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TRANSFORMATION OF PLASMODIUM ACTIN POLYMERS AT HIGH TEMPERATURES

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SUMMARY

Plasmodium G-actin polymerizes to F-actin on the addition of 70 mM KCl and to another polymeric state, termed "Mg-polymer", on the addition of 2 mM $MgCl_2$ or of 1.5 mM $MgCl_2$ and 70 mM KCl.

The Mg-polymer of plasmodium actin is transformed rapidly (in 20 min) into F-actin by raising the temperature to around 55° in the presence of 70 mM KCl, 1.5 mM $MgCl_2$, 1.5 mM ATP and 15 mM Tris–maleate buffer (pH 7.0). The kinetic analysis showed that this transformation is of the first order, suggesting that it is not an interpolymer process but an intrapolymer process; namely one filament of the Mg-polymer transforms into one filament of F-actin.

INTRODUCTION

Actin was extracted and purified from plasmodium of the myxomycete, *Physarum polycephalum*[1,2]. It is in the state of dispersed monomers (G-actin) in the absence of salts. On the addition of KCl it polymerizes to F-actin, while on the addition of $MgCl_2$ it polymerizes to the other kind of polymer which has been termed Mg-polymer[3]. The viscosity of the Mg-polymer is much lower than that of F-actin though the sedimentation coefficient is almost the same as that of F-actin. F-actin has no ATPase activity at room temperature, while the Mg-polymer shows the activity accompanied by the exchange of bound ADP with ATP in the solution, suggesting the conformational change in Mg-polymer[4]. These polymers can be depolymerized into G-actin reversibly by dialyzing out the salts. Moreover, Mg-polymer transforms to F-actin when it is dialyzed against the Mg^{2+}-free salt solution at room temperature, *e.g.* the 0.1 M KCl solution, and F-actin transforms to Mg-polymer when it is dialyzed against the 2 mM $MgCl_2$ solution at room temperature[3]. However, the rates of these transformations are very slow. It takes about two days. Therefore, the condition was sought in which the transformation can take place more rapidly. As a result, it was found that the incubation of the Mg-polymer at a fairly high temperature (around 55°) in the presence of KCl and ATP can induce its rapid transformation into F-actin. The kinetic analysis showed that this transformation is of the first order, suggesting that it is not an interpolymer process but an intrapolymer process. Such a transformation between Mg-polymer and F-actin may have a physiological significance, since recently

by electronmicroscopy the conformational changes of F-actin-like microfilaments in plasmodium[5] were observed in connexion with the regulation of protoplasmic flow.

MATERIALS AND METHODS

Plasmodia

Plasmodia of the myxomycete, *Physarum polycephalum*, were cultured by the method of CAMP[6], improved by HATANO AND OOSAWA[1], to obtain a large amount of plasmodia.

Plasmodium G-actin

Plasmodium G-actin was prepared from plasmodia according to the method of HATANO AND OOSAWA[1] with the slight modification where 3 mM cysteine was substituted by 0.2 mM borate buffer (pH 8.1)[4].

Formation of Mg-polymer

Plasmodium G-actin (0.5–4 mg/ml) was pre-incubated with 2 mM $MgCl_2$ at 0° for 10 min and then polymerized by the addition of KCl to 70 mM and of Tris–maleate buffer (pH 7.0) to 15 mM at 22°. The final $MgCl_2$ concentration was 1.5 mM. The pH of the solution containing 15 mM Tris–maleate buffer used here decreased from 7.0 to 6.8 as the temperature increased from 22 to 55°.

Protein concentration

Protein concentration was measured by the biuret method[7] using the absorbance 0.068 at 540 nm for 1 mg/ml plasmodium actin.

Viscosity

Viscosity was measured by Ostwald-type capillary viscometers, flow times of which for buffer solutions were around 35 sec at 22° and around 19 sec at 55°.

