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Mg-POLYMER OF ACTIN FORMED UNDER THE INFLUENCE OF β-ACTININ

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SUMMARY

When rabbit actin was polymerized by 2–3 mM $MgCl_2$ in the presence of β-actinin, 5–10% of actin by weight, aggregates very similar to the Mg-polymer of plasmodium actin were formed: reduced viscosity was low, 1–2 dl/g, and the sedimentation coefficient was 31–33 S. The ATPase activity, although lower than that of the plasmodium Mg-polymer, was clearly demonstrated.

Electron microscopic observations revealed that the Mg-polymer consisted of globular aggregates and random aggregates of short F-actin particles in negatively stained samples. However, when preparations were fixed with glutaraldehyde and then negatively stained with uranyl acetate, short F-actin particles alone were observed. It is considered that the Mg-polymer consists of short fragile F-actin particles.

The Mg-polymer was transformed into short F-actin filaments when incubated for 5 min at 45° in the presence of ATP. When incubated for 25–30 min at 55°, elongation of F-actin particles occurred. This was explained by partial inactivation of β-actinin, where some recombination of F-actin particles must have taken place.

INTRODUCTION

HATANO and his collaborators[1,2] have shown that actin prepared from plasmodia of the myxomycete, *Physarum polycephalum*, forms a special kind of polymer in the presence of Mg^{2+} or other divalent cations, which has a low viscosity (0.5–1.5 dl/g), but the same sedimentation constant (30 S) as that of the usual F-actin polymerized by KCl. This polymer shows ATPase activity under conditions where F-actin has no activity at all[3]. The Mg-polymer thus designated appears under the electron microscope as a globular aggregate with a diameter of 100–600 Å, and it can be transformed into double-stranded helical polymer (usual F-actin)[4] by the addition of ATP and KCl[5]. HATANO has emphasized the role of the Mg-polymer of plasmodium actin in the protoplasmic streaming of plasmodium (*cf.* refs. 6, 7).

β-Actinin, one of the regulatory proteins of striated muscle, was shown to retard the polymerization process of actin at a low Mg^{2+} concentration[8]. Recently we have been able to purify β-actinin from plasmodium and to show its possible presence in HATANO's plasmodium actin preparations as a contaminant[9]. This finding led us to examine the possibility that the Mg-polymer is formed under the influence of β-actinin. The present study has revealed that the Mg-polymer is not specific to plas-

modium actin, and even rabbit actin can form the Mg-polymer in the presence of Mg^{2+} and β-actinin.

EXPERIMENTAL

Preparation of proteins

Rabbit actin was prepared according to the method of MOMMAERTS[10] with a slight modification[11]. β-Actinin was purified from rabbit skeletal muscle[12] and from acetone-treated plasmodium[9]. Unless otherwise specified, rabbit β-actinin was used in the present study.

Physical techniques

Viscosity was measured at 25°, using an Ostwald-type viscometer (flow time of water, approx. 60 sec). Flow birefringence was determined in an apparatus of the Edsall type (Rao Instrument Co.). Sedimentation patterns were photographed in a Beckman Model E ultracentrifuge. Electron microscopic observations were performed with a Hitachi 11A electron microscope, as described before[11]. Negative staining with uranyl acetate was occasionally carried out after the treatment with 2.5 % glutaraldehyde. Sonication was performed for 15 sec at 15° in a Branson sonifier.

Biochemical techniques

ATPase measurements were carried out in a solution containing 10 mM Tris–maleate buffer, pH 7.0, 1 mM ATP and varied concentrations of $MgCl_2$ at 45°. Usually actin concentration was 1 mg/ml. Inorganic phosphate was determined by the method of TAKAHASHI[13] after isobutanol extraction. Protein concentration was measured by the Biuret method.

RESULTS

Viscosity

As already reported in an earlier paper[8], the increase in viscosity of a polymerizing actin solution induced by 0.1 M KCl became appreciably smaller when β-actinin had been added before the addition of KCl. Thus the final values of reduced viscosity were approx. 4.2 and 6.6 dl/g with and without 10 % β-actinin, respectively (Fig. 1). On the other hand, when 5 mM $MgCl_2$ was added to polymerize actin, the viscosity values were 2.0 and 6.9 dl/g with and without 10 % β-actinin, respectively (Fig. 1). The difference is remarkable when the viscosity with β-actinin in 0.1 M KCl is compared with that in 5 mM $MgCl_2$. It is to be noted that the low value in $MgCl_2$ did not increase at all upon further addition of 0.1 M KCl and 1 mM ATP at 25°. Another experiment shown in Fig. 2 confirmed the results mentioned above: the viscosity of actin with 5 % β-actinin was 4.4 dl/g in 0.1 M KCl and 2.4 dl/g in 2.5 mM $MgCl_2$, respectively, and upon sonication the viscosity in 0.1 M KCl was remarkably decreased to a level lower than that in $MgCl_2$, whereas that in 2.5 mM $MgCl_2$ did not change significantly.

