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NATURAL F-ACTIN

III. NATURAL F-ACTIN AS INACTIVE POLYMER

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SUMMARY

1. Natural F-actin was found to be short in length, and the length distribution was not changed by storage demonstrating that an end-to-end interaction between natural F-actins was lacking.

2. Natural F-actin never accelerated the G–F transformation of Straub G-actin, but a great acceleration was produced by sonic treatment.

3. Depolymerization, sonic ATPase and the exchange of bound divalent cations of natural F-actin occurred more slowly than with Straub F-actin demonstrating that natural F-actin was more stable than Straub F-actin.

4. The effect of β-actinin, tropomyosin and myosin on Straub F-actin was examined, but all the characteristics of natural F-actin could not be explained by their presence.

5. To explain the behavior of natural F-actin, it was postulated that some additional factors other than the blocking by β-actinin of the ends of natural F-actin existed.

INTRODUCTION

As reported in the first paper in this series[1], natural F-actin isolated directly from myofibrils without the use of an organic solvent or of the depolymerization process shows different characteristics from the Straub F-actin. The length of natural F-actin is shorter than that of Straub F-actin and corresponds to the unit length of the I-filament in a sarcomere (approx. 1 μ). Moreover, if ATP and Mg^{2+} or Ca^{2+} are present, the length of natural F-actin is constant for a long time (*e.g.*, few days) after being extracted from the muscle[2]. This stability of natural F-actin is closely related to its inactivity.

It has been found that natural F-actin does not accelerate the polymerization of Straub G-actin, while Straub F-actin does[3]. Sonicated natural F-actin, however, greatly accelerates the polymerization of Straub G-actin. The polymerizing activities

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of sonicated natural F-actin and of Straub F-actin are similar[4]. In addition, it has been found that the structure of the natural F-actin polymer is stable; that is, F-actin resists depolymerization, shows a reduced exchange rate of bound divalent cations[5], and has low sonic ATPase activity, *etc.*[6].

MARUYAMA[7–9] found a protein factor named β-actinin which regulates the length of F-actin. The factor can shorten Straub F-actin and can weaken the interaction between filaments, but the complete behavior of natural F-actin cannot be explained by the presence of β-actinin in Straub F-actin.

EXPERIMENTAL METHODS

Material

Natural F-actin was prepared and purified from the glycerinated back and psoas rabbit muscle according to the method previously described[1,10].

Tropomyosin-free Straub actin was prepared from rabbit skeletal muscle as described before[11].

Myosin A was prepared according to the usual procedure except that the final clarification was made by centrifugation at $10^5 \times g$ for 2 h (ref. 12).

Tropomyosin was prepared according to BAILEY[13].

β-Actinin was prepared by the method of MARUYAMA[8].

Trypsin (EC 3.4.4.4), the trypsin inhibitor and ATP were purchased from Sigma Chemical Co.

Method

The flow birefringence of an Edsall type apparatus (Rao Instrument Co.) was used for measuring the extinction angle and the amount of flow birefringence at various velocity gradients.

Viscosity measurements were carried out by Ostwald type viscometers whose flow times for water were about 30–40 sec at 20°.

Sonic vibration was performed in a sonic generator, made by Kubota (Japan) or by Branson (U.S.A.), at 10 kcycles/sec.

The ATPase activity was determined by measuring the liberation of P_i by the method of TAUSSKY AND SHORR[14].

The protein concentration was determined by a biuret reaction[15] and was checked by the Kjeldahl method.

RESULTS

(1) *Stability of natural F-actin*

When natural F-actin is stored for a long time in the absence of nucleotides, it gradually denatures[2]. A small increase in the extinction angle and a marked decrease in the birefringence are observed. Natural F-actin is, however, protected from denaturation by the presence of nucleotides and divalent cations[2]. As shown in Fig. 1, aging F-actin for a few days after preparation results in a slight increase in the concentration dependence of the extinction angle, but extrapolation of the extinction angle to zero concentration is not changed, demonstrating that the length of natural F-actin does not change in the aging process. These results are consistent with observations using the electron micrograph[10]. However, an increase in the concentration

dependence of the extinction angle of aged natural F-actin means that the interaction between filaments increases; thus, the factors inhibiting the interaction between filaments were removed or denatured slightly during storage. This phenomenon can be explained to some extent by the release of β-actinin from the F-actin[7]. As a whole, the structure of natural F-actin is stable and never shows a tendency to become longer. These facts suggest that the interaction between natural F-actin filaments is either weak or nonexistent.