ATPase activity

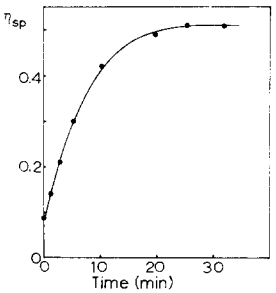
ATPase activity was determined by measuring liberated inorganic phosphate by the method of MARTIN AND DOTY[8].

RESULTS

Viscosity increase

The Mg-polymer obtained at 22° after the addition of 70 mM KCl to G-actin incubated in 2 mM $MgCl_2$ at 0° gave the reduced viscosity of around 0.7 to 1.5 dl/g. When this Mg-polymer was incubated at relatively high temperatures (50–63°) in the presence of 70 mM KCl, 1.5 mM ATP, 1.5 mM $MgCl_2$ and 15 mM Tris–maleate buffer (pH 7.0), its viscosity markedly increased to almost the same level as that of F-actin, *i.e.* the reduced viscosity of around 3 dl/g, as shown in Fig. 1. Then, the increased viscosity was ascertained to be a result of the transformation of Mg-polymer to F-actin. For this viscosity increase, both 70 mM KCl and 1.5 mM ATP were found to be necessary. In the presence of 1.5 mM $MgCl_2$, 1.5 mM ATP and 15 mM Tris–maleate buffer (pH 7.0) at 55°, if KCl of 70 mM was not present in the solution, the viscosity

increase of the Mg-polymer was small. In the presence of 70 mM KCl, 1.5 mM $MgCl_2$, and 15 mM Tris–maleate buffer (pH 7.0) at 55°, if the concentration of ATP was low (*e.g.* 50 μM), the viscosity of Mg-polymer decreased within several minutes to the level



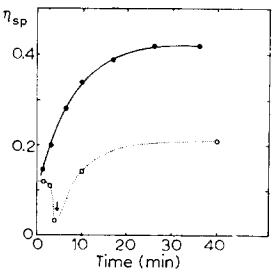


Fig. 1. Viscosity increase when Mg-polymer was incubated at 55°. 1.85 mg/ml protein; in 1.5 mM $MgCl_2$, 70 mM KCl, 1.5 mM ATP and 15 mM Tris–maleate buffer (pH 7.0).

Fig. 2. Effect of ATP on the viscosity increase of Mg-polymer. Mg-polymer was incubated at 55° in the presence of 1.5 mM ATP (●—●) or 50 μM ATP (○···○). In the latter case, ATP was added at the time indicated by the arrow in the figure to the concentration of 1.5 mM. Other conditions: 1.1 mg/ml protein, 70 mM KCl, 1.5 mM $MgCl_2$ and 15 mM Tris–maleate buffer (pH 7.0).

of G-actin, as shown in Fig. 2. This is probably due to the loss of ATP in the solution by the ATPase activity of Mg-polymer because at high temperatures of around 55° both F-actin and Mg-polymer were found to have the ATPase activity as in the case of muscle F-actin[9]. If the concentration of ATP was increased to 1.5 mM within a few min after the depolymerization of Mg-polymer at 55°, a partial increase of viscosity was observed, suggesting that a part of actin was still alive in the absence of ATP for at least a few min at 55°. In the presence of 70 mM KCl, 1.5 mM $MgCl_2$ and 15 mM Tris–maleate buffer (pH 7.0), if 1.5 mM ADP was added instead of 1.5 mM ATP, the viscosity of Mg-polymer was not changed by raising the temperature to 55°. Namely, ADP cannot induce the transformation although it can prevent denaturation of Mg-polymer at such a high temperature.

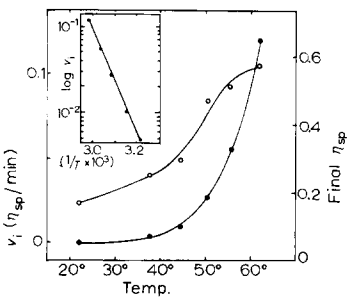
This viscosity increase is induced in the temperature range from 40 to 63°. As shown in Fig. 3, at around 40° only a slight increase (to about 150 % of Mg-polymer) is observed. The complete increase of viscosity is induced at around 55°. When the Mg-polymer is transferred to 55°, after it was incubated at 45° for several minutes, the viscosity of the Mg-polymer begins to increase at a higher rate than at 45°, to reach the level corresponding to pure F-actin. At temperatures higher than 63°, the viscosity of the Mg-polymer rapidly decreased to the level of G-actin even in the presence of 70 mM KCl, 1.5 mM ATP, 1.5 mM $MgCl_2$ and 15 mM Tris–maleate buffer (pH 7.0), showing that the protein was denatured.