These observations suggest that the Mg-polymer of actin with low viscosity could be formed under the influence of β-actinin. In fact, when 2 mM $MgCl_2$ and 0.1 M KCl were added simultaneously, low viscosity material was formed. This was

also the case with 2.5 mM $CaCl_2$ instead of $MgCl_2$. The latter two observations are in good agreement with the results obtained with plasmodium actin[1,2]. When β-actinin was added to preformed F-actin in 2 mM $MgCl_2$, its high viscosity was only slightly decreased. Hence β-actinin must be added to G-actin before polymerization with Mg^{2+} in order to obtain the low viscosity material. This will be called the Mg-polymer.

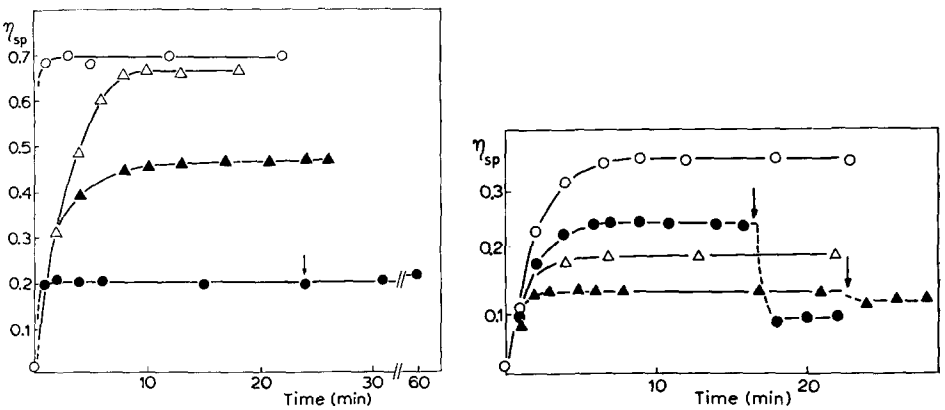

Fig. 1. Viscosity increase of actin polymerized by $MgCl_2$ and KCl in the presence of β-actinin. G-actin, 1.0 mg/ml. 10 mM Tris buffer, pH 7.2, 25°. β-Actinin, 0.1 mg/ml, when added. Additions: ○, 5 mM $MgCl_2$; ●, β-actinin + 5 mM $MgCl_2$; △, 0.1 M KCl; ▲, β-actinin + 0.1 M KCl. Arrow indicates the addition of 0.1 M KCl and 1 mM ATP.

Fig. 2. Effect of sonication on the viscosity of the Mg-polymer and F-actin polymerized in the presence of β-actinin. G-actin, 0.8 mg/ml. 10 mM Tris buffer, pH 7.2, 25°. ○, 5% β-actinin + 0.1 M KCl; ●, 10% β-actinin + 0.1 M KCl; △, 5% β-actinin + 2.5 mM $MgCl_2$; ▲, 10% β-actinin + 2.5 mM $MgCl_2$. Arrow indicates sonication.

Sedimentation pattern

HATANO *et al.*[2] first showed that the sedimentation coefficient of plasmodium actin is about 30 S, both in 0.1 M KCl and in 2 mM $MgCl_2$. The sedimentation patterns of the Mg-polymer and F-actin formed under the influence of β-actinin were very similar, although the peak of the former appeared to be somewhat less sharp than that of the latter (Fig. 3a). The sedimentation coefficient was approx. 31–32 S for the Mg-polymer, and 35 S for F-actin at a protein concentration of 0.6 mg/ml. It should be mentioned that the presence of G-actin was negligible in 2 mM $MgCl_2$ (see Fig. 3a), although the peak due to G-actin was noticeable in 1 mM $MgCl_2$. The sedimentation patterns of the Mg-polymer (31 S, with β-actinin) and of F-actin formed by 2 mM $MgCl_2$ (about 60 S, without β-actinin) are shown in Fig. 3b. The area of the control F-actin was much smaller than that of the Mg-polymer.

Flow birefringence

Samples which were subjected to sedimentation and viscosity measurements were also examined by the flow birefringence technique. The results are summarized in Figs. 4 and 5. The amounts of flow birefringence at varied velocity gradients are just like the values of viscosity (Fig. 4): birefringence of KCl-polymerized F-actin with 10% β-actinin was much higher than that of the Mg-polymer, especially at low velocity gradients. Upon sonication both were lowered to the same level, which was

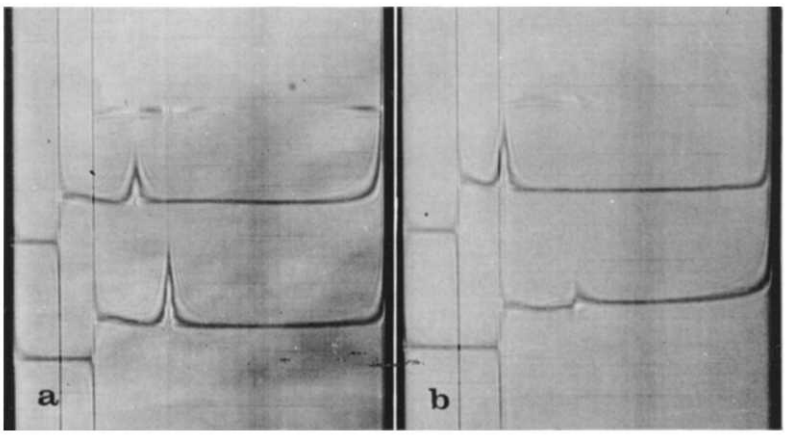

Fig. 3. Sedimentation pattern of the Mg-polymer of actin. Actin, 0.6 mg/ml. β-Actinin, 0.06 mg/ml. 10 mM Tris buffer, pH 7.2, 20°. (a) With β-actinin: Upper, 2 mM $MgCl_2$ + 0.1 M KCl; Lower, 0.1 M KCl. (b) 2 mM $MgCl_2$: Upper, with β-actinin; Lower, without β-actinin. Photographs were taken 20 min (a) and 8 min (b) after reaching full speed (29500 rev./min).

