BBA 45996

ATPase ACTIVITY OF PLASMODIUM ACTIN POLYMER FORMED IN THE PRESENCE OF Mg^{2+}

TSUYOSHI TOTSUKA AND SADASHI HATANO

Institute of Molecular Biology, Faculty of Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya (Japan)

(Received April 20th, 1970)

SUMMARY

1. In the presence of Mg^{2+}, plasmodium actin forms a polymer different from F-actin. This polymer, termed Mg-polymer, has an ATPase activity. Its specific activity is about one-hundredth of the activity of myosin from muscle or plasmodium and of the same order as that of muscle F-actin under sonic vibration.

2. The formation of Mg-polymer and its ATPase activity are not due to modification of the protein in the preparation procedure. The ATPase activity is not due to contamination in the preparation but is closely related to the amount of Mg-polymer formed.

3. The ATPase activity of Mg-polymer is markedly inhibited by high concentration of ATP (about 1 mM) in the presence of KCl; but it is not inhibited in the absence of KCl.

4 Mg-polymer releases its bound [^{14}C]ADP in a cold ATP solution. The half time of the release is about 10 min at 22°, which is of the same order as the rate of ATP splitting. This is understandable if both the ATP splitting and the ADP exchange are associated with the same cyclic process of intrapolymer reaction occurring everywhere along the Mg-polymer.

5. Copolymers of plasmodium and muscle G-actins are formed in the presence of Mg^{2+}. The ATPase activity of copolymers decreases rapidly with increasing content of muscle actin; it is suggested that interaction of neighboring plasmodium actin molecules in the polymer is required for the ATPase activity.

INTRODUCTION

Plasmodium G-actin polymerizes to F-actin on the addition of monovalent salt solutions, *e.g.* 0.1 M KCl[1,2]. Electronmicroscopy shows that plasmodium F-actin has a two stranded-helical structure[3] which is similar to that of muscle F-actin[4]. Plasmodium G-actin, however, polymerizes to another state of actin polymer, termed Mg-polymer, on the addition of $MgCl_2$ or of $MgCl_2$ and KCl, that is, in the presence of Mg^{2+}. Mg-polymer has a much lower viscosity than F-actin, though its sedimentation coefficient is almost the same as that of F-actin. A characteristic property of this Mg-polymer is the ATPase activity, whereas plasmodium F-actin has no ATPase activity.

It has been reported by WOHLFARTH-BOTTERMANN[5], RHEA[6] and NAGAI AND KAMIYA[7] that in the gel layer of plasmodium there are bundles of microfilaments having a structure similar to that of F-actin. TAKATA *et al.*[8] and KAMIYA[9] presented some evidence from electronmicroscopy that the microfilaments can transform to another state of polymer in plasmodium and that the transformed polymer resembles Mg-polymer in its appearance. Therefore, the polymorphism of polymers of plasmodium actin discovered *in vitro* may have a physiologically important role in protoplasmic flow.

The purpose of this work was to investigate a few characteristics of the ATPase activity of Mg-polymer and propose a possible model of the ATPase reaction.

MATERIALS AND METHODS

Plasmodium

Plasmodium of the myxomycete *Physarum polycephalum* was cultured by the method of CAMP[10] with small modification to obtain large amounts of plasmodia as described in the previous paper[1].

Plasmodium G-actin

Plasmodium G-actin was prepared from plasmodia by the method of HATANO AND OOSAWA[1] using specific binding of plasmodium actin to muscle myosin A. Although in the previous work extraction and purification of plasmodium actin were carried out in the presence of 3 mM cysteine, in this work cysteine was replaced by 0.2 mM borate buffer (pH 8.1) without loss of activity of actin.

Muscle G-actin

Muscle G-actin was prepared from rabbit skeletal muscle by the methods described by MOMMAERTS[11] AND DRABIKOWSKY AND GERGELY[12].

Protein concentration

Protein concentration was measured by the biuret method[13] using the absorbance 0.068 at 540 nm for plasmodium actin at 1 mg/ml and the absorbance 0.07 for muscle actin at 1 mg/ml.

Viscosity

Viscosity was measured by Ostwald type capillary viscometers, in which the flow time was around 30 sec for buffer solutions.

ATPase activity

ATPase activity was determined by measuring liberated inorganic phosphates by the method of MARTIN AND DOTY[14].

RESULTS

Polymerization of plasmodium G-actin and ATP-splitting

As shown in Fig. 1, by the simple addition of monovalent salts, plasmodium G-actin is polymerized to F-actin, which has a high viscosity (η_{sp}/c: 5 dl/g) and a

high sedimentation coefficient (30 S). In the presence of Mg^{2+}, however, it was polymerized into Mg-polymer which gave a much lower viscosity (0.7 to about 1.5 dl/g) and a sedimentation coefficient of the same order (30 S). In electronmicrographs F-actin appeared as a two-stranded helical polymer, while Mg-polymer appeared as globular or sometimes flexible polymers[3].

Similarly to muscle G-actin, plasmodium G-actin binds an equimolar amount of ATP. During polymerization into either F-actin or Mg-polymer, this ATP is split into ADP and inorganic phosphate[3], thus

$$\text{G-actin–ATP} \xrightarrow[\text{0.1 M KCl}]{} \text{F-actin–ADP} + P_i$$

$$\text{G-actin–ATP} \xrightarrow[\text{2 mM } MgCl_2 \text{ (and 0.1 M KCl)}]{} \text{Mg-polymer–ADP} + P_i$$

F-actin-ADP itself cannot split ATP in the solution, whereas Mg-polymer can do so even after polymerization is complete. Fig. 2 shows that Mg-polymer can hydrolyze ATP added from outside but cannot hydrolyze ADP. The specific activity of this ATPase is of the order of 1 nmole/min per mg protein.