Therefore, in the following experiments, the heat-treatment was carried out almost always at 55°, except when the temperature is indicated otherwise.

Viscosity of transformed F-actin did not change when the temperature was decreased to 22° even in the presence of 1.5 mM $MgCl_2$. The reverse transformation of F-actin to Mg-polymer was not induced by simply changing the condition to that favorable to the Mg-polymer.

When transformed F-actin was depolymerized to G-actin by dialysis against a

solution containing 50 μM ATP and 0.2 mM borate buffer (pH 8.1), the resultant G-actin of the reduced viscosity of 0.15 dl/g was ascertained to repolymerize to F-actin of the reduced viscosity of 3.5 dl/g on the addition of KCl to 70 mM.



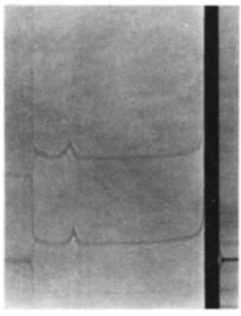


Fig. 3. The viscosity increase and its rate as a function of temperature. Mg-polymer was incubated at the temperature range from 22° to 63°. The initial rates of viscosity increase (V_i, ●—●) and the final viscosities (η_{sp}, ○—○) were plotted against the temperature. The insert is the logarithmic plot of V_i against the reciprocal of the temperature (Arrhenius plot). An activation enthalpy of the viscosity increase was calculated as 28 kcal/mole. 1.85 mg/ml protein; in 1.5 mM ATP, 1.5 mM $MgCl_2$, 70 mM, KCl and 15 mM Tris–maleate buffer (pH 7.0).

Fig. 4. Sedimentation pattern. Upper: Mg-polymer. Lower: Mg-polymer heated at 55° for 25 min in the presence of 70 mM KCl, 1.5 mM $MgCl_2$, 1.5 mM ATP and 15 mM Tris–maleate buffer (pH 7.0). 1 mg/ml protein; in 1.5 mM $MgCl_2$, 70 mM KCl, 1.5 mM ATP and 15 mM Tris–maleate buffer (pH 7.0). Photograph was taken at 15 min after a speed of 37020 rev./min was reached at 15°.

Sedimentation pattern, electronmicroscopy and ATPase activity

After the Mg-polymer was incubated at 55° for 25 min in the presence of 70 mM KCl, 1.5 mM $MgCl_2$, 1.5 mM ATP and 15 mM Tris–maleate buffer (pH 7.0), the sedimentation pattern of the transformed F-actin was observed at 15°. As shown in Fig. 4, it gave a single peak of about 30 S, which was almost the same as that of the F-actin polymerized from G-actin on the addition of KCl to 70 mM. Moreover, the sedimentation pattern became similar to that of F-actin; namely the peak is sharper than that of the orginal Mg-polymer.

Electron micrographs also showed that many F-actin filaments having a two-stranded helical structure appeared after the incubation of the Mg-polymer at 55° (see Figs. 5a and 5b).

After transformation at 55°, the ATPase activity of the polymer was measured at 22°. As shown in Fig. 6, the activity was found to be very low, which is about one-tenth of that of the original Mg-polymer, showing that almost all the Mg-polymer was transformed and maintained in the state of F-actin. The F-actin could combine with muscle myosin A to form actomyosin which showed a viscosity drop upon the addition of ATP.