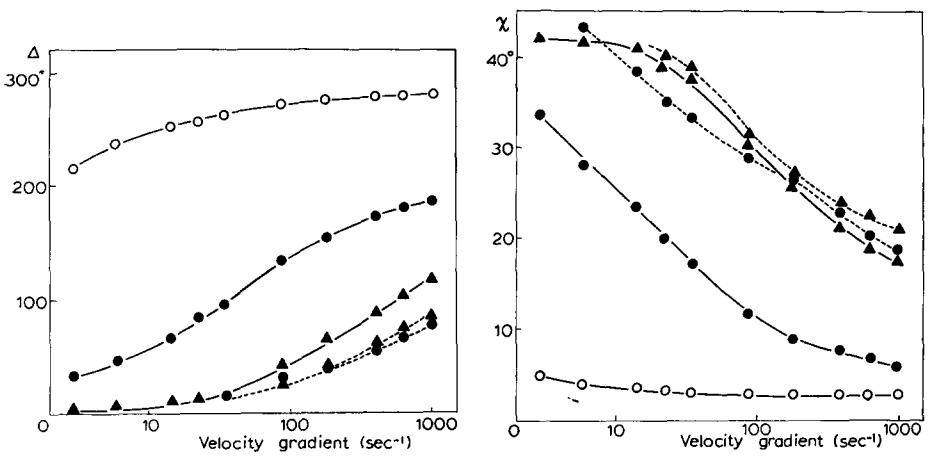

Fig. 4. Flow birefringence of actin polymerized by $MgCl_2$ and KCl in the presence of β-actinin. Conditions as in Fig. 2, except that 10% β-actinin was present. ○, control (0.1 M KCl); ●, β-actinin + 0.1 M KCl; ▲, β-actinin + 2 mM $MgCl_2$; — — —, sonicated.

Fig. 5. Extinction angle of actin polymerized by $MgCl_2$ and KCl in the presence of β-actinin. Conditions and symbols as in Fig. 4.

not so different from that of the intact Mg-polymer. The extinction angles at varied velocity gradients are shown in Fig. 5. It is evident that apparent particle lengths of F-actin particles polymerized by KCl in the presence of β-actinin are much larger (1.0–1.7 μm) than those of the Mg-polymer (0.4–0.7 μm), which are similar to those of sonicated KCl-F-actin. Naturally, control F-actin is longer (1.6–8 μm) than the F-actin with β-actinin.

The effect of increasing amounts of β-actinin on the flow birefringence of F-actin polymerized in the presence of 1 mM $MgCl_2$ is shown in Fig. 6. Already 2.5 % β-actinin decreased the value of birefringence by more than 50 %. 10 % β-actinin exerted a full

effect. When 1 mM ATP and 0.1 M KCl were added to the Mg-polymer and incubated for 5 min at 45°, the Mg-polymer was transformed into short F-actin filaments (see Fig. 6). Thereafter the birefringence was measured, and a remarkable increase was noticed. Upon sonication, it was decreased but not to the initial level of the Mg-polymer, except in the case with 20% β-actinin. The relationship between the degree of birefringence and the amount of β-actinin added was exactly the same as in the case where F-actin is sonicated in 0.1 M KCl[11].

The effect of increasing concentrations of $MgCl_2$ on the flow birefringence of F-actin with 10% β-actinin was investigated. As shown in Fig. 7, up to 2 mM, the birefringence of the Mg-polymer is much lower than the value obtained when the Mg-polymer is transformed into F-actin particles. The difference, *e.g.* at 1 mM $MgCl_2$, could be explained in two ways: (i) at a low $MgCl_2$ concentration in the presence of β-actinin, the formation of the Mg-polymer is so slow that some G-actin is still present, and the monomers are quickly polymerized by the addition of KCl at 45°, (ii) the size of the Mg-polymer formed at low $MgCl_2$ concentrations is so small that it is not well orientated under the velocity gradient measured. Therefore, the value of birefringence is small. On the addition of KCl, elongation of particles takes place to give a larger value of birefringence. Experimental evidence is in favor of the first assumption, because a peak due to actin monomers was observed in the sedimentation pattern in 1 mM $MgCl_2$ and not in 2 mM $MgCl_2$ (see Fig. 3). Furthermore, the extinction angle, *e.g.* at a velocity gradient of 1000 sec^{-1}, was the same in 1 mM $MgCl_2$ as in 2 mM $MgCl_2$.

