1968; Katsura and Noda, 1973*a*, *b*) and those of kinetic experiments done by Davis in vitro (1981*a*, *b*).

Preliminary reports on the present work have been presented at the 6th Taniguchi International Symposium on Biophysics (Ishiwata, 1981*a*) and the 19th and the 20th annual meetings of the Biophysical Society of Japan (1981 and 1982).

MATERIALS AND METHODS

Muscle Fiber

A bundle of rabbit glycerinated psoas muscle fibers was prepared according to a previous method (Ishiwata et al., 1985). Muscle bundles with a sarcomere length of 2.4 or 2.75 μm were prepared by applying a slight stretch before a glycerol treatment. Those of 3.2 μm were prepared by stretching a glycerinated muscle bundle with a sarcomere length of 2.75 μm in a relaxing solution (0.1 M KCl, 5 mM $MgCl_2$, 4 mM ATP, 4 mM EGTA and 10 mM MOPS [pH 7.0]) at 0°C. A glycerinated muscle bundle (composed of ~10 fibers) set in an optical cell, was first immersed in a rigor buffer containing 60 mM KCl, 5 mM $MgCl_2$ and 10 mM Tris-Maleate buffer (pH 6.8) for ~10 min. Then, it was immersed further for about 10 min in a solution containing 60 mM KCl, 5 mM $MgCl_2$, 10 mM PPi, 1 mM EGTA or 100 μM $CaCl_2$ and 10 mM Tris buffer, which has a suitable pH value for the subsequent experiments (preincubation). Tris-maleate (pH 6.2 and 6.5) or Tris-HCl (pH 7.0, 7.5, and 8.0) was used as a pH buffer. When the effects of pH 7.5 and 8.0 were examined, Tris-HCl of pH 7.0 was used for this preincubation because there was a possibility that thick filaments were partially dissociated at high pH even at 60 mM KCl. The dissociation of thick filaments was initiated by replacing the above preincubation solution with that containing high KCl concentration and an appropriate pH buffer. The pH value of solution was readjusted for each condition. The experiments at 5 and 15°C were performed in a cold room and those at 25°C were done in a dark room. The temperature was maintained within ±2°C. The ATP was purchased from Boehringer Mannheim Co. Ltd. (Indianapolis, IN) and potassium PPi and other chemicals were of reagent grade.

[1] *Abbreviations used in this paper:* EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*',*N*'-tetraacetic acid; PPi, pyrophosphate.

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Optical Diffraction and Stiffness

The apparatus and method of measurement were essentially those described in the preceding paper (Ishiwata et al., 1985). The intensities of the first- and the second-order diffraction lines were measured simultaneously by use of two photodiodes fixed at each diffraction peak in the meridional plane. Diffracted light was focused on a photodiode by a convex lens whose diameter and focal length were, respectively, 1.2 and 2.1 cm. The distance between the fiber and the photodiode was 10 cm. The intensity of each diffraction line in a meridional plane was measured. The length of thick filaments was estimated by the intensity of the first- and the second-order diffraction lines according to models *a* or *b* in the preceding paper. The stiffness of a muscle fiber was measured by a tension transducer (UL 2, Shinkoh, Nagano Prefecture, Japan) according to the procedure in the preceding paper. Intensities of diffraction lines and stiffness were recorded in parallel by a 3 pen-recorder (VP-6633A; National Instrument Co., Inc., Baltimore, MD).

Electron Microscopy

A JEM 100 CX (JEOL USA, Electron Optics Division, Peabody, MA) electron microscope was used at an operating voltage of 80 kV. A muscle bundle composed of ~30 fibers was set in the optical cell. Thick filaments were dissociated in 0.35 M KCl, 5 mM $MgCl_2$, 1 mM EGTA, 10 mM PPi and 20 mM sodium cacodylate buffer (pH 7.0). The degree of dissociation of thick filaments was estimated from the intensity of diffraction lines. After thick filaments were partially dissociated, the above solution was replaced by the same solution containing 3% glutaraldehyde. This prefixation was performed for 40 min. The prefixed muscle bundle was then extensively washed with 120 mM cacodylate buffer (pH 7.2). Intensities of diffraction lines were recorded during the above prefixation and washing. A muscle bundle was then taken out from the optical cell and tied to a platinum wire of 0.3 mm in diameter. This was postfixed with 1% OsO_4 in 120 mM cacodylate buffer (pH 7.2) for 90 min and gradually dehydrated with ethanol and acetone. A muscle bundle cut off from the platinum wire was then embedded in Epon 812. Thin sectioning was done by use of an ultramicrotome (JUM 5A, JEOL USA, Analytical Instruments Division, Cranford, NJ). The section was stained sequentially with 1% uranyl acetate and lead citrate. All procedures were done at 20°C.

RESULTS

Time Course of the Intensity Change of Diffraction Lines Due to the Disassembly of Thick Filaments

We examined the time course of the intensity change after the increase of KCl concentration stepwise from 60 mM to some specified one, as shown in Fig. 1. Because the diameter of a muscle bundle was ~150 μm, time needed for a solution in a muscle fiber to be exchanged through diffusion would take several seconds if diffusion coefficients of inorganic ions were assumed to be of the order of 10^{-5} cm^2/s. Therefore, the intensity change observed during the very initial stage of dissociation might have some error.