Kinetics of the transformation

Viscosity increase

Solutions of the Mg-polymer at various concentrations were obtained by dilution of a concentrated Mg-polymer solution (3.5 mg/ml) and incubated at 55° under the salt conditions of 70 mM KCl, 1.5 mM $MgCl_2$, 1.5 mM ATP and 15 mM Tris–maleate buffer (pH 7.0). Then, the increase of the viscosity of each solution was followed. Half-

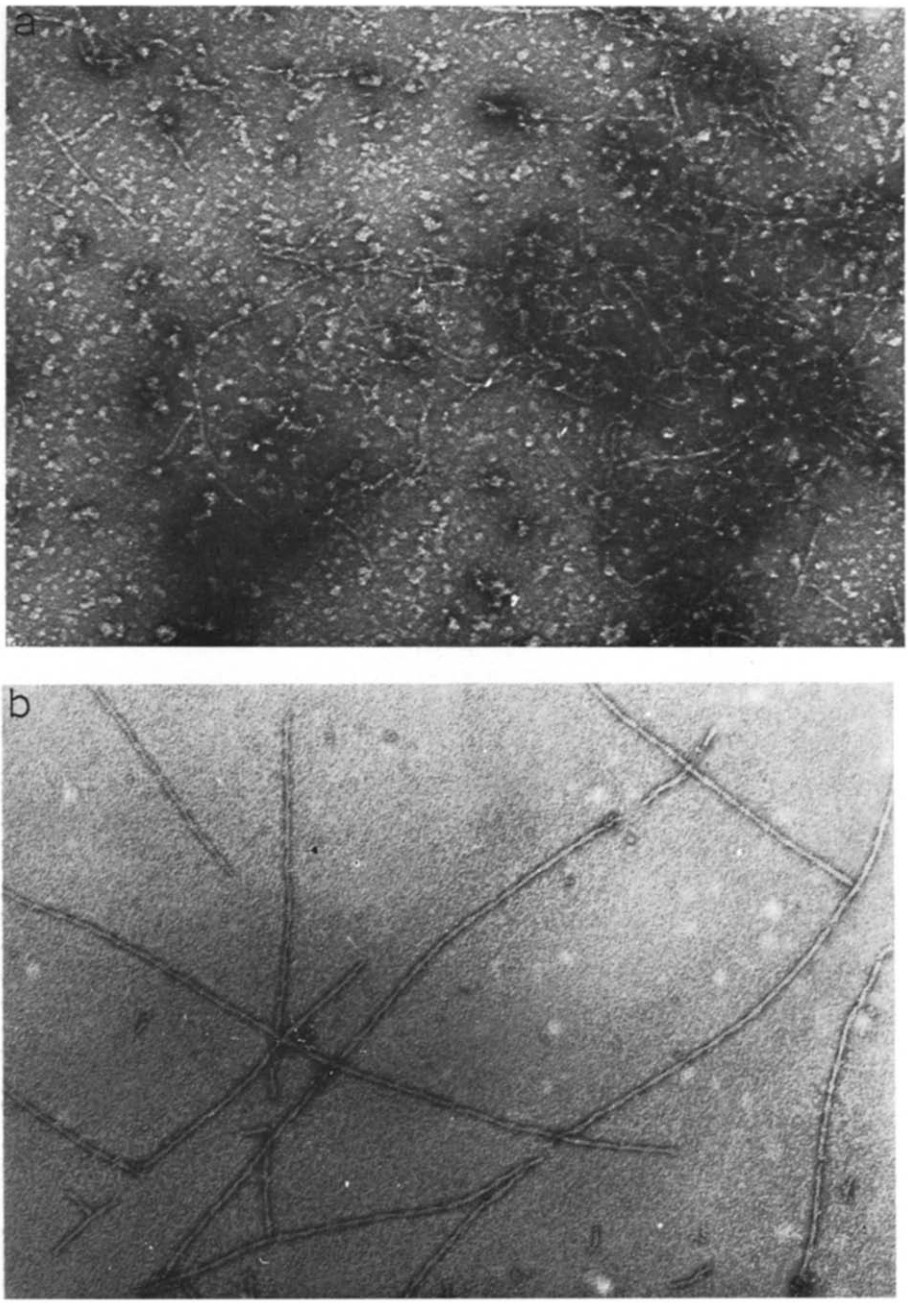


Fig. 5. Electron micrographs of plasmodium Mg-polymer (a) and the transformed F-actin (b). Negative staining with uranyl acetate. × 81 000. (a) Mg-polymer seems to be amorphous or to be shaped like irregularly bended filaments. Artifacts may be included in such an appearance because the Mg-polymer is less stable than F-actin against negative staining. (b) F-actin obtained by incubation of the Mg-polymer at 55° for 25 min in the presence of 70 mM KCl, 1.5 mM $MgCl_2$, 1.5 mM ATP and 15 mM Tris–maleate buffer (pH 7.0). These micrographs were kindly taken by Dr. S. Higashi-Fujime.

time $t_{1/2}$ of the viscosity increase was defined as the time necessary for the viscosity to reach the middle value of the initial viscosity (η_{in}) and the final viscosity (η_{fin}). The initial rate of increase of the reduced viscosity was also determined.

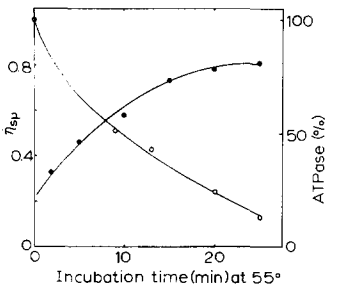
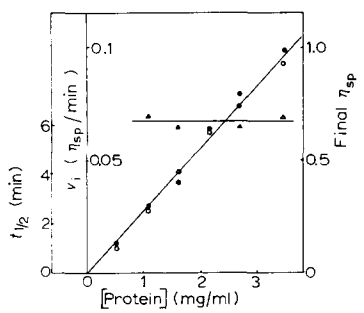