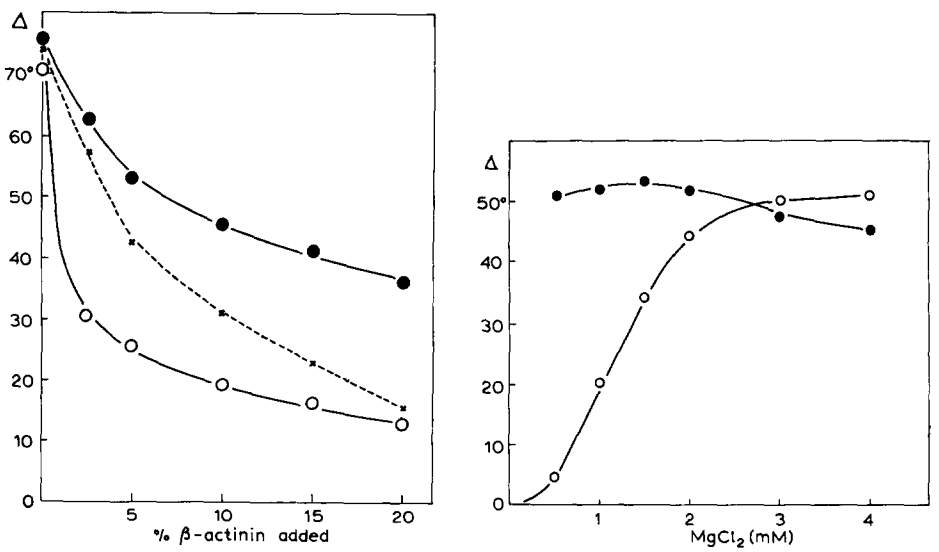

Fig. 6. Effect of increasing the amount of β-actinin on the flow birefringence of the Mg-polymer of actin. G-actin, 0.23 mg/ml, was polymerized by 1 mM $MgCl_2$ in the presence of varied amounts of β-actinin. 10 mM Tris buffer, pH 7.2. 2 h at 25° after the addition of $MgCl_2$, birefringence was measured at a velocity gradient of 1000 sec^{-1} (○). Then 1 mM ATP and 0.1 M KCl were added and incubated for 5 min at 45° (●). Subsequently, the samples were subjected to sonication (×).

Fig. 7. Effect of $MgCl_2$ concentration on the flow birefringence of the Mg-polymer formed under the influence of β-actinin. G-actin, 0.16 mg/ml, was polymerized by varied concentrations of $MgCl_2$ in the presence of 10% β-actinin. Other conditions as in Fig. 6. ○, $MgCl_2$; ●, after incubation with 1 mM ATP and 0.1 M KCl at 45°.

ATPase activity

One of the characteristic features of plasmodium Mg-polymer is its ATPase activity, approx. 1 nmole/mg per min at 25° (ref. 3). It was found that the Mg-polymer with 8 % β-actinin split ATP at a rate of 0.4 nmole/mg per min at 30° and pH 7.2 in the presence of 3 mM $MgCl_2$ and 1 mM ATP. Control F-actin did not hydrolyze ATP at all. Since the ATPase was very low at 30°, other experiments were performed at 45° (ref. 14). At 45° control F-actin showed some ATPase activity, about 0.4 nmole/mg per min, in the presence of 1 mM $MgCl_2$, which is in good agreement with the previous finding of ASAKURA AND OOSAWA[15]. β-Actinin activated the ATPase by about 160 %, as shown in Fig. 8. About 10 % β-actinin was enough for the full activation. Addition of 0.1 M KCl resulted in complete loss of the ATPase activity.

As already mentioned, at 1 mM $MgCl_2$ there is some monomeric G-actin whose interaction with the Mg-polymer may be the cause of the apparent ATPase activity[15]. Therefore, the dependence of the ATPase activity upon $MgCl_2$ concentration was examined. As summarized in Fig. 9, there was certainly a tendency for the ATPase activity in the presence of β-actinin to decrease as the Mg^{2+} concentration was elevated. It is of some interest that plasmodium β-actinin activated the ATPase activity 3 times even at 5 mM $MgCl_2$, whereas rabbit β-actinin did not enhance it at all. So far as the action of β-actinin is concerned, this is the only difference between β-actinins from rabbit skeletal muscle and myxomycete plasmodia[9].

Electron microscopic observations

The Mg-polymer negatively stained with uranyl acetate was seen in various forms under the electron microscope. Typical forms are presented in Figs. 10a, b and c. The globular shapes, 200–600 Å in diameter, are shown in Fig. 10a. Fig. 10b shows aggregates of F-actin-like filaments at random. These types of aggregates were most

