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THE EFFECT OF TEMPERATURE ON THE INTERACTION BETWEEN F-ACTIN AND TROPOMYOSIN

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SUMMARY

The effect of temperature on the binding between F-actin and tropomyosin was studied by means of flow birefringence and ultracentrifugation. Tropomyosin bound to F-actin at room temperature is reversibly dissociated from F-actin by raising temperature. The dissociation temperature is about 35–40° at physiological salt concentration and decreases with deviation of the salt concentration from physiological. The saturated amount of bound tropomyosin at room temperature is also dependent on the salt concentration. The suppression of the ATPase activity of F-actin by bound tropomyosin observed at room temperature is released at high temperature.

INTRODUCTION

Many investigations have been made to elucidate the physiological function of tropomyosin in muscle and it has been suggested that this protein performs an important role in regulating the structure of actin which interacts with myosin in the force generating mechanism. The binding of tropomyosin to F-actin has been demonstrated *in vitro* by means of viscometry[1,2], ultracentrifugation[1–3] and flow birefringence[4] studies. This binding has also been found to slow down the rate of exchange of nucleotides and divalent cations bound to F-actin[5,6].

In this work, special attention is paid to the effect of temperature on the association–dissociation equilibrium between F-actin and tropomyosin at different salt conditions. The analyses are made by measuring the degree of flow birefringence, the amount of ultracentrifuged sediments and also the activity of the steady ATPase of F-actin.

MATERIALS

Actin. A dried powder of rabbit skeletal muscle was prepared by essentially the same method as that of STRAUB[7], except that tropomyosin and new proteins discovered by EBASHI and co-workers[8–10] were carefully removed before the acetone treatment of myosin-extracted minced muscle by its incubation in distilled water for 2 h at room temperature. G-actin was extracted with 20 ml of distilled water/g of the powder at

low temperature (2°). For polymerization, KCl was added to the extracted solution to a final concentration of 30 mM. The F-actin solution thus obtained was sedimented by ultracentrifugation at 78000 × *g* for 3 h. The pellet was dissolved in 100 mM KCl and 5 mM Tris–HCl (pH 8.0) and again ultracentrifuged, 107000 × *g* for 2 h. The pellet was then dissolved in 0.5 mM ATP and 5 mM Tris–HCl (pH 8.0) in the absence of salt. After depolymerization, it was clarified by centrifugation at 107000 × *g* for 1 h. G-actin thus obtained was polymerized with 100 mM KCl and ultracentrifuged again at 107000 × *g* for 2 h.

Tropomyosin. The mince muscle incubated in water at room temperature for 2 h was filtered. Tropomyosin was purified from the supernatant by the method of BAILEY[11], using isoelectric precipitation at pH 4.6, followed by $(NH_4)_2SO_4$ separation between 48 and 52% saturation. The purification procedure was repeated (usually three cycles or more) until all traces of nucleoprotein had been removed; the removal of the nucleoprotein was checked by ultraviolet absorption spectra of solutions obtained after dialysis with water. Tropomyosin was stored in the frozen state until use.

METHODS

Flow birefringence (Δn) was measured by a Rao-type home made apparatus with temperature control by circulating water at low shear rate of 16 sec^{-1} (ref. 12). Viscometry was carried out in a thermostat by an Ostwald viscometer in which the flow time of water was about 30 sec at 20°. Ultracentrifugation for the preparation of actin was performed in a Hitachi preparative ultracentrifuge. Sedimentation measurements at high temperature were carried out with a Hitachi analytical ultracentrifuge and at low temperature with a Spinco Model E with temperature control. Sonic vibration at 10 kHz was supplied with a sonic generator KMS 100 of Kubota, Japan. The optical rotation of tropomyosin was measured with Jasco autospectrophotometer UV-5 with temperature control by circulating water.

Protein concentrations were measured by the biuret method. The concentration of F-actin was determined by the degree of flow birefringence. The spectrophotometric method was also applied to determine the concentration of tropomyosin[13]. The concentration of inorganic phosphate in the solution was measured by the method of MARTIN AND DOTY[14]. Deproteinization was done with 12% $HClO_4$ at 0°.