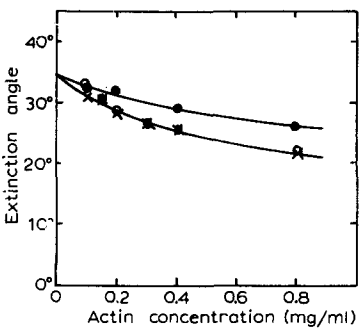

Fig. 1. Change in concentration dependence of extinction angle of natural F-actin. Extinction angle was measured after standing. Natural F-actin was stored at 0.8 mg/ml in 0.1 M KCl, 1 mM $MgCl_2$, 0.5 mM ATP, and diluted by the same solvent before measurement. Shear rate was 9.8 sec^{-1}. ●, 0 time; ○, left standing at 20° for 20 h; ×, left standing at 0° for 40 h.

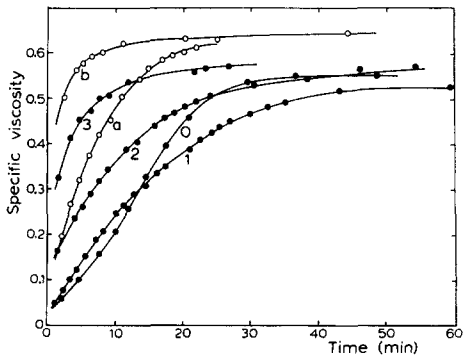

Fig. 2. Acceleration of polymerization of Straub G-actin by natural F-actin and Straub F-actin. Polymerization is shown by specific viscosity (η_{sp}). Final condition: 0.8 mg/ml Straub G-actin, 0.08 mg/ml F-actin added, 10 mM KCl, 0.1 mM $MgCl_2$, 2 mM ATP, 5 mM Tris–HCl buffer (pH 7.5). 0, Straub G-actin + salt (control); 1, Straub G-actin + 1/10 vol. of natural F-actin (0.8 mg/ml, 0.1 M KCl, 1 mM $MgCl_2$, 0.5 mM ATP); 2, Straub G-actin + 1/10 vol. of sonicated natural F-actin (the same as above); 3, Straub G-actin + equal volume of sonicated natural F-actin (0.16 mg/ml, 20 mM KCl, 0.2 mM $MgCl_2$, 10 mM Tris–HCl buffer (pH 7.5), 4 mM ATP). a, Straub G-actin + 1/10 vol. of Straub F-actin (0.8 mg/ml, 0.1 M KCl, 1 mM $MgCl_2$, 0.5 mM ATP); b, Straub G-actin + equal volume of sonicated Straub F-actin (0.16 mg/ml, 20 mM KCl, 0.2 mM $MgCl_2$, 4 mM ATP, 10 mM Tris–HCl buffer (pH 7.5)). Temp. 20°.

(2) No acceleration of polymerization

When Straub F-actin was added to G-actin in the presence of salts, polymerization was greatly accelerated[3]. This phenomenon is explained by assuming that F-actin acts as a seed for polymerization. Similar experiments were made using natural F-actin.

The result reported in the previous section showed that the polymerizing ability at the end of natural F-actin was lacking. If this was true, natural F-actin might not have an accelerating ability for the polymerization of G-actin. To clarify this point, an experiment was made with viscometry in low salt concentrations where the polymerization of G-actin, without the addition of seed was slow. It was appropriate to examine the acceleration effect. As shown in Fig. 2, natural F-actin did not accelerate the polymerization. This result demonstrates that natural F-actin lacks the seeding ability for polymerization; however, Straub F-actin having an average length a few times longer than that of natural F-actin[10,16] shows marked seeding ability.

The previous experiment was examined by using a higher ATP concentration (2 mM). In the presence of a small amount of ATP, the depression of the final value of

viscosity was observed. In this case, as shown in Fig. 3a, ATP in solution was almost completely dephosphorylated within 10 min, and polymerization ceased. This might be due to the denaturation of G-actin by the ATPase contaminating the natural F-actin[1].