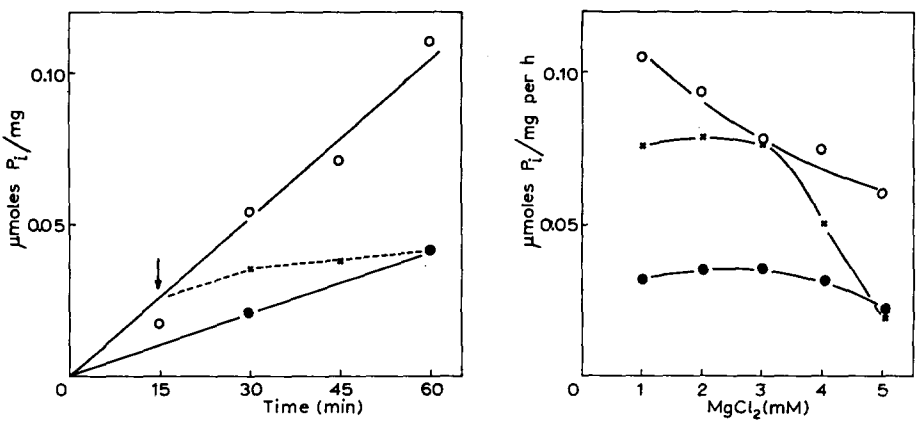

Fig. 8. The ATPase activity of the Mg-polymer formed under the influence of β-actinin. The reaction mixture contained actin, 1.0 mg/ml, 10 mM Tris buffer, pH 7.2, and 1 mM ATP. Incubated at 45°: ●, 1 mM $MgCl_2$; ○, 7.5% β-actinin + 1 mM $MgCl_2$; ×, 7.5% β-actinin + 1 mM $MgCl_2$ + 0.1 M KCl. Arrow shows the addition of KCl.

Fig. 9. The ATPase activity of the Mg-polymer formed under the influence of rabbit and plasmodium β-actinins at varied $MgCl_2$ concentrations. β-Actinin was added in 10% of actin. Incubation for 1 h. Other conditions, as in Fig. 8. ●, control without β-actinin; ○, plasmodium β-actinin; ×, rabbit β-actinin.

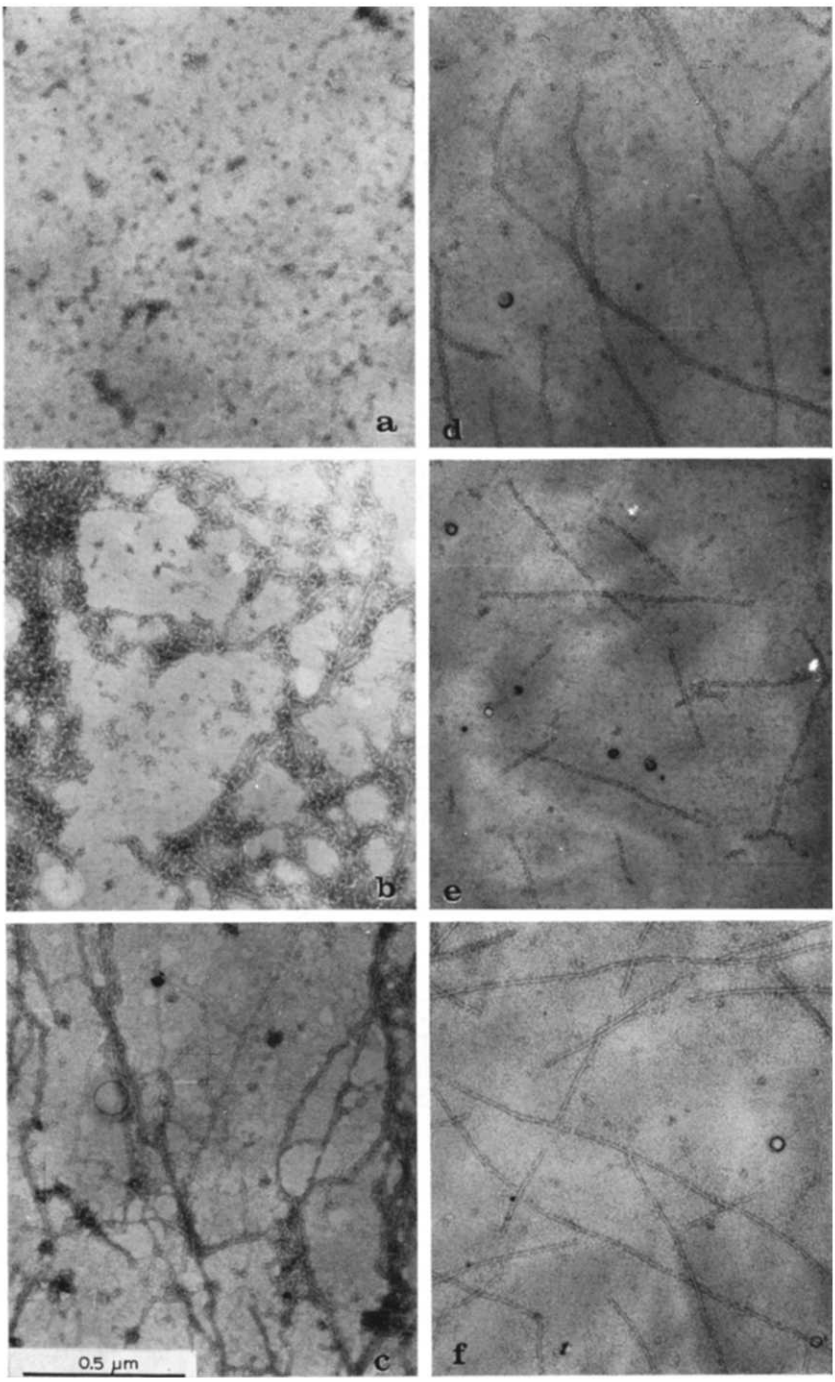

Fig. 10. Electron micrographs of the Mg-polymer formed under the influence of β-actinin. (a)(b) 5% β-actinin, 2 mM $MgCl_2$; (c) 5% β-actinin, 1 mM $MgCl_2$; (d) control, 1 mM $MgCl_2$; (e) 5% β-actinin, 1 mM $MgCl_2$, but prefixed with glutaraldehyde; (f) control, 1 mM $MgCl_2$, but prefixed with glutaraldehyde.