RESULTS

Polymerization of actin at different temperatures in the presence and absence of tropomyosin

When neutral salt is added to G-actin, the degree of flow birefringence increases with polymerization. The time course of polymerization was followed by measuring the degree of flow birefringence under various conditions. First, G-actin was polymerized under the salt condition: 100 mM KCl, 5 mM Tris–HCl (pH 8.0) and 0.5 mM ATP at room temperature (25°) in the presence and absence of tropomyosin. As shown in Fig. 1, in the presence of tropomyosin, the birefringence Δn increases and attains a larger value than in its absence. As tropomyosin alone can not contribute to Δn at low shear rates because of its small size, the above result indicates the interaction between F-actin and tropomyosin. It is likely that tropomyosin bound to F-actin

contributes to Δn by directing the long axis of its rodlike part in parallel to that of F-actin. In Fig. 1a, during polymerization, Δn in the presence of tropomyosin is always larger than in its absence by a nearly constant ratio and also the half polymerization time is almost equal in both cases. This suggests that the binding of tropomyosin takes place in parallel to polymerization of actin, and tropomyosin has no effect on the rate of polymerization of G-actin.

On the other hand, when G-actin was polymerized at 45°, the time course of po ymerization of G-actin in the presence of tropomyosin was almost the same as in its absence. Tropomyosin has no appreciable effect on the polymerization of actin

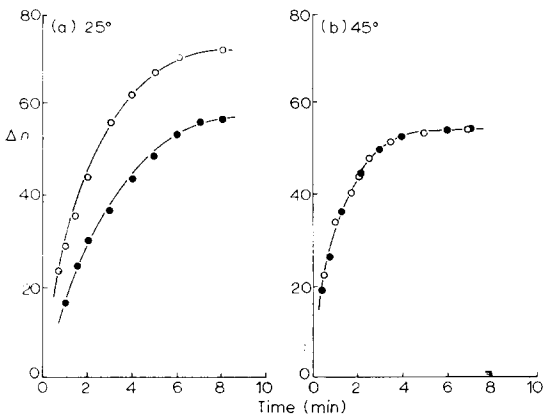


Fig. 1. Polymerization of actin in the presence and absence of tropomyosin at different temperatures. (a) At 25°. Actin (0.8 mg/ml) was polymerized with salt in the presence and absence of tropomyosin (0.16 mg/ml). Solvent: 100 mM KCl, 5 mM Tris–HCl (pH 8.0) and 0.5 mM ATP. ○, in the presence of tropomyosin; ●, in its absence. (b) At 45°. Conditions and symbols are the same as above. The degree of flow birefringence of 1 mg/ml F-actin is about 77° with the flow birefringence apparatus used.

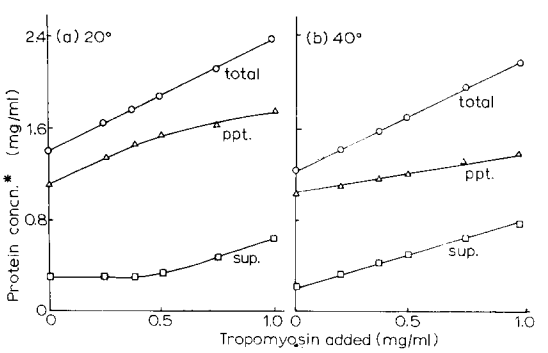


Fig. 2. Analysis of the binding by ultracentrifugation at 20° (a) and 40° (b). Ultracentrifugation was performed by a Hitachi preparative ultracentrifuge with a temperature control. Samples (4 ml) contain constant amounts of F-actin (a: 1.4 mg/ml and b: 1.2 mg/ml) and various amounts of tropomyosin as shown on the abscissa. Solvent condition is the same as that in Fig. 1. In these figures sup. means the amount of supernatant left by centrifugation, ppt. that of sediments, and total the sum of sup. and ppt. *For comparison of ppt. with sup., sediments were dissolved in the initial volume (4 ml) and concentrations were represented in mg/ml unit.

at such a high temperature (Fig. 1b). Thus, the interaction between F-actin and tropomyosin strongly depends on temperature. Temperature dependence is also found when tropomyosin is added after polymerization of actin.