When aged natural F-actin was used as a seed marked deceleration of the polymerization sometimes was observed, but the final value of polymerization did not decrease as shown in Fig. 3b. This may be due to the release of β-actinin as described above.

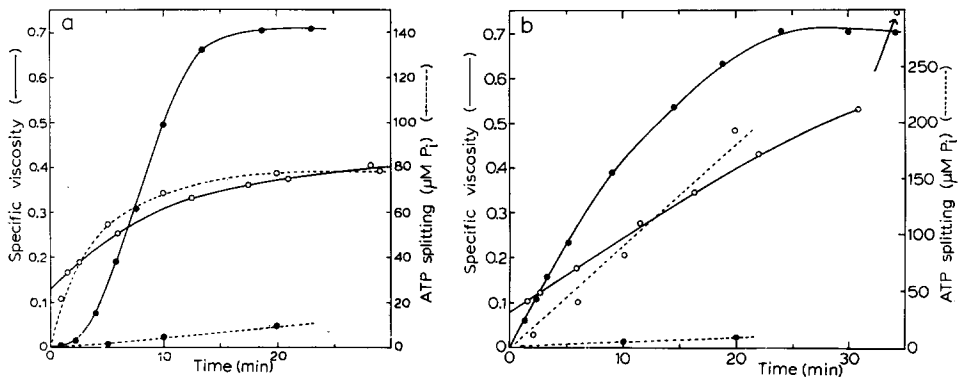

Fig. 3. a. Relation between ATPase activity and acceleration of polymerizations by natural F-actin. Condition: 0.6 mg/ml Straub G-actin, 20 mM KCl, 0.2 mM $MgCl_2$, 5 mM Tris–HCl buffer (pH 8.0), 0.07 mM ATP at 20°. ———, viscosity; – – – – –, ATP splitting; ●, control (no natural F-actin); ○, Straub G-actin + 1/5 vol. of natural F-actin (final concn. 0.12 mg/ml). b. The same experiment as a. Condition: 0.6 mg/ml Straub G-actin, 20 mM KCl, 0.2 mM $MgCl_2$, 5 mM Tris–HCl buffer (pH 8.0), 1.5 mM ATP at 20°. Symbols are the same as in a.

From these results, two factors can be considered to exist. One inactivates the end-to-end interaction between filaments or the seed ability (we say ending factor in this report) of natural F-actin. The other factor decelerates polymerization. The supernatant solution of natural F-actin obtained by ultracentrifugation sometimes had this latter function. The deceleration of polymerization was caused by the solution of natural F-actin after dialysis against distilled water. By this treatment, actin molecules were almost denatured. This factor was observed in a fraction soluble in 25% $(NH_4)_2SO_4$ and precipitated in 37.5% $(NH_4)_2SO_4$ followed by dialysis against distilled water. When this factor was added to sonicated Straub F-actin, the increase in viscosity after sonication was slightly inhibited. The behavior of this factor is very similar to that of β-actinin[7,9]. The existence of β-actinin in the supernatant of ultracentrifuged natural F-actin was estimated previously[1].

(3) Appearance of acceleration effect

(a) Sonicated natural F-actin. When we added sonicated Straub F-actin as a seed for polymerization, the acceleration effect was greatly increased according to the increase in the filament ends[4].

Similarly, sonicated natural F-actin which was broken into small fragments (about 0.1 μ, see the next report[10]) was added to a solution of G-actin. A great acceleration in polymerization was observed (Fig. 2). This acceleration disappeared rapidly

when the solution was left standing after sonication (Fig. 4). The disappearance of acceleration could be due to a return to the original states caused by the end-to-end polymerization between fragmented natural F-actin[10]. Compared with the result obtained in high salt and protein concentrations, when diluted natural F-actin was sonicated in dilute salt and was used as a seed, a large acceleration was observed (Fig. 2). This observation is believed to be due to the slow decrease in new active ends of fragmented F-actin; thus, the polymerization between fragmented F-actin in diluted solution is slow because the probability of collision is decreased. Because mixing sonicated actin requires a few seconds, the polymerization of fragmented F-actins occurs, in concentrated solutions, before the addition of G-actin.