Time Course of Length Change of Thick Filaments

Fig. 2 *a* shows that the lengths of thick filaments estimated independently from the intensities of the first- and the second-order diffraction lines according to model *a* in the preceding paper coincided with each other. This indicates that in the absence of Ca^{2+} the dissociation of thick filaments occurs from both ends maintaining the thickness of the filaments constant (model *a*). In the presence of Ca^{2+} (Fig. 2 *b*), however, the dissociation seems to occur according to model *b* (the thickness of the filaments also decreases in proportion to their shortening). The results can be interpreted as follows. Even in the presence of Ca^{2+}, thick filaments dissociate from both ends according to model *a* but a considerable part of dissociated myosin molecules bind to thin filaments (see Discussion in the preceding paper). Therefore, dissociation observed by optical diffraction became consistent with model *b*. This interpretation was supported by the fact that, at a low temperature, e.g., 5°C, where dissociated myosin molecules hardly bind to thin filaments, the dissociation of thick filaments occurred according to model *a* irrespective of Ca^{2+} concentrations (see Fig. 7).

We examined the effects of KCl concentrations, pH and temperature, not in the presence but in the absence of Ca^{2+}, because the results in the absence of Ca^{2+} can be simply analyzed by model *a*. For the same reason, we examined the effects of Ca^{2+} only at a low temperature (5°C). The error bars in Fig. 2 *a* show standard deviation estimated from the results of seven experiments. Such error bars were unavoidable even if short muscle bundles obtained by cutting a long bundle were examined.

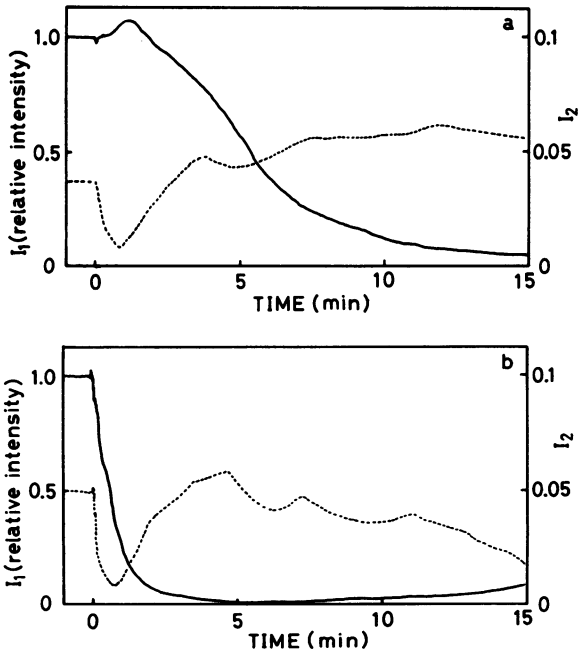

FIGURE 1 Time course of intensity change of diffraction lines after the addition of high salt and Mg-PPi. Intensities of the first-order (I_1; ——) and the second-order (I_2; – – –) diffraction lines are shown in a relative value. At time zero, rigor buffer with PPi was replaced by a solution containing 0.35 M KCl, 5 mM $MgCl_2$, 10 mM PPi, 10 mM Tris-HCl (pH 7.0) and 1 mM EGTA (*a*) or 100 μM $CaCl_2$ (*b*). Temperature, 25°C. Sarcomere length, 2.75 ± 0.1 μm.

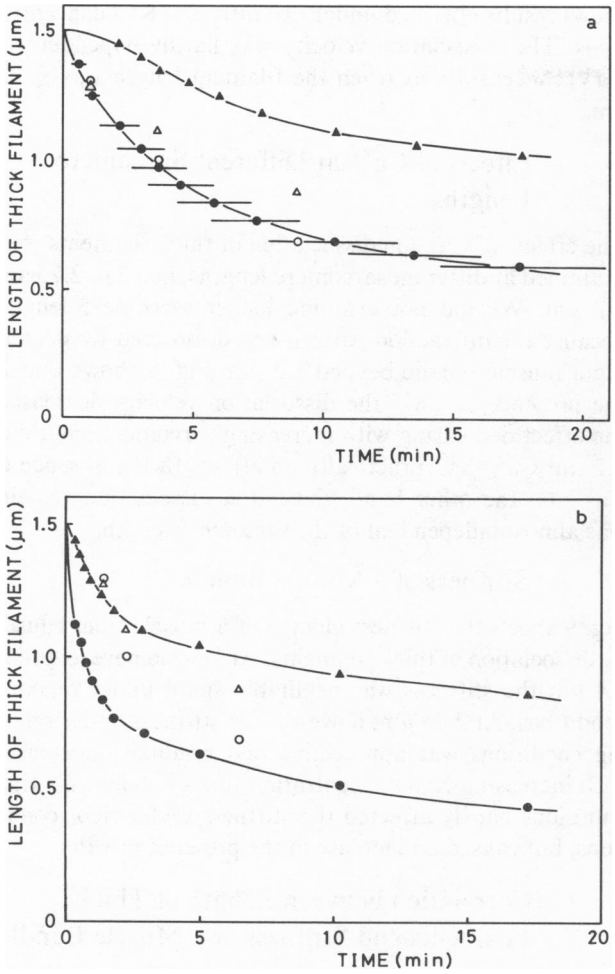

FIGURE 2 Time course of length change of thick filaments estimated according to models described in the preceding paper (Ishiwata et al., 1985). Conditions for disassembly of thick filaments in *a* and *b* are the same as in Fig. 1 *a* and *b*, respectively. The number of muscle bundles examined were 11 in *a* and 5 in *b*. Data points were obtained by averaging seven and three measurements, respectively; data on bundles that showed extremely fast or slow dissociation were omitted (four and two measurements, respectively). Error bars show standard deviation obtained at each length. Lengths of thick filaments were estimated according to model *a* (●, ○) or model *b* (▲, △) by comparing the intensities of the first-order (closed symbols) or the second-order (open symbols) diffraction line.