Analysis by ultracentrifugation

The effect of temperature on the interaction was studied by means of centrifugation at different temperatures. Mixtures of various ratios of tropomyosin to F-actin were prepared at room temperature in a solution containing 100 mM KCl, 5 mM Tris–HCl (pH 8.0) and 0.5 mM ATP, where the binding of two proteins was confirmed in flow birefringence studies. These solutions were then centrifuged at 107000 $\times$ *g* for 3 h at different temperatures. The amount of proteins in the sediments and supernatants was measured by the Biuret method. As shown in Fig. 2a, when centrifugation was performed at low temperature (20°), tropomyosin bound to F-actin co-sedimented with F-actin and the excess amount of tropomyosin was left in the supernatant. At small ratios of tropomyosin to F-actin, almost all the tropomyosin added was bound to F-actin, and at high ratios, the amount of bound tropomyosin increased only slightly with a further increase of tropomyosin.

On the other hand, when centrifugation was performed at high temperature (initially at 42° then down to 38° during centrifugation), dissociation of tropomyosin from F-actin took place and only small amounts of tropomyosin co-sedimented with F-actin, as shown in Fig. 2b. (In these experiments, a small amount of actin was observed in the supernatant even in the absence of tropomyosin. This might be due to the incomplete sedimentation by centrifugation and/or the partial depolymerization of F-actin).

The temperature dependence of the interaction was also confirmed from the sedimentation pattern (Fig. 3) in an analytical ultracentrifuge at different temperatures. At low temperature (16.5°), tropomyosin co-sedimented with F-actin while the tropomyosin peak was found near the meniscus when F-actin was absent. At high temperature (40°), the tropomyosin peak was observed near the meniscus both

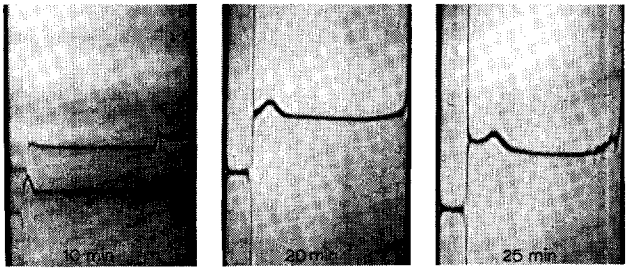


Fig. 3. Sedimentation pattern of mixtures of F-actin and tropomyosin at different temperatures. Left: Ultracentrifugation was performed by a Spinco Model E analytical ultracentrifuge with temperature control at 16.5°. The upper sample contains 0.9 mg/ml tropomyosin and 4.2 mg/ml F-actin and the lower sample, 0.9 mg/ml tropomyosin only. Photographs were taken at the time shown in the figures after reaching a maximum speed of 59780 rev./min; bar angle, 70°. The solvent is 120 mM KCl, 5 mM Tris–HCl (pH 8) and 0.2 mM ATP. Middle: Ultracentrifugation was performed by a Hitachi analytical ultracentrifuge with temperature control at 40°. The sample contains 0.88 mg/ml tropomyosin. The solvent is 60 mM KCl, 5 mM Tris–HCl (pH 8), 0.2 M urea and 2.5 mM ATP. Bar angle, 60°. Right: Procedures and condition are the same as that in Middle except for the presence of 4.5 mg/ml F-actin. Interaction at low temperature was recognized by measuring the degree of flow birefringence just prior to the measurement.

in the presence and absence of F-actin. In this way the results obtained by the flow birefringence method were confirmed by centrifugation.