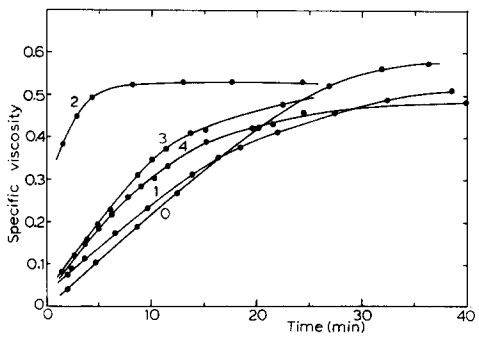

Fig. 4. Disappearance of seed ability after sonication of natural F-actin. Natural F-actin (0.16 mg/ml, 20 mM KCl, 0.2 mM $MgCl_2$, 4 mM ATP, 10 mM Tris–HCl buffer (pH 7.5)) was sonicated and equal volume of Straub G-actin was added. Final condition: 0.8 mg/ml Straub G-actin, 0.08 mg/ml natural F-actin, 10 mM KCl, 0.1 mM $MgCl_2$, 2 mM ATP, 5 mM Tris-HCl buffer (pH 7.5). Temp. 20°. 0, Straub G-actin only; 1, Straub G-actin + natural F-actin before sonication; 2, Straub G-actin + natural F-actin just after sonication; 3, Straub G-actin + natural F-actin 45 min after sonication; 4, Straub G-actin + natural F-actin 3 h after sonication.

Similar behavior was observed with Straub F-actin. However, the acceleration effect of Straub F-actin never disappeared although the acceleration effect decreased with time following sonication because of the polymerization of sonicated fragments[17].

This difference between natural F-actin and Straub F-actin is related to the fact that sonicated natural F-actin recovers its original length and does not become significantly longer[10]. These facts also indicate that some factor inhibiting further polymerization in natural F-actin exists.

(*b*) *Other modified natural F-actin.* When natural F-actin was subjected to a trypsin or a cyclic treatment of depolymerization and polymerization, it tended to be like the Straub F-actin[2,10]. This tendency was observed in the acceleration effect of polymerization of Straub G-actin, but the effect was not large.

Natural F-actin increased in length when treated with trypsin. Following the treatment with trypsin natural F-actin was able to accelerate the polymerization of G-actin to some extent, but the degree of acceleration was lower than that with Straub F-actin. Natural F-actin treated by cyclic depolymerization and polymerization showed a slight seeding ability, and the length distribution changed similarly to KI–F-actin[10].

The effect of trypsin was slightly larger than that of repolymerization. These observations suggest that the factor regulating the length and inhibiting the seeding

activity in natural F-actin was partly digested by a trypsin treatment or released from natural F-actin through the depolymerization–repolymerization cycle.

(4) *Stability of natural F-actin*

(a) *Depolymerization.* If F-actin is diluted under a low salt condition, the depolymerization can be observed by a decrease in the viscosity or in the flow birefringence[18]. For the purpose of elucidating the stability of natural F-actin, its depolymerization process was compared with that of Straub F-actin by measuring viscosity. As shown in Fig. 5, depolymerization of natural F-actin is slower than that of Straub F-actin. In particular, the initial rapid decrease in the viscosity was not observed in natural F-actin, and the final value was high which suggests that the critical concentration of natural F-actin is smaller than that of Straub F-actin when it is assumed that the formation of both actin filaments is a kind of condensation phenomenon[19,20].

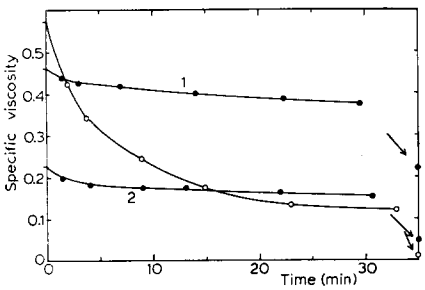

Fig. 5. Depolymerization of natural F-actin. 4.7 mg/ml natural F-actin: in 20 mM KCl, 5 mM Tris–HCl buffer (pH 8.0), 0.5 mM ATP was diluted to 0.94 mg/ml and 0.47 mg/ml in 4 mM KCl, 5 mM Tris–HCl buffer (pH 8.0), 0.5 mM ATP. Straub F-actin: 5.2 mg/ml in 20 mM KCl was diluted to 0.52 mg/ml in 4 mM KCl, 5 mM Tris–HCl buffer (pH 8.0), 0.5 mM ATP. Temp. 20°. Decrease of viscosity was followed. ●, natural F-actin: 1, 0.94 mg/ml; 2, 0.47 mg/ml. ○, Straub F-actin.