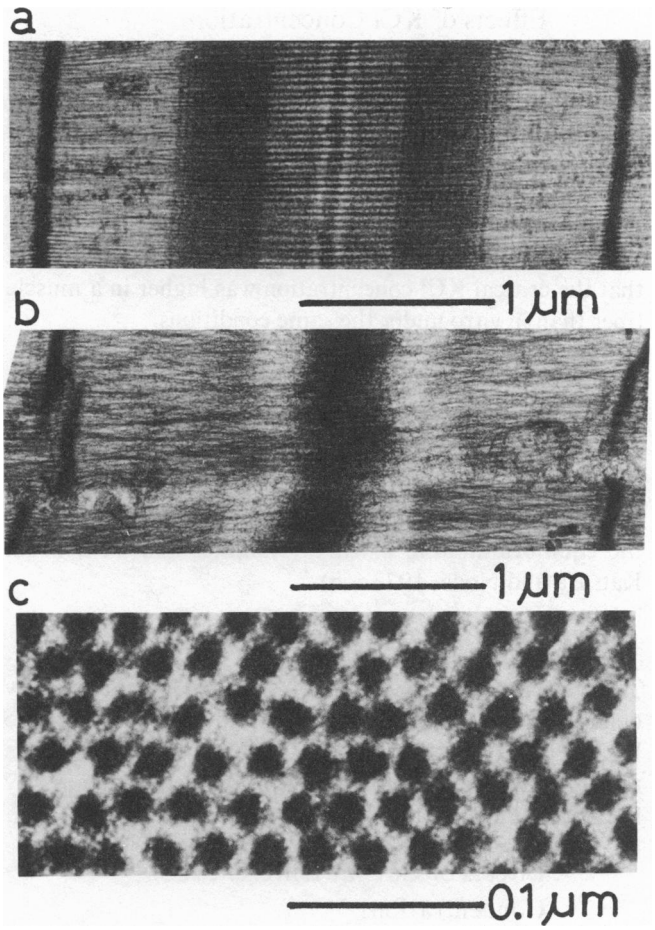

FIGURE 3 Electron micrographs of a muscle fiber before and after partial dissociation of thick filaments. (*a*) A bundle of muscle fibers was fixed in 60 mM KCl, 5 mM $MgCl_2$, 20 mM cacodylate buffer (pH 7.0), and 3% glutaraldehyde for 40 min at 20°C. (*b*) and (*c*) A bundle of muscle fibers was immersed in a high salt solution containing 0.35 m KCl, 5 mM $MgCl_2$, 10 mM PPi, 1 mM EGTA and 20 mM cacodylate buffer (pH 7.0) for 4 min and fixed in the same solution with 3% glutaraldehyde for 40 min at 20°C. (*a*) and (*b*) longitudinal section; (*c*) cross section.

Electron Microscopy

To confirm the conclusions drawn from the optical diffraction, we studied the structure of partially dissociated thick filaments in a muscle fiber by electron microscopy (Fig. 3). Treatment with 0.35 M KCl for 4 min at 20°C produces dissociation from both ends of thick filaments whose length became ~0.5 μm (see Hanson and Huxley, 1955). In cross section the filaments are essentially unchanged and no filaments disappear, although the lattice constant is smaller, probably because of the shrinkage of a muscle fiber. These observations support model *a* for the disassembly mechanism in the preceding paper.

The length of thick filaments estimated from the intensity of diffraction lines before prefixation was ~0.8 μm. It is reasonable, however, to consider that thick filaments were further dissociated from 0.8 to 0.5 μm during the prefixation, which was performed under the dissociating conditions to suppress filament reformation and to minimize the binding to thin filaments of dissociated myosin molecules. From Fig. 6, it is to be expected that if dissociation continued during fixation the filaments would have shortened by a further 0.2–0.3 μm at 20°C.

It was impossible to estimate the length of thick filaments during prefixation, because the intensities of the first- and the second-order diffraction lines increased on addition of glutaraldehyde, a finding which could not be interpreted in terms of simple dissociation from both ends of the thick filaments. Optical properties of muscle fibers would have been changed in a complicated manner by the fixation procedure.

Effects of KCl Concentration

Fig. 4 shows that the velocity of dissociation of thick filaments sharply increases on increasing the KCl concentration from 0.3 to 0.5 M. The critical concentration of KCl above which dissociation of thick filaments occurred was dependent on pH and temperature as shown below. For example, at 0.2 M KCl, the dissociation hardly occurred at pH 7.0 but did occur at pH 8.0. It is to be noted that the critical KCl concentration was higher in a muscle fiber than in vitro under the same conditions.