Dependence of the interaction on temperature and salt concentration

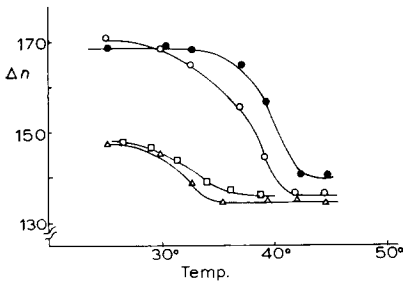
To obtain full details of the effect of temperature on the interaction, the change of flow birefringence of the mixed solution of tropomyosin and F-actin was investigated when the temperature was raised from 25 to 45° and then cooled to 25°. The value of Δn of the mixture prepared in a salt solution of 100 mM KCl, 5 mM Tris–HCl (pH 8.0) and 0.5 mM ATP decreased to that of pure F-actin with increasing temperature and returned to almost initial value on cooling, *i.e.* the binding between F-actin and tropomyosin is reversible in this temperature range.

One can see from Fig. 4 that, at physiological salt concentration, dissociation occurs at around 38°. Such dissociation always occurs with increasing temperature over the whole range of the salt concentration. But, the critical temperature for dissociation depends on the salt concentration. It is highest at around the physiological salt concentration, and becomes lower as the salt concentration deviates from physiological.

The dissociation of tropomyosin at lower and higher salt concentrations was also studied at a constant temperature (20°) by means of centrifugation. Mixtures of tropomyosin and F-actin at various salt concentrations were centrifuged at 20°. In Fig. 5 the values of Δn and the amounts of sediments by centrifugation at 20° are plotted against the log salt concentration. Partial dissociation of tropomyosin from F-actin was observed when the salt concentration deviates from physiological, *e.g.* at 40 mM and 400 mM KCl (Fig. 5). The physiological concentration is optimal for the binding of tropomyosin to F-actin.

The ATP splitting

F-Actin was found to have a temperature-dependent ATPase activity and some structural changes were assumed to be required for this ATP splitting[15]. There-



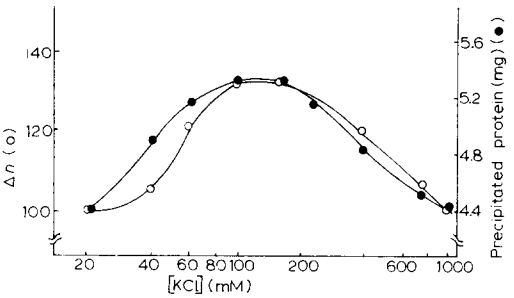


Fig. 4. Temperature dependence of the binding. Mixtures contain 1.7 mg/ml F-actin and 0.5 mg/ml tropomyosin. The solvent is 400 mM (□), 100 mM (●), 60 mM (○), 40 mM (△) KCl, 5 mM Tris–HCl (pH 8) and 0.5 mM ATP.

Fig. 5. Salt concentration dependence of the binding. All mixtures (4 ml) contain 1.1 mg/ml F-actin and 0.4 mg/ml tropomyosin. The left ordinate represents the degree of flow birefringence and the right one the amount of sediment obtained by centrifugation. The other solvent condition is 5 mM Tris–HCl (pH 8) and 0.5 mM ATP at 20°. ○, degree of flow birefringence; ●, the amount of sediment by centrifugation.

fore, to study the effect of tropomyosin on the structure of F-actin, ATP splitting in the mixture of tropomyosin and F-actin was measured at various temperatures. Mixtures of a constant amount of F-actin and various amounts of tropomyosin were prepared in a salt solution of 100 mM KCl, 5 mM Tris–HCl (pH 8.0) and 0.5 mM ATP at 30°. The binding of tropomyosin to F-actin was confirmed by measurement of Δn just prior to the measurement of ATPase activity. The result in Fig. 6 shows that the ATPase activity of the mixtures at 30° decreases with increasing amount of bound tropomyosin.

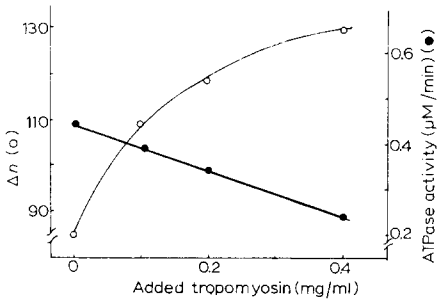


Fig. 6. ATPase activity of F-actin in the presence of tropomyosin. Solutions contain a constant amount of F-actin (1.1 mg/ml) and various amounts of tropomyosin. ATP was added just prior to the ATPase activity measurement. The left ordinate indicates the degree of flow birefringence and the right the rate of ATP splitting. The solvent is 100 mM KCl, 10 mM Tris–HCl (pH 8.0) and 0.5 mM ATP. ○, degree of flow birefringence; ●, ATPase activity.