(b) *ATPase under sonic vibration.* F-Actin shows marked ATPase activity under sonic vibration[6]. Measurement of sonic ATPase is difficult because natural F-actin itself shows ATPase activity (referred to as intrinsic ATPase here) without sonic vibration as described in Section 2.

Therefore, sonic ATPase is estimated by subtracting the intrinsic ATPase from the total ATPase under sonic vibration. However, a decrease in the intrinsic ATPase after sonic vibration was observed so the estimation of sonic ATPase has some error. The value obtained by this method was a few tenths of that of Straub F-actin.

As in the other method for estimating sonic ATPase, ATPase was measured in the presence of EDTA. Under such conditions, the intrinsic ATPase of natural F-actin is almost inhibited[21], but sonic ATPase of Straub F-actin is little changed. The result is consistent with the above observation; that is, the sonic ATPase of natural F-actin is low. The result is shown in Table I.

(c) *Exchange and release of bound divalent cations.* Natural F-actin has specific divalent cations as does Straub F-actin[1]. The amount of divalent cation is about 1.5 moles/60000 g of actin[22], *i.e.*, 1 mole/40000 g of actin, and more Mg^{2+} than Ca^{2+} is found.

TABLE I

SONIC ATPASE

Sample solution (0.1 M KCl, 10 mM Tris–HCl buffer (pH 8.0), 1 mM ATP, 1 mM EDTA) was sonicated at 20°, and splitting of ATP was followed for 10 min. ATPase was determined from the initial gradient of P_i liberation.

Sample		*Concn. (mg/ml)*	*Other protein*	*Concn. (mg/ml)*	*ATPase (nmoles/mg actin)*
Straub F-actin		1	None	0	6.0
			Myosin	0.05	6.2
				0.1	3.7
			β-Actinin	0.05	5.0
				0.13	4.9
				0.5	4.5
Natural F-actin	1	0.5	—	—	1.3
	2	1.0	—	—	0.8

These divalent cations are slowly released by aging natural F-actin, as in the case of Straub F-actin[5,22], but the release rate seems to be somewhat smaller than that of Straub F-actin (Fig. 6). In this experiment ATP was added to the solvent to prevent denaturation, but slight denaturation was observed.

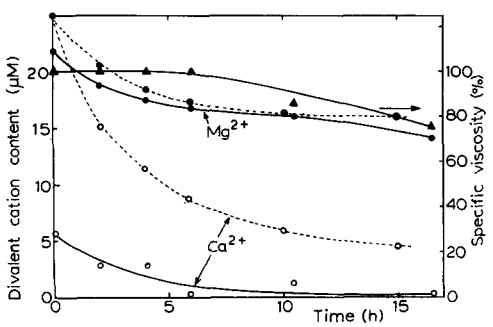

Fig. 6. Release of bound divalent cation from natural F-actin compared with that of Straub F-actin. F-Actin was left standing in a divalent cation-free solvent at 35.4°. Free divalent cations were removed by Dowex 50-W treatment[22]. Condition: 1 mg/ml actin, 0.1 M KCl, 0.5 mM ATP, 10 mM Tris–HCl buffer (pH 8.0). ———, natural F-actin: ●, Mg^{2+} content; ○, Ca^{2+} content; ▲, viscosity. – – – –, Straub F-actin; ●, Mg^{2+} content of Mg^{2+}–F-actin; ○, Ca^{2+} content of Ca^{2+}–F-actin.