Effects of pH

The pH from 6.2 to 8.0 was examined at 0.35 M KCl in the absence of Ca^{2+} at 25°C (Fig. 5). The higher the value of pH, the higher the initial velocity of dissociation of thick filaments. This property is consistent with that observed in the equilibrium state in vitro (Kaminer and Bell, 1966; Katsura and Noda, 1973*a*, *b*).

Effects of Temperature

The velocity of dissociation of thick filaments strongly depends on temperature; the lower the temperature, the higher the velocity (Fig. 6). Note, however, that the temperature dependence of the dissociation velocity became small when the filament length became $\lesssim 0.6$ μm.

Effects of Ca^{2+} at Different KCl Concentrations

As already indicated, micromolar concentrations of Ca^{2+} lowered the velocity of dissociation under all conditions we examined at the sarcomere length of 2.75 ± 0.1 μm. Fig. 7 shows results obtained under two different KCl concentrations. The dissociation velocity was hardly dependent on Ca^{2+} concentrations when the filament length was $\lesssim 0.6$ μm.

Effects of Ca^{2+} at Different Sarcomere Lengths

The effects of Ca^{2+} on dissociation of thick filaments were examined at different sarcomere lengths, i.e., 2.4, 2.75 and 3.2 μm. We did not examine longer sarcomere lengths because the diffraction pattern was disordered by stretching a muscle bundle beyond 3.2 μm. Fig. 8 shows that in the presence of Ca^{2+} the dissociation velocity decreased, the effect decreasing with increasing sarcomere length; at 3.2 μm Ca^{2+} had practically no effect. In the absence of Ca^{2+}, on the other hand, the initial dissociation velocity was almost independent of the sarcomere length.

Stiffness of a Muscle Bundle

Fig. 9 shows the stiffness change of a muscle fiber caused by dissociation of thick filaments. At a sarcomere length of 2.4 μm the stiffness was negligibly small under relaxing conditions. At 2.75 μm, however, the stiffness under relaxing conditions was appreciable and gradually decreased with increasing KCl concentration (data not shown). Calcium ions hardly affected the stiffness under rigor conditions, but caused an increase in the presence of PPi.

Correlation between Length of Thick Filaments and Stiffness of a Muscle Bundle

It was difficult to compare the time courses of the length change of thick filaments and of the stiffness change of a

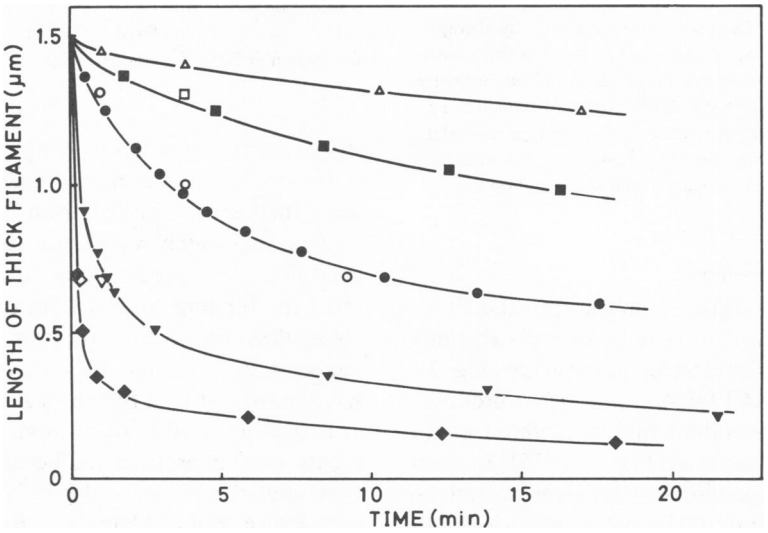

FIGURE 4 Effect of KCl concentration on the dissociation of thick filaments. Medium, 5 mM $MgCl_2$, 10 mM PPi, 10 mM Tris-HCl (pH 7.0), 1 mM EGTA and various concentrations of KCl (M); 0.25 (△), 0.30 (■, □), 0.35 (●, ○), 0.40 (▼, ▽) and 0.50 (◆, ◇). Temperature, 25°C. Sarcomere length, 2.75 ± 0.1 μm. In this and the following figures, in averaging data, bundles that showed extremely fast or slow dissociation were omitted (see legend to Fig. 2). The length of thick filaments was estimated by the intensity of the first-order (closed symbols) or the second-order (open symbols) diffraction line according to model *a*.