On the other hand, when measurements were performed at 45°, both the ATPase activity and the degree of flow birefringence of F-actin in the presence and absence of tropomyosin were the same (Table I).

TABLE I

THE ATPASE ACTIVITY OF F-ACTIN IN THE PRESENCE AND ABSENCE OF TROPOMYOSIN AT 45°

F-actin, 1.0 mg/ml; tropomyosin, 0.5 mg/ml. The temperature values denoted as 25° are the values before the incubation at 45°. Procedures and solvent are the same as in Fig. 6.

Temp.	*Sample*	Δn	*ATPase activity* ($\mu M/min$)
25°	F-actin	75	—
	F-actin + tropomyosin	110	—
45°	F-actin	78	2.4
	F-actin + tropomyosin	78	2.4

F-actin solutions in the presence and absence of tropomyosin were prepared at 30° and then one half of the mixture was incubated at 45° for 15 min. The remaining half and the solution of F-actin only were left standing at 30°. After the dissociation of tropomyosin from F-actin had been confirmed at 45° by measuring the degree of flow birefringence, the mixture was cooled to 30° and its ATPase activity was

compared with that of the solutions kept at 30°. The result in Table II shows that the ATPase activity of F-actin in the presence of tropomyosin was suppressed by the binding of tropomyosin to F-actin to the same extent in the two solutions. The ATPase activity of F-actin was reversibly suppressed by tropomyosin.

TABLE II

THE REVERSIBILITI OF THE EFFECT OF THE INTERACTION ON THE ATPASE ACTIVITY OF F-ACTIN

F-actin, 1.0 mg/ml; tropomyosin, 0.5 mg/ml.

Sample	*Temp.*	$\triangle n$	*ATPase activity* ($\mu M/min$)
F-actin	30°	82	0.55
F-actin + tropomyosin	30°	106	0.40
F-actin + tropomyosin	*30°	106	—
	↓		
	45°	82	—
	↓		
	30°	104	0.40

* The temperature is changed as shown by the arrows (see text).

TABLE III

THE ATPASE ACTIVITY OF F-ACTIN IN A SONIC FIELD

Measurements were performed at various KCl concentrations at 25° in a sonic field. Other solvents are the same as in Fig. 1. F-actin, 1.6 mg/ml; tropomyosin, 0.5 mg/ml.

[*KCl*] (mM)	*Sample*	*ATPase activity* ($\mu M/min$)
20	F-actin	7.5
	F-actin + tropomyosin	7.5
100	F-actin	6.7
	F-actin + tropomyosin	3.4
1000	F-actin	6.7
	F-actin + tropomyosin	6.7

In this way, the interaction of tropomyosin with F-actin can be observed by the ATPase activity of F-actin. Under a sonic field, the interaction of shortened F-actin with tropomyosin was studied by measuring the ATPase activity. Sonic vibration was applied to F-actin in the presence and absence of tropomyosin under various salt concentrations at 25°. The results in Table III show that the ATPase activity of F-actin both in the presence and absence of tropomyosin was enhanced by sonic vibration. However, the ATPase activity was suppressed by tropomyosin at physiological salt concentration, while at salt concentrations differing from physiological (both lower and higher) no suppression was observed.

DISCUSSION

The dissociation of tropomyosin from F-actin was previously demonstrated by raising and lowering salt concentration[1,2,4,17]. In addition it was found here that the dissociation occurs with increasing temperature and the temperature for dissociation depends on the salt concentration. A scheme of the results is shown in Fig. 7 on the three dimensional plot, where the three coordinates are temperature, degree of flow birefringence and log salt concentration. This figure shows that the optimal condition for the binding between F-actin and tropomyosin is in the range of the physiological salt concentration and at room temperature.