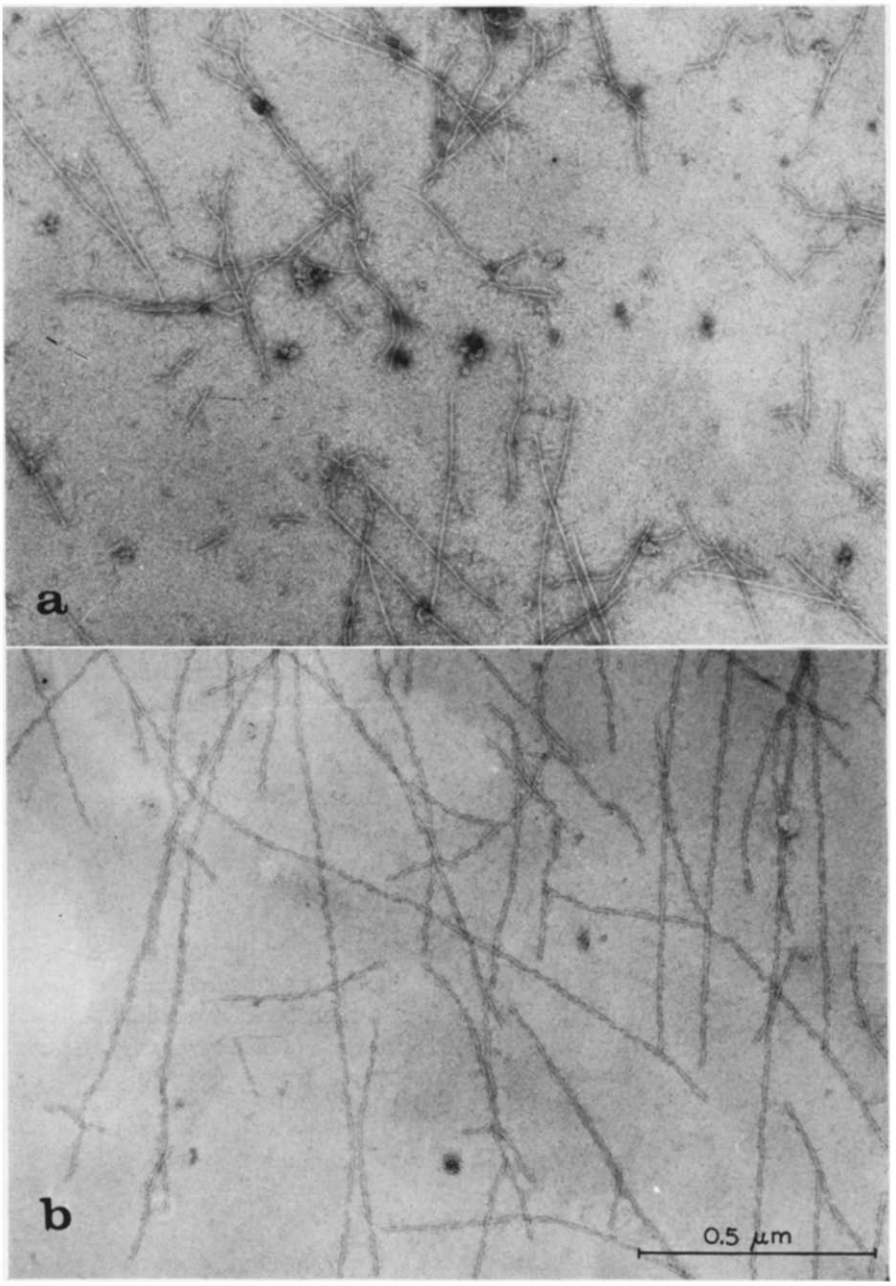

Fig. 11. Electron micrograph of the transformed F-actin. (a) Mg-polymer containing 10% β-actinin in 2 mM $MgCl_2$ was incubated for 5 min at 45°, in the presence of 1 mM ATP. (b) The above was further incubated for 30 min at 55°.

abundant in the Mg-polymer of actin formed under the influence of β-actinin. Sometimes a more filamentous structure was observed among the aggregates mentioned above, as seen in Fig. 10c. These forms are in good agreement with those described by TOTSUKA and co-workers[2,3,5] on plasmodium actin. Control F-actin, polymerized by 1–2 mM $MgCl_2$, showed F-actin filaments, somewhat deteriorated and twisted (Fig. 10d).

When the Mg-polymer was fixed for 5 min with 2.5 % glutaraldehyde on a mesh, it turned into short, but straight F-actin filaments (Fig. 10e). The length distribution of such fixed filaments is as follows: number average length, $\langle l \rangle_n = 0.22$ μm; weight average length, $\langle l \rangle_w = 0.43$ μm; the ratio $\langle l \rangle_w / \langle l \rangle_n = 1.95$ and the maximal length, $l_{max} = 1.15$ μm for the total number of filaments measured, $n = 329$. This result was obtained with the Mg-polymer formed in the presence of 16 % β-actinin and 2 mM $MgCl_2$. However, the length distribution did not significantly change when the amounts of β-actinin added were 5 % and 8 %, respectively. The fixed control F-actin (Fig. 10f) showed a typical double-stranded helical structure which is obtained by polymerization in 0.1 M KCl[4].