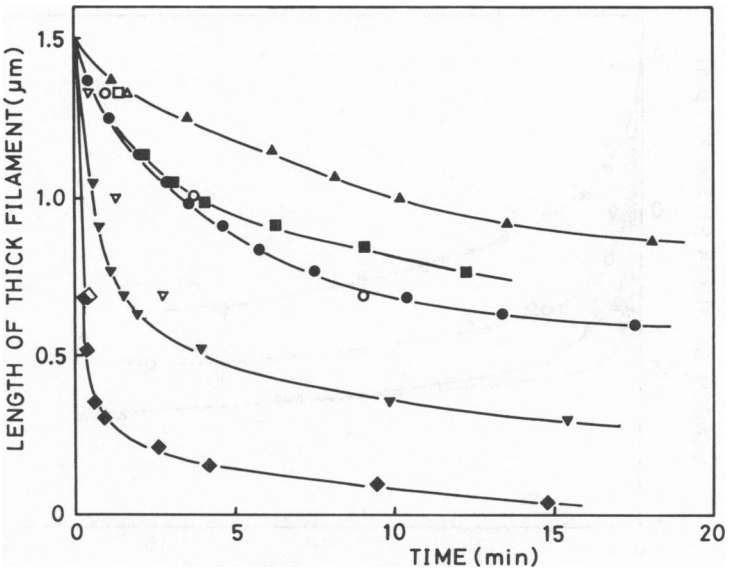

FIGURE 5 Effect of pH on the dissociation of thick filaments. Medium, 0.35 M KCl, 5 mM $MgCl_2$, 10 mM PPi, 1 mM EGTA and various pH buffers (see Materials and Methods); pH 6.2 (▲, △), 6.5 (■, □), 7.0 (●, ○), 7.5 (▼, ▽) and 8.0 (◆, ◇). Closed and open symbols, as in Fig. 4. Temperature, 25°C. Sarcomere length, 2.75 ± 0.1 μm.

muscle bundle at a sarcomere length of 2.75 μm, because there was ambiguity in subtracting a base line to obtain the stiffness due solely to cross-bridge formation. Therefore, we compared the above quantities at a sarcomere length of 2.4 μm. Fig. 10 shows that the stiffness changes nearly in proportion to the length of thick filaments irrespective of the Ca^{2+} concentration, although the stiffness decreases a little faster during the initial stage of dissociation of the filaments.

DISCUSSION

The dissociation kinetics of myosin filaments has not been studied in vitro, except by Davis (1981*a*, *b*) who investigated the dissociation process due to the pressure jump at 0.15 M KCl and pH 8.1 at different protein concentrations. Therefore, we cannot quantitatively compare the dissociation velocity of thick filaments in a muscle fiber—~0.1 μm/min at 0.3 M KCl and pH 7.0 (Fig. 4)—with

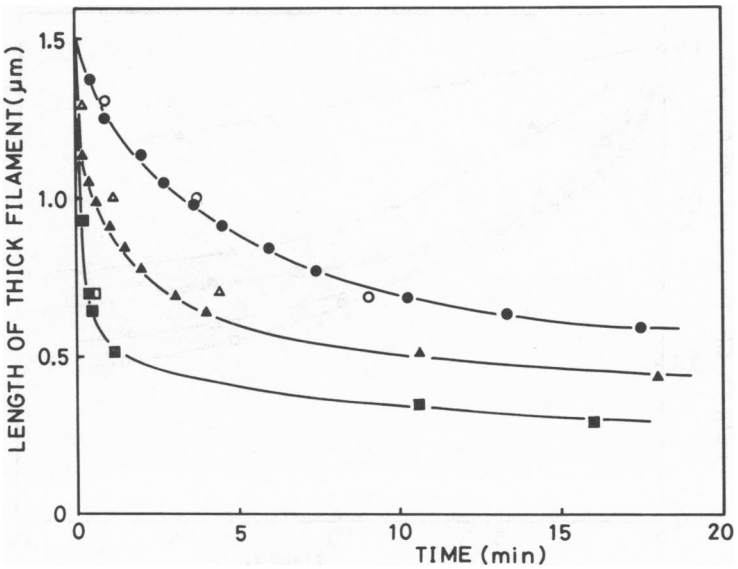

FIGURE 6 Effect of temperature on the dissociation of thick filaments. Medium, 0.35 M KCl, 5 mM $MgCl_2$, 10 mM PPi, 10 mM Tris-HCl (pH 7.0) and 1 mM EGTA. Temperature, 25 (●, ○), 15 (▲, △), and 5°C(■, □). Closed and open symbols, as in Fig. 4. Sarcomere length, 2.75 ± 0.1 μm.

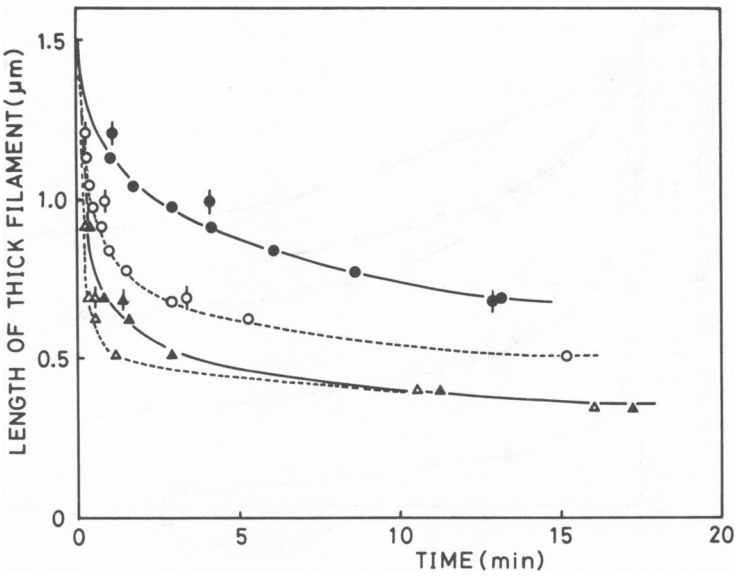

FIGURE 7 Effect of Ca^{2+} on the dissociation of thick filaments at two different concentrations of KCl. Medium, 5 mM $MgCl_2$, 10 mM PPi, 10 mM Tris-HCl (pH 7.0) and 0.3 M KCl (●, ◖, ○, ◔) or 0.35 M KCl (▲, ◭, △, ◬). Closed symbols, + 100 μM $CaCl_2$; open symbols, + 1 mM EGTA. The length of thick filaments was estimated by the intensity of the first-order (●, ○, ▲, △) or the second-order (◖, ◔, ◭, ◬) diffraction line according to model *a*. Temperature, 5°C. Sarcomere length, 2.75 ± 0.1 μm.