Fig. 6. Decrease of the ATPase activity in the process of the transformation at 55°. The ATPase activity (○—○) of the polymer partially transformed into F-actin during incubation at 55° in the presence of 70 mM KCl, 1.5 mM $MgCl_2$, 1.5 mM ATP and 15 mM Tris–maleate buffer (pH 7.0) was measured at 22°. The abscissa is the time of incubation. The ordinate, the ATPase activity, is proportional to the amount of remaining Mg-polymer. 1.4 mg/ml protein. Before the measurement of the ATPase activity, each solution was mixed with Dowex 1 (Cl^- type, 200–400 mesh) for 3 min in the cold and the resin was removed by centrifugation 10000 × *g* for 3 min, and then, ATP was added to the final concentration of 0.13 mM.

The $t_{1/2}$'s and the initial rates were both almost independent of the protein concentration. They were 6 min and 0.3 dl/g per min in the case of Fig. 7. Moreover, the final reduced viscosities of F-actin obtained at various protein concentrations were also constant. These facts suggest that the transformation is of the first order and that the final state of the polymers is the same.

ATPase activity

The change of the ATPase activity during the transformation was examined. As described earlier, F-actin once transformed from Mg-polymer at 55° was not transformed reversibly to Mg-polymer even when the temperature was decreased to room temperature. Therefore, during the incubation of the Mg-polymer at 55° in the pre-



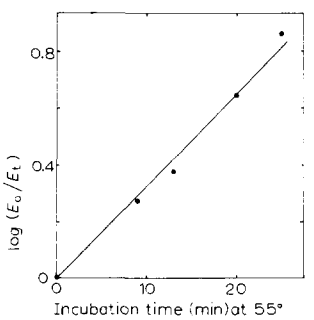


Fig. 7. Kinetics of viscosity increase. ○—○, final specific viscosity; ●—●, the initial rate of increase of specific viscosity; △—△, half-time of viscosity increase. Solvent condition: 70 mM KCl, 1.5 mM $MgCl_2$, 1.5 mM ATP and 15 mM Tris–maleate buffer (pH 7.0). Temperature, 55°.