To see if the dissociation of tropomyosin from F-actin was correlated with the change of the state of tropomyosin, the viscosity and the optical rotation of tropomyosin solutions were measured at various temperatures. With increasing temperature, tropomyosin polymers depolymerize and the helical content decreases, as shown in Fig. 8. However, the temperature range of these changes does not always

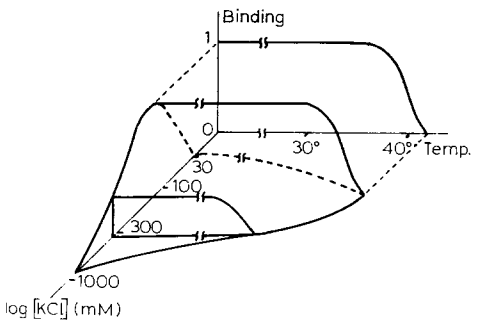


Fig. 7. Scheme of the dependence of the binding on the salt concentration and the temperature. Three coordinates represent degree of flow birefringence (in this scale birefringence where saturated amount of tropomyosin binds to F-actin is normalized to 1 and that of pure F-actin to 0), the temperature and the log salt concentration. A projection chart of this model to the Δn–temperature plane shows the temperature dependence of the binding, *e.g.* cross-sections parallel to the Δn-temperature plane show the temperature dependence of the binding at various salt concentration (Fig. 4) and those to the Δn–[KCl] plane, salt concentration dependence (Fig. 5).

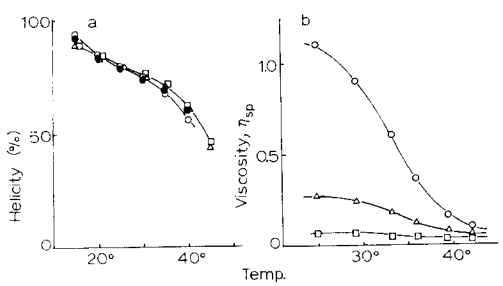


Fig. 8. Temperature dependences of the helical content (left) and the viscosity (right) of tropomyosin at various salt concentrations. (a) Samples contain 1 mg/ml tropomyosin in 5 mM Tris–HCl (pH 8.0) and various KCl concentrations (mM): ○, 20; ●, 70; △, 170; □, 270. (b) Samples contain 2 mg/ml tropomyosin in 5 mM Tris–HCl (pH 8.0) and various KCl concentrations (mM): ○, 40; △, 100; □, 400.

correspond to that of dissociation, as shown in Fig. 9; moreover, the helical content is not much influenced by the salt concentration, whereas the dissociation temperature changes with the salt concentration.

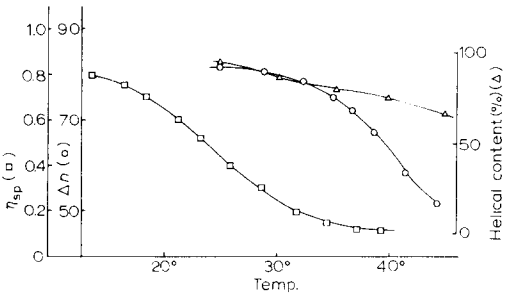


Fig. 9. Comparison of the change of the binding of tropomyosin to F-actin with changes of the helical content and the viscosity of tropomyosin with increasing temperature. ○, the binding with F-actin (F-actin, 0.65 mg/ml; tropomyosin, 0.25 mg/ml); △, the helical content of tropomyosin (1 mg/ml); □, the viscosity of tropomyosin (2.6 mg/ml). The solvent is the same as in Fig. 1.

As shown in Fig. 4, at room temperature (25°), the birefringence of the mixture of F-actin and tropomyosin becomes lower with deviation of the salt concentration from the optimum. At such salt concentrations, the value of birefringence showed no increase with further lowering of temperature. This suggests that the saturated amount of bound tropomyosin and/or the mode of binding may depend on the salt concentration. Other experiments[19,20] showed that there may be two steps in the stoichiometry of binding of tropomyosin to F-actin and these steps may have different dependences on the environmental conditions.