that of myosin filaments in vitro under the same dissociating conditions. Preliminary work, however, showed that the dissociation velocity in a muscle fiber is less than one thousandth that in vitro (data not shown). It is certain that the structure of thick filaments is stabilized in a muscle fiber.

The dissociation velocity of thick filaments increased with increasing the KCl concentration from 0.25 to 0.5 M (Fig. 4), with increasing pH value from 6.2 to 8.0 (Fig. 5) and with decreasing temperature from 25 to 5°C (Fig. 6). We compare these results with those in in vitro experiments done at equilibrium. Josephs and Harrington (1968) and Katsura and Noda (1973*b*) obtained an empirical formula that showed that the monomer concentration of myosin coexisting with filaments increases with increasing pH and KCl concentration. That is, myosin filaments tend to dissociate at high pH or high KCl concentrations. This trend is consistent with our results. On the other hand, the effect of temperature apparently contradicts the result obtained in vitro, that is, the stability of myosin filaments

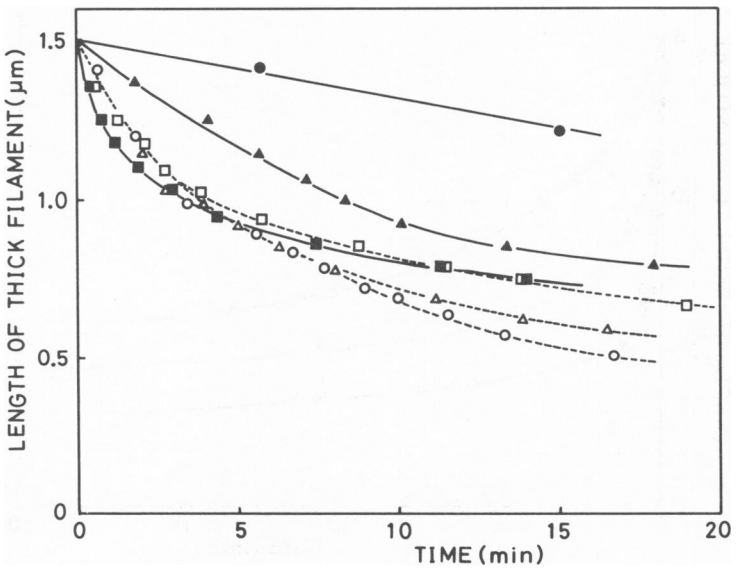

FIGURE 8 Effect of Ca^{2+} on the dissociation of thick filaments at three different sarcomere lengths. Medium, 0.275 M KCl, 5 mM $MgCl_2$, 10 mM PPi, 10 mM Tris-HCl (pH 7.0) and 100 μM $CaCl_2$ (closed symbols) or 1 mM EGTA (open symbols). Temperature, 5°C. Sarcomere length, 2.4 (●, ○), 2.75 (▲, △) and 3.2 (■, □) ± 0.1 μm.

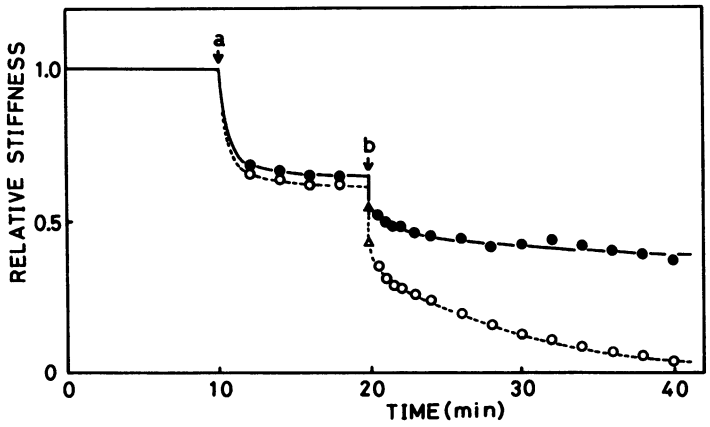

FIGURE 9 Time course of stiffness change of a muscle bundle after change of medium. The value of stiffness was normalized to that under rigor conditions, because the diameter of a muscle bundle was different from preparation to preparation. (*a*) The rigor buffer was replaced by 60 mM KCl, 5 mM $MgCl_2$, 10 mM PPi and 10 mM Tris-HCl (pH 7.0). (*b*) The medium was replaced by 0.275 M KCl, 5 mM $MgCl_2$, 10 mM PPi, 10 mM Tris-HCl (pH 7.0) and 100 μM $CaCl_2$ (●) or 1 mM EGTA (○). (▲, △) extrapolated points. Temperature, 5°C. Sarcomere length, 2.4 ± 0.1 μm.