Transformation from Mg-polymer to F-actin

HATANO[6], and more recently TOTSUKA[5], have shown that the plasmodium Mg-polymer is transformed into F-actin by thermal treatment in the presence of 0.1 M KCl and 1 mM ATP. We have confirmed their experiments using the Mg-polymer formed under the influence of β-actinin. When the Mg-polymer was incubated for 5 min at 45° after the addition of 0.1 M KCl and 1 mM ATP, the Mg-polymer was completely transformed into short F-actin filaments (Fig. 11a) which did not show ATPase activity. The length distribution is shown in Table I; the number average length was as short as 0.26 μm, which was not different from the length of glutaraldehyde-fixed Mg-polymer. Then this short F-actin was further incubated for 25–30 min at 55°, as described by TOTSUKA[5]. Certainly, $\langle l \rangle_n$ became longer (0.57 μm), although it was shorter than that of F-actin polymerized by Mg^{2+} in the absence of β-actinin (0.93 μm), (Fig. 11b).

It was found that ATP was absolutely necessary to the transformation of the Mg-polymer at 55°; otherwise F-actin was precipitated as random aggregates. ATP must protect F-actin from thermal inactivation[14], whereas KCl was not necessary. In the case of the transformation at 45°, the presence of ATP was also required, but the presence of KCl was favorable.

TABLE I

PARTICLE LENGTH DISTRIBUTION OF THE Mg-POLYMER TRANSFORMED INTO F-ACTIN

Actin (0.3 mg/ml) was polymerized for 1 h at 25° in the presence of 5% β-actinin (0.015 mg/ml) and 2 mM $MgCl_2$ at pH 7.2 (10 mM Tris buffer). The resultant Mg-polymer was incubated for 5 min at 45° after the addition of 1 mM ATP and 0.1 M KCl. Then the sample was further incubated for 25 min at 55°. The control lacked β-actinin and was not subjected to the thermal treatment.

Treatment	n	$\langle l \rangle_n$ (μm)	$\langle l \rangle_w$ (μm)	$\langle l \rangle_w / \langle l \rangle_n$	l_{max} (μm)
45°, 5 min	518	0.26	0.47	1.81	1.75
55°, 25 min	601	0.57	1.02	1.79	3.9
Control	750	0.93	2.00	2.15	7.9

The flow-birefringence properties of the Mg-polymer before and after the heat treatment at 55° are shown in Fig. 12. It was quite evident that elongation of F-actin particles occurred.

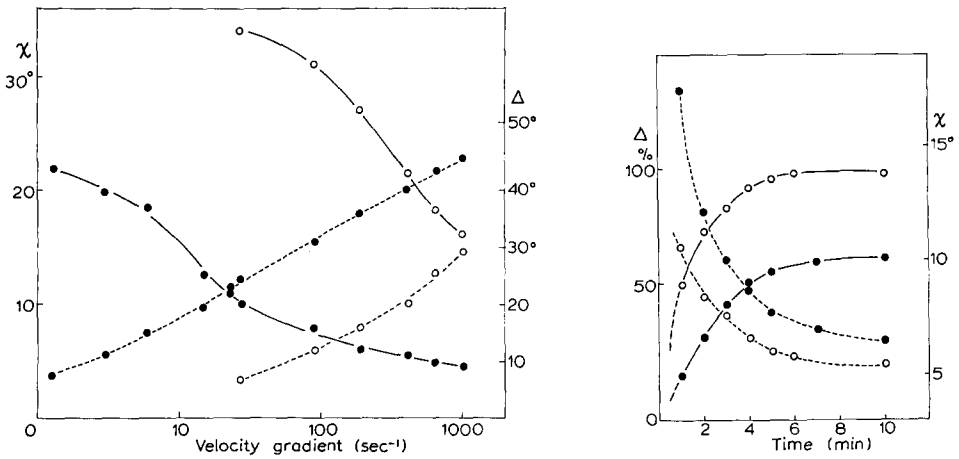

Fig. 12. Transformation of the Mg-polymer into F-actin by thermal treatment. G-actin, 0.25 mg/ml, was polymerized by 2 mM $MgCl_2$ in the presence of 7% β-actinin, then incubated for 30 min at 55° after the addition of 1 mM ATP. ○, Mg-polymer; ●, transformed F-actin; ———, extinction angle; – – – –, birefringence.

Fig. 13. Recovery process of Mg–F-actin after sonication. G-actin, 0.25 mg/ml, was polymerized either by 2 mM $MgCl_2$ or by 0.1 M KCl for 2 h at 25° and pH 7.2 (10 mM Tris buffer). After sonication, the birefringence and extinction angle were continuously measured at a velocity gradient of 100 sec^{-1}. Temperature, 25°. ○, 0.1 M KCl; ●, 2 mM $MgCl_2$; ———, extinction angle; – – – –, birefringence.