Fig. 8. Kinetics of the decrease of the ATPase activity in the process of the transformation at 55°. E_0; the initial value of the ATPase activity. E_t; the ATPase activity after incubation at 55° for *t* min. Other conditions are the same as those in Fig. 6. Experimental procedures were the same as in Fig. 6.

sence of 70 mM KCl, 1.5 mM $MgCl_2$, 1.5 mM ATP and 15 mM Tris–maleate buffer (pH 7.0), parts of the solution were transferred to 22° at intervals. Then, free nucleotides were removed with Dowex 1 (Cl^- type) (200–400 mesh) and the ATPase activity was measured after the addition of ATP to 0.13 mM in the presence of 70 mM KCl, 1.5 mM $MgCl_2$ and 15 mM Tris–maleate buffer (pH 7.0). The ATPase activity at 22° was expected to be proportional to the concentration of Mg-polymer which remained in the solution during the incubation. In the case of the pure Mg-polymer, it was confirmed previously that the ATPase activity is proportional to the concentration of the Mg-polymer.

As the viscosity of the Mg-polymer solution increased at 55°, the ATPase activity of the solution measured by the above procedure decreased, reaching in 25 min about one-tenth of the initial ATPase activity of the Mg-polymer, as shown in Fig. 6.

As analyzed later, the time-course of the decrease of the ATPase activity is well understood by assuming the intrapolymer transformation of Mg-polymer to F-actin.

DISCUSSION

It was found in this experiment that the Mg-polymer is rapidly transformed to F-actin in the temperature range from 50° to 63°. In the experiment of the decrease of the ATPase activity, as shown in Fig. 8, $\log(E_0/E_t)$ was found to be almost proportional to the time of incubation, where E_0 is the ATPase activity of the initial Mg-polymer at 22° and E_t is that measured at 22° after incubation at 55° for t min. As the ATPase activity of the Mg-polymer is proportional to the concentration of the Mg-polymer, this means that $\log(c_0/c_t)$ was almost proportional to the time of incubation, where c_0 is the initial concentration of Mg-polymer and c_t is the concentration of Mg-polymer after incubation for t min. This fact and the experiment of the viscosity increase both suggest that the transformation is of the first order. Moreover, the experiment of the viscosity increase showed that the reduced viscosities of the F-actins formed from the Mg-polymer are independent of its concentration, suggesting that the F-actin's are in the same state. In relation to this phenomenon, it is to be noted that when F-actin solutions of various protein concentrations were obtained by dilution of a concentrated F-actin solution, these solutions showed almost the same reduced viscosity as that of the original F-actin solution. Whereas, when F-actin solutions were obtained by polymerization of various concentrations of G-actin, the reduced viscosity of the F-actin decreased with the increase of the actin concentration.

These facts are well understood if it is assumed that the rate-determining process of the transformation of Mg-polymer to F-actin at 55° involves the intrapolymer. The Mg-polymer was previously assumed to be a folded polymer or an interrupted helical polymer. If so, the transformation is due to straightening and strengthening of such a polymer to the two-stranded helical one.

In Fig. 4, the F-actin-type polymer which was converted from the Mg-polymer, similarly to the F-actin polymerized from G-actin, showed a sharper peak and a slightly faster rate of sedimentation than the Mg-polymer. However, under such a strong centrifugal field certain structural changes in polymers may be expected to occur, for example, the weakening of the bonds between monomers in F-actin to a certain extent or on the contrary the strengthening of those in the Mg-polymer. Moreover, the interpolymer interaction of the F-actin is expected to be different from that

of the Mg-polymer and the sharper peak and the faster rate of sedimentation of the F-actin than those of the Mg-polymer may reflect such a difference.

For this transformation, ATP was necessary and ADP could not be substituted for ATP though it could stabilize the Mg-polymer at 55°. The transformation was irreversible at least under the condition tested here. However, as reported previously[3], it was indeed found that mutual transformations occurred reversibly between the Mg-polymer and F-actin at room temperature although the rates were very slow. Now, the mild conditions which induce rapid mutual transformation between the Mg-polymer and F-actin are under investigation.

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