in vitro hardly depends on temperature between 1 and 16°C (Josephs and Harrington, 1968), whereas it appears that the stability of thick filaments in a muscle fiber increased with increasing temperature (Fig. 6). This apparent discrepancy is attributable to the stabilization of the structure of thick filaments in a muscle fiber by cross-bridges. This interpretation is supported by the following facts. First, in vitro experiments show that the association constant between myosin and actin increases; that is, the number of cross-bridges increases with increasing temperature under appropriate conditions (Highsmith, 1977; Ishiwata et al., 1979). Second, the temperature effect became small when the length of thick filaments became $\lesssim$ 0.6 μm, where there was no overlap between thick and thin filaments, i.e., no cross-bridges (see Fig. 6). And finally, there was practically no temperature effect on the dissociation velocity of thick filaments in a muscle fiber under relaxing conditions (Higuchi H., and S. Ishiwata, unpublished work).

Also, the effects of Ca^{2+} shown in Fig. 7 can be interpreted similarly as already discussed in the preceding paper (Ishiwata et al., 1985). However, the reason why the thick filament structure was stabilized by Ca^{2+} on decreasing sarcomere lengths may be a little more complicated (see Fig. 8). On the one hand, cross-bridge formation stabilizes the structure of thick filaments and the number of cross-bridges increases with decreasing sarcomere length. On the other hand, if the cross-bridge formed by a

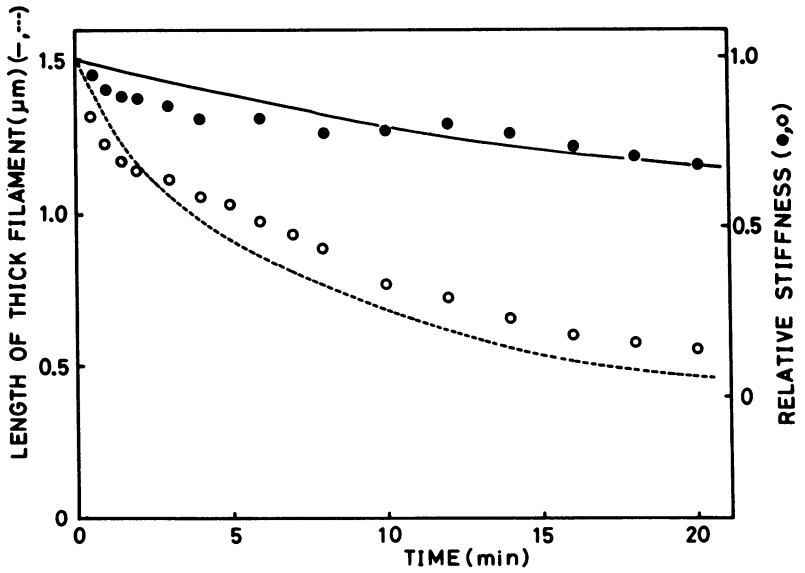

FIGURE 10 Correlation between stiffness of a muscle bundle and length of thick filaments. Length data (—, ---) in the presence or absence of Ca^{2+} taken from Fig. 8. (●, ○) relative values of stiffness in the presence or absence of Ca^{2+}. The value 1 corresponds to the extrapolated value (▲, △) in Fig. 9 before the filaments began to dissociate. It was assumed that the stiffness became 0 when the thick filaments were shortened to 0.4 μm and the sarcomere length was 2.4 μm, so that the overlap between thick and thin filaments disappeared.

myosin molecule independently stabilizes the myosin-myosin binding in the tail part of the molecule and if the cross-bridges are formed uniformly, the initial dissociation velocity should be independent of sarcomere length. Thus the Ca^{2+} effects may be explained as follows: The stability of the whole structure of a thick filament depends on the number of cross-bridges formed on it and not on the density (number/unit length). Otherwise, cross-bridges formed in the central part of a thick filament would stabilize the structure at the tips of the filament.

During the dissociation of thick filaments the stiffness of a muscle bundle decreases nearly in proportion to the decrease of overlap between thick and thin filaments, whether or not Ca^{2+} is present (Fig. 10). This is consistent with the conclusions that (*a*) thick filaments dissociate from both ends and (*b*) cross-bridges that independently contribute to the stiffness are distributed uniformly on thick filaments.

The optical diffraction method is unable to detect whether or not a core structure of thick filaments is disorganized in a muscle fiber. That is, if cross-bridges are preserved as arrow-head structures the intensity of diffraction lines will be scarcely changed even if the core structure of thick filaments is disorganized from both ends, because the protein concentration in the A-band region does not change. Therefore, if we used the optical diffraction method only, we could not exclude the possibility that thick filaments in a muscle fiber dissociate independently of Ca^{2+} concentration or temperature but the number of cross-bridges are regulated by these factors, as the intensity of diffraction lines would appear to be regulated. In this case, the stiffness should decrease at a rate independent of Ca^{2+} concentrations or temperature, despite the fact that the dissociation velocity of thick filaments estimated by the optical diffraction method depended on the Ca^{2+} concentration or on temperature. The results in Fig. 10 rule out this possibility. That is, the shortening of thick filaments estimated by the optical diffraction method and the decrease of stiffness occurs in parallel, with or without Ca^{2+}. Thus, the results in Fig. 10 strongly support the conclusion that thick filaments dissociate from both ends and cross-bridges stabilize the binding between myosin molecules so as to suppress the dissociation of the filament. Also, the results in Fig. 10 suggest that during the dissociation of thick filaments the structure of the remaining central part of the filaments is not disorganized even partially.