In summarizing the above analyses, it is difficult to discover any specific force responsible for the binding between F-actin and tropomyosin. It may be reasonable to assume cooperation of many kinds of binding forces, *e.g.* the coulomb force, the van der Waals force, other entropy forces. The binding is a result of the delicate balance of different kinds of forces.

It is important that the effect of tropomyosin on the ATPase activity of F-actin appears in parallel with the binding estimated by means of flow birefringence and centrifugation. Two possibilities on the origin of the ATPase activity of F-actin have been proposed. One is that actin polymers themselves can catalyze the splitting of ATP[15,18]; the other is that the cyclic dissociation and association of G-actin at the ends of polymers may cause the splitting of ATP bound to G-actin[16]. According to the latter mechanism, the splitting of ATP is induced when actin polymers shortened by sonication repolymerize (or recombine each other). As shown in Fig. 1, tropomyosin had no influence on the rate of polymerization of G-actin, while the ATPase activity of F-actin was suppressed by the tropomyosin binding. If we were to explain the suppression of the ATPase activity by the latter mechanism, the rate of polymerization of actin should be delayed by tropomyosin. Therefore it is difficult to consider that only the latter mechanism is responsible for the ATP splitting of F-actin. Tropomyosin may bind to F-actin with its long axis parallel to that of actin polymers and make F-actin more rigid. This would result in the decrease of ATPase activity of F-actin.

Finally, it is worthwhile to note again that because tropomyosin cannot bind to F-actin at high temperatures, the centrifugation of F-actin at such high temperatures may be very useful for its pure preparation.

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REFERENCES

1 W. DRABIKOWSKI AND J. GERGELY, *J. Biol. Chem.*, 237 (1962) 3412.
2 A. MARTONOSI, *J. Biol. Chem.*, 237 (1962) 2795.
3 K. LAKI, K. MARUYAMA AND D. R. KOMINZ, *Arch. Biochem. Biophys.*, 98 (1962) 323.
4 K. MARUYAMA, *Arch. Biochem. Biophys.*, 105 (1964) 142.
5 S. KITAGAWA, W. DRABIKOWSKI AND J. GERGELY, *Arch. Biochem. Biophys.*, 125 (1968) 706.
6 M. KASAI AND F. OOSAWA, *Biochim. Biophys. Acta*, 172 (1969) 300.
7 F. B. STRAUB, *Stud. Inst. Med. Chem. Univ. Szened*, 1 (1941) 5.
8 S. EBASHI, F. EBASHI AND K. MARUYAMA, *Nature*, 203 (1965) 645.
9 S. EBASHI AND F. EBASHI, *J. Biochem. (Tokyo)*, 58 (1965) 7.
10 S. EBASHI AND K. MARUYAMA, *J. Biochem. (Tokyo)*, 58 (1964) 20.
11 K. BAILEY, *Biochem. J.*, 4 (1948) 271.
12 M. KASAI, H. KAWASHIMA AND F. OOSAWA, *J. Polym. Sci.*, 44 (1960) 51.
13 T. OOI, *Biochemistry*, 6 (1967) 2433.
14 J. B. MARTIN AND D. D. DOTY, *Anal. Chem.*, 21 (1949) 965.
15 K. TAWADA AND H. ASAI, *J. Mol. Biol.*, 20 (1966) 403.
16 Y. NAKAOKA AND M. KASAI, *J. Mol. Biol.*, 5 (1969) 376.
17 W. DRABIKOWSKI AND E. NOWAK, *Eur. J. Biochem.*, 5 (1968) 376.
18 S. ASAKURA, M. TANIGUCHI AND F. OOSAWA, *J. Mol. Biol.*, 7 (1963) 55.
19 W. DRABIKOWSKI, D. KOMINZ AND K. MARUYAMA, *J. Biochem. (Tokyo)*, 63 (1968) 802.
20 S. ISHIWATA AND S. FUJIME, *J. Phys. Soc. Japan*, 30 (1971) 302.