Incorporation of Ca^{2+} in natural F-actin was also examined by the use of radioactive $^{45}Ca^{2+}$. Under sonic vibration slow incorporation was observed. About half of the divalent cations were exchanged with $^{45}Ca^{2+}$ after 20 min of sonication at room temperature, while almost all divalent cations of Straub F-actin were exchanged in this condition as reported in the previous papers[11,23,24]. The above experiments show that the behavior of natural F-actin is the same, on the whole, as that of Straub F-actin; however, natural F-actin is more stable or less reactive than Straub F-actin.

(5) *Effect of other proteins on Straub F-actin*

In the previous section, the differences in depolymerization, in sonic ATPase activity and in the divalent cation exchange between natural F-actin and Straub F-actin were shown. In addition the differences in length and seeding ability between the two F-actins were discussed.

It is expected that these differences are due either to a difference in the actin molecule itself or to the coexistence of other muscle proteins and the manner in which they are organized.

Existence of other muscle proteins in natural F-actin was expected, β-actinin was considered to be included as described in Section 2 (refs. 1,2), and tropomyosin was expected to be present (less than 10%)[25]. These proteins are believed to bind F-actin[1,2,25,26]. α-Actinin present in the original natural F-actin solution may not bind to the F-actin, because its content is greatly decreased by purification[26]. Troponin is not considered to be present at least as active molecules[26].

In this section, the effect of other muscle proteins on Straub F-actin will be examined.

(*a*) *ATPase and polymerization.* As reported in Section 2, natural F-actin shows ATPase activity which is gradually decreased by further purification by ultracentrifugation. Therefore, ATPase is considered to be due to the contamination of another protein[1].

As shown in Table II, ATPase of natural F-actin was activated by Ca^{2+} and Mg^{2+} and was inhibited by EDTA. The relative effects of these modifiers did not change remarkably with purification. Because myosin or H-meromyosin show ATPase under these conditions, the effect of either the metals or EDTA on myosin or H-meromyosin were compared. If the intrinsic ATPase of natural F-actin is explained by the contamination of these proteins, the amount of them expected to be present will be about 1/20–1/50 weight of actin. As seen in Table II, the effects of the metals

TABLE II

ATPASE ACTIVITY OF NATURAL F-ACTIN AND MYOSIN (OR H-MEROMYOSIN)

ATPase was followed in 0.1 M KCl, 0.5 mM ATP, 10 mM Tris–HCl buffer (pH 8.0) at 23°.

Sample	*Concn. (mg/ml)*	*ATPase (nmoles/min)*			
		*o**	*$MgCl_2$ (1 mM)*	*$CaCl_2$ (1 mM)*	*EDTA (1 mM)*
Natural F-actin	1	6.5	13.0	30.0	0
Natural F-actin twice purified	1	2.33	3.41	11.2	0
Straub F-actin	1				
+ myosin 1	0.05	18.9	13.8	23.2	0.2
+ myosin 1	0.1	40.4	35.2	50.0	0.8
+ myosin 2	0.05	9.5	7.4	21.4	0.8
+ H-meromyosin	0.05	6.2	6.5	22.0	2.0
Myosin 1	0.05	0.2	0	21.9	10.3
Myosin 2	0.05	8.7	1.7	26.0	11.5
H-Meromyosin	0.05	2.0	0.7	15.0	19.2

* ATPase under no divalent cation is not so reliable because of contamination of divalent cation in sample solution.

and of EDTA are very similar. In the previous paper[1], it was reported that this ATPase was not a myosin type but rather some granular ATPase because ATPase of natural F-actin was inhibited by EDTA. However, the ATPase of actomyosin was found to be inhibited by EDTA while that of myosin was activated. This result is consistent with that reported by BARVON *et al.*[21]. These results indicate that myosin or H-meromyosin are not excluded as the protein contaminating natural F-actin.

When we added myosin or H-meromyosin to the solution of G-actin, in the absence of free ATP, the depression of the final level of polymerization was observed, but the acceleration of polymerization also occurred. Therefore, the total feature caused by these proteins was different from that of natural F-actin.

As described in Section 2, some factor inhibiting polymerization similar to β-actinin was obtained. The effect of β-actinin on polymerization was examined under the conditions of Section 2. As shown in Fig. 7, β-actinin decelerated the polymerization to some extent, but the final level of viscosity was not changed. Much more deceleration was observed when the amount of β-actinin was increased. However, when the Straub F-actin containing β-actinin was used as a seed for polymerization, the acceleration of polymerization was observed.