Finally, we analyzed the dissociation process of thick filaments more quantitatively. We replotted in Fig. 11 the time course of the length change of thick filaments shown in Fig. 4 against the logarithm of time (t). We found the following empirical formula, $b(t) = -C_1 \ln(C_2 t + C_3)$, which can simulate the time course of length change, where $b(t)$ is the length of thick filament at time t and C_1, C_2 and C_3 are constants that depend on dissociation conditions. (This formula also simulates the results in Figs. 5, 6, 7, and 8.) This formula is equivalent to $-db/dt = v = v_0 \exp(\alpha b)$, and integration gives the relationships $C_1 = 1/\alpha$, $C_2 = \alpha v_0$, and $C_3 = \exp(-\alpha b_0)$, where b_0 is the initial length of thick filaments in each region of dissociation (see Table I). For analyzing the data, we divided the length of thick filaments into two regions, depending on b: 1; $1.5 \geq b \gtrsim 0.5$ μm and 2; $b \lesssim 0.5$ μm. The values of constants in the empirical formula obtained in each region by the least squares method are summarized in Table I. The results show that v_0 strongly depends on the KCl concentration but α does not (at least in region 1; the accuracy of data in region 2 was relatively low). Recently, on the basis of the kinetic measurement of turbidity, Davis (1981*b*) showed that the dissociation velocity of reconstituted myosin filaments in vitro is proportional to $\exp(\beta l)$, where β is a constant and l is a length of myosin filaments. Our results are consistent with those obtained by Davis. If the inverse of the dissociation velocity is considered to be a measure of the structural stability at the tip of the filaments, our results indicate that the stability at the tip decreases as the length of the filaments increases (see Trinick and Cooper, 1980; Davis, 1981*b*; Ishiwata, 1981*b*). This property of thick filaments may play an important part in the mechanism of the length determination of the filaments. Also, it should be noted that the parameters of this stabilization may change at 0.4–0.5 μm filament length because the values of constants in the empirical formula change there (see Fig. 11 and Table I). This length may correspond to that of the central part of thick filaments in which the mode of binding of myosin molecules may differ from that of the end part of the filament (see Maw and Rowe, 1980;

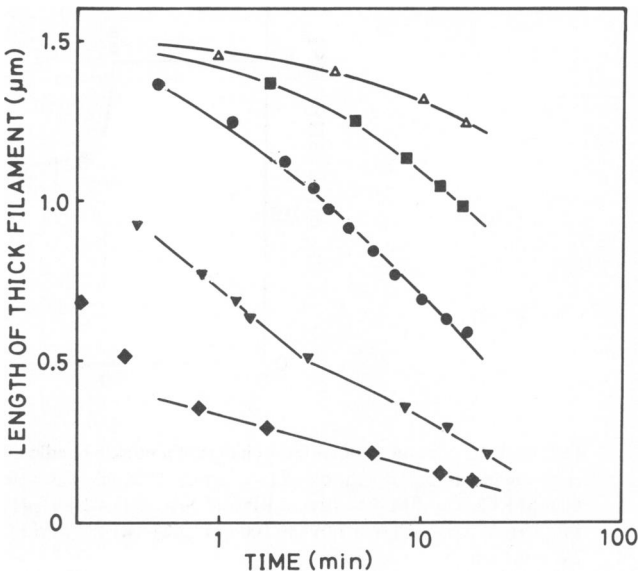

FIGURE 11 Length of thick filaments (b) vs. log t (dissociation time). Data points replotted from Fig. 4; symbols as in Fig. 4. Solid lines were drawn according to the empirical formula $b = -C_1 \ln(C_2 t + C_3)$, where the constants, C_1, C_2, and C_3 were determined by a least-squares method, separately for $b > 0.5$ μm and $b < 0.5$ μm (see Table I, and see text).

TABLE I
VALUES OF CONSTANTS IN THE EMPIRICAL FORMULA

KCl	Region 1			Region 2		
	v_0	α	b_0	v_0	α	b_0
M	$\mu m/min$	μm^{-1}	μm	$\mu m/min$	μm^{-1}	μm
0.25	4.9×10^{-6}	5.9	1.5	—	—	—
0.30	4.9×10^{-4}	3.5	1.5	—	—	—
0.35	3.1×10^{-3}	3.2	1.5	—	—	—
0.40	9.7×10^{-3}	4.3	1.5	1.2×10^{-2}	5.9	0.5
0.50	—	—	—	8.7×10^{-3}	15	0.4

See Fig. 11. The values of v_0, α, and b_0 were calculated by using the values of C_1, C_2 and C_3, which were determined by the least-squares method. The length of thick filaments was divided into two regions, 1 and 2, depending on whether $b_0 > 0.4$ or 0.5 μm; in each region the data points were fitted to the empirical formula. The data points obtained at <30 s of dissociation time were omitted, because the accuracy was not satisfactory. Dashes in the table show that the value could not be obtained.

Reisler et al., 1980; Squire, 1981; Niederman and Peters, 1982).

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