DISCUSSION

HATANO and his colleagues have repeatedly emphasized that the formation of the Mg-polymer is the characteristic property of plasmodium actin, and that the reversible transformation of the Mg-polymer into F-actin is deeply involved in the protoplasmic streaming in the myxomycete plasmodia[6]. On the other hand, ADELMAN AND TAYLOR[16] pointed out that the Mg-polymer formation could not be clearly observed with their actin preparations from plasmodium purified by a method[17] different from that of HATANO *et al.*[1,2]. They have suggested that β-actinin and other regulatory proteins may exist in plasmodia[16], although any possibility of relating β-actinin to the Mg-polymer formation was not mentioned. It could be the case that β-actinin was not contaminating the preparation of ADELMAN AND TAYLOR. However, the fact that the reduced viscosity of F-actin (in 0.1 M KCl) was as low as 4 dl/g, and that the sedimentation coefficient of the main peak was 30 S, suggest the possible presence of β-actinin in their preparations. It has been shown that β-actinin is very probably contained in HATANO's actin preparations[9].

The characteristics of the Mg-polymer of plasmodium actin are as follows: the reduced viscosity is as low as 0.5–1.5 dl/g, whereas the sedimentation constant is the same as that of plasmodium F-actin (approx. 30 S)[2]. The Mg-polymer of rabbit actin formed in the presence of β-actinin showed a viscosity of 1–2 dl/g, and a sedi-

mentation constant of 31–33 S. This value is slightly lower than that of F-actin (approx. 35 S). Plasmodium Mg-polymer showed ATPase activity, approx. 1 nmole/mg per min (ref. 3). The rabbit Mg-polymer also had ATPase activity, but the rate was less than half that of the plasmodium Mg-polymer. In this respect, it is noteworthy that plasmodium β-actinin was more effective in inducing the ATPase action of the Mg-polymer of rabbit actin at various $MgCl_2$ concentrations than rabbit β-actinin (see Fig. 9).

The size and shape of negatively stained Mg-polymer under the electron microscope is remarkably variable, from a globular shape to random filamentous aggregates. It is of interest that the glutaraldehyde-fixed Mg-polymer is changed into F-actin filaments. There is a possibility that the Mg-polymer is flexible[18], easily deteriorated by uranyl acetate, and upon fixation it becomes rigid enough not to suffer from the action of the stain.

The low viscosity of the Mg-polymer formed under the influence of β-actinin (1–2 dl/g) may be explained by the short particle length of the glutaraldehyde-fixed specimen: approx. 0.22 μm for $\langle l \rangle_n$, which is not significantly different from the value (0.26 μm) of short F-actin particles incubated at 45° in the presence of ATP and 0.1 M KCl. In fact, the viscosity was only slightly increased by this treatment. The difference in s values between Mg-polymer (approx. 32 S) and F-actin polymerized by 0.1 M KCl in the presence of β-actinin (approx. 35 S) may also be explained by the difference in particle length (the latter being approx. 0.4 μm for $\langle l \rangle_n$). In an experiment shown in Fig. 2, the viscosity of the latter was 3.1 dl/g and was decreased to 1.2 dl/g upon sonication and the particle length became 0.2 μm after sonication. On the other hand, there was no change in viscosity (1.5 dl/g) of the Mg-polymer by sonication. These considerations do not support the idea that the Mg-polymer exists in solution as random aggregates seen in the electron micrograph. As already mentioned, the Mg-polymer is thought to be a short fragile polymer.

The transformation of the Mg-polymer into stable F-actin requires ATP at 45°. Addition of 0.1 M KCl alone was not so effective as that of ATP. Incubation for 30 min at 55° in the presence of ATP resulted in the elongation of F-actin from 0.2 to 0.5 μm for $\langle l \rangle_n$. Evidently this must have been due to partial inactivation of β-actinin[12]. Therefore, recombination of short F-actin particles occurred.

With respect to the rather fragile structure of the Mg-polymer of actin with β-actinin, we should mention that the so-called Mg-actin polymerized by a low mmolar concentration of $MgCl_2$ alone is also less rigid than usual F-actin (Ca-actin)[19]. To demonstrate this, the process of recombination of fragmented F-actin after sonication was investigated with the Mg-actin and the Ca-actin. As shown in Fig. 13, the rate of recombination of fragments was slower in Mg-actin than in Ca-actin, and also the final recovery was incomplete with Mg-actin, very probably due to random aggregate formation. The former fact was electron microscopically verified: in 1 min after sonication, $\langle l \rangle_n$ of the Ca-actin was elongated from 0.16 to 0.54 μm while that of the Mg-actin remained 0.27 μm (see ref. 11).

The mode of action of β-actinin in the formation of the Mg-polymer of actin is completely unknown. The only fact we know is that β-actinin is more bound to actin in the presence of $MgCl_2$ than in the presence of KCl (T. ISHII AND K. MARUYAMA, unpublished results). Probably the bound β-actinin may result in the fragile structure of F-actin.

Finally we should mention that most of the characteristic properties of the plasmodium Mg-polymer are reproduced in the Mg-polymer of actin formed under the influence of β-actinin (5–10 % of actin by weight), but some discrepancies remain, namely lower viscosity and higher ATPase activity of the plasmodium Mg-polymer. It is quite possible that plasmodium actin may have a stronger tendency to form Mg-polymer than rabbit actin. It is worthwhile to examine this point, using pure actin from plasmodia. Hatano's sample could be freed from β-actinin by repeating thermal treatment at 55°. Unfortunately, plasmodium β-actinin is resistant to tryptic action[9] so that trypsin treatment to remove β-actinin[8] is not applicable.

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