This fact shows that natural F-actin may contain β-actinin. Some properties of natural F-actin are explained by this observation, but the nature of natural F-actin which does not work as a seed is not explained by the presence of β-actinin.

(*b*) *Sonic ATPase.* As described in Section 3, sonic ATPase of natural F-actin is smaller than that of Straub F-actin. This difference is closely related to the stability of natural F-actin and was expected to be due to the specific interaction between coexisting proteins and F-actin. The effects of β-actinin, myosin and tropomyosin on the sonic ATPase of Straub F-actin was examined (Table I). In this case, the sonic

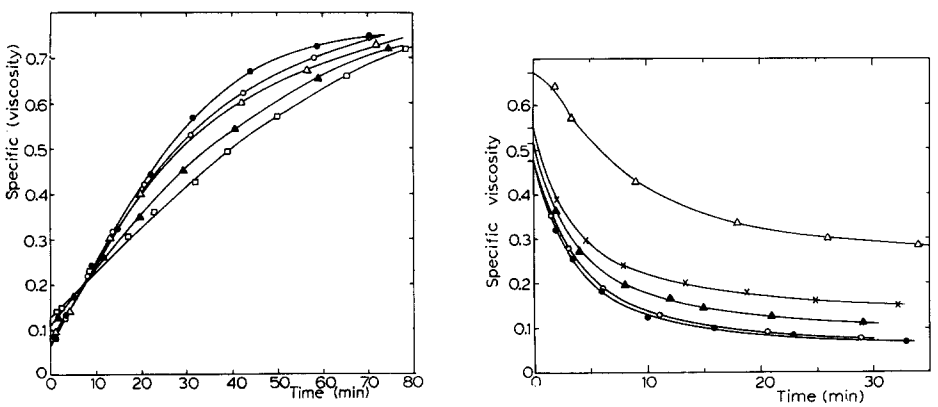

Fig. 7. Effect of β-actinin on polymerization of Straub G-actin. Polymerization condition: 0.73 mg per ml Straub G-actin, 3.3 mM Tris–HCl buffer (pH 8.0), 20 mM KCl, 0.2 mM $MgCl_2$, at 20°. ●, G-actin only; ○, + β-actinin 0.017 mg/ml (1/43 of actin); △, + β-actinin 0.034 mg/ml (1/21 of actin); ▲, + β-actinin 0.068 mg/ml (1/17 of actin); □, + β-actinin 0.14 mg/ml (1/5 of actin).

Fig. 8. Effect of muscle proteins on depolymerization of Straub F-actin. Condition: 5.5 mg/ml Straub F-actin in 50 mM KCl, 0.5 mM ATP, 5 mM Tris–HCl buffer (pH 8.0) was diluted to 0.46 mg per ml in 4 mM KCl, 0.5 mM ATP, 5 mM Tris–HCl buffer (pH 8.0). ●, control; ○, + β-actinin 1/15 wt. of F-actin; ▲, + tropomyosin 1/3 wt. of F-actin; △, + tropomyosin 2/3 wt. of F-actin; ×, + myosin 1/20 wt. of F-actin.

ATPase was measured in the presence of EDTA in order to inhibit the intrinsic ATPase. This result shows that these proteins partially inhibit the sonic ATPase of Straub F-actin.

(*c*) *Depolymerization.* The depolymerization of natural F-actin was much slower than that of Straub F-actin. The effects of tropomyosin, β-actinin and myosin on depolymerization were examined. As shown in Fig. 8, these proteins inhibit the depolymerization to a certain extent. A large quantity of these proteins is necessary, however, in order to account for the behavior of F-actin, and it seems unlikely that natural F-actin contains sufficient quantities for the behavior to be completely attributed to these proteins. In addition, significant differences exist between natural F-actin and Straub F-actin, even in the presence of high concentrations of the proteins, when one considers the decrease in viscosity at the beginning of depolymerization. In conclusion, these factors may strengthen the structure of natural F-actin but do not explain all of the characteristics attributed to natural F-actin.

(*4*) *Exchange or release of divalent cation*

The effects of tropomyosin, myosin and β-actinin on the exchange ability of bound Ca^{2+} of Straub F-actin were examined. As reported in the previous paper[5], these proteins make Straub F-actin less reactive. The rate of exchange or release of Ca^{2+} becomes slower.

DISCUSSION

The inactivity and stability of natural F-actin have been described. These characteristics can be summarized as follows: (1) Natural F-actin shows no acceleration effect on the polymerization of G-actin. (2) Sonic vibration results in natural F-actin gaining the ability to accelerate polymerization, but this effect disappears in a few minutes following sonication. At this time, the average length of the natural F-actin filaments recover their original length[7]. These characteristics suggest that some factor other than actin itself binds to the ends of actin polymers and blocks the ends activities. If this is the case, the results described in this paper can be easily explained. For example, the appearance of seeding ability by sonication is due to the new ends which are produced by sonic vibration and are not blocked by this factor. Following sonic vibration, the active ends rebind each other, and the seeding ability disappears. This assumption means that the average length of natural F-actin is determined by the ratio of the concentration of actin and of the ending factor. There must be a very strong interaction between the F-actin and the ending factor, and, in addition, there must be a very small amount of the ending factor since, for example, 1 mole per 370 moles of actin if thin filaments contain 1 mole of the ending factor. In the case of natural F-actin, the length distribution is very broad, and its average length is about 0.5 μ. This may be interpreted in the process of preparation as thin filaments are destroyed to some extent (or partly solubilized), and ratio of ending factor to actin is changed.

What is the ending factor? β-Actinin is considered to be such a protein. As described by MARUYAMA[7], Straub F-actin can be shortened by sonic vibration in the presence of β-actinin; however, it requires very large amounts of β-actinin, and Straub F-actin *plus* β-actinin become longer during storage following sonication.

Moreover as described in this paper, F-actin containing β-actinin works as a seed for polymerization. This behavior of Straub F-actin containing β-actinin is different from natural F-actin.

β-Actinin, however, lowers the interaction between Straub F-actin and prevents network formation[7,9]. This is also observed in natural F-actin. As described in Fig. 1, the appearance of a concentration dependence of the extinction angle during storage may be considered to correspond to some release of β-actinin from natural F-actin. Moreover, the deceleration of polymerization is also explained by the existence of β-actinin. Therefore, natural F-actin may contain β-actinin, but the inactivity of natural F-actin cannot be explained by the presence of β-actinin alone.

The exchange of a bound divalent cation or nucleotide is considered to occur at the ends of the actin polymers[5]. If all ends are blocked, the exchange is expected to be abolished. The results, however, show that a slow exchange reaction occurs. Probably, therefore, all of the polymers are not blocked and the exchange occurs at the free ends. The number of free ends may be so small that the acceleration of polymerization is not observed; possibly the ends may be blocked completely, but a spontaneous destruction or loosening of the polymer structure at the middle of filaments leads to the formation of new ends.

Stability of natural F-actin appeared in depolymerization (except the initial slow decrease of viscosity). Sonic ATPase and divalent cation exchange or release can be explained by the coexistence of muscle proteins such as tropomyosin and/or β-actinin. In addition to the above effect, the ending factor, if it exists, also might explain this stability. The slow decrease in the viscosity at the initial period after dilution is interpreted by the inhibition of depolymerization by the ending factor.

In conclusion, natural F-actin is the same protein as Straub F-actin and contains β-actinin and tropomyosin in small amounts. The stability of natural F-actin can be explained by the presence of these proteins. However, the lack of seeding activity is not explained by the existence of these proteins. By assuming the existence of an ending factor, all phenomena described in this paper are understood consistently. This factor may be a new protein or a protein related to β-actinin. For instance, β-actinin acts as an ending factor in natural F-actin, but prepared β-actinin does not make Straub F-actin an inactive polymer. Such different actions may be expected *in vivo* where β-actinin exists in a different state which is not attained by isolated β-actinin. Otherwise, the ending factor as a protein may not exist, and these characteristics may be due to the nature of actin itself. At any rate, the ending factor does not determine the length, but it protects the filaments from being lengthened or shortened and preserves the determined length (I-filaments to be 1 μ). The ending factor may be indispensable to form actin filaments of a muscle fiber.

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