Comparison with muscle actin prepared by the same method

Plasmodium actin has a property different from muscle actin in constructing the Mg-polymer having the ATPase activity. Since plasmodium actin was prepared by a method different from that usually applied to muscle actin, it must be ascertained whether or not the property of actin was modified in the preparation procedure. Therefore, the method of preparation of plasmodium actin was applied to muscle and the property of muscle actin obtained was compared with that of plasmodium actin.

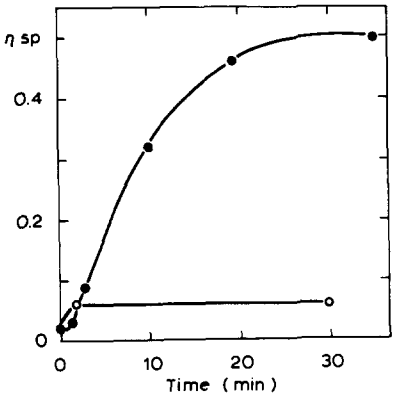

Fig. 1. Polymerization of plasmodium actin on the addition of 0.1 M KCl in the presence (o–o) or the absence (●–●) of 2 mM $MgCl_2$. Protein concentration 0.9 mg/ml, 50 μM ATP and 3 mM cysteine (pH 7.0).

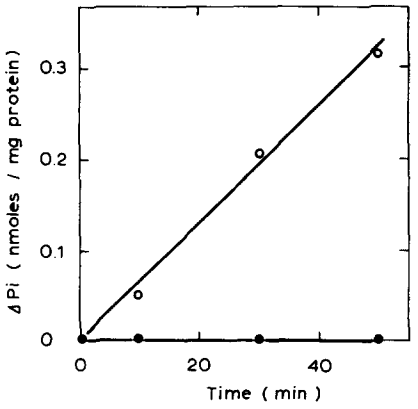

Fig. 2. Time-course of ATP splitting by Mg-polymer. ATPase activity was measured under the ollowing conditions. Protein concentration 2 mg/ml, 2 mM $MgCl_2$ and 15 mM Tris–maleate buffer (pH 7.0). 0.2 mM ATP (○–○) or 0.2 mM ADP (●–●) was added at 0 min to a Mg-polymer solution from which free nucleotides had been removed by Dowex-1.

An appropriate amount of muscle myosin A was added to a water extract of the acetone-dried powder of rabbit skeletal muscle to form the actomyosin complex. The complex was precipitated in 0.05 M KCl and the washed precipitate was treated with acetone and dried in air. From this dried powder of the complex, G-actin was extracted with water and purified by isoelectric precipitation at pH 4.7 followed by salting-out with $(NH_4)_2SO_4$ in the range from 15 to 35% satn. The yield of muscle G-actin by this method was almost the same as that by the usual method of ultracentrifugation in the state of F-actin. On the addition of monovalent salts, this G-actin from muscle was polymerized into F-actin which gave a high viscosity (10 dl/g). Unlike plasmodium actin, even in the presence of Mg^{2+} it was polymerized into F-actin having the same order of viscosity. This suggests that the formation of Mg-polymer is not an artifact due to the special method of preparation but is really characteristic of plasmodium actin.

Relation between the state of polymers and the ATPase activity

It was confirmed that plasmodium actin shows no ATPase activity in the state of G-actin.

Plasmodium actin polymerizes to F-actin on the addition of monovalent salts. This F-actin can be transformed to Mg-polymer by the addition of Mg^{2+}. The transformation, however, is very slow, taking 1 day or longer. The viscosity of F-actin does not appreciably decrease in a few hours after the addition of Mg^{2+}. That is, F-actin once formed is rather stable even in the presence of Mg^{2+}. On the other hand, if Mg^{2+} is added to G-actin directly, Mg-polymer is formed. Therefore, we can have F-actin and Mg-polymer at the same salt concentration, for example, at 70 mM KCl and 1.5 mM $MgCl_2$. In this condition Mg-polymer can split ATP but F-actin can not. This means that the special structure of Mg-polymer is required for the activity of the steady ATPsplitting. The ATPase activity found in the solution of Mg-polymer is not due to contamination in the preparation of actin.

Formation of Mg-polymer at various Mg^{2+} concentrations

G-actin was preincubated at 0° for 10 min in various concentrations of $MgCl_2$. Some Mg^{2+} was bound to G-actin but, at such a low temperature, polymerization did not take place. Polymerization was induced by raising the temperature and adding KCl to 70 mM. As shown in Fig. 3, with increasing concentration of $MgCl_2$ during incubation, the viscosity of polymers formed decreased rapidly; and at a very low concentration of $MgCl_2$ (of the order of 0.1 mM) it reached almost the same value as that of Mg-polymer formed in 2 mM $MgCl_2$. Pure Mg-polymer is formed at the low concentration of Mg^{2+}.

The ATPase activity of the same sample solutions was measured under a constant salt condition, namely in 70 mM KCl and 1.5 mM $MgCl_2$. As remarked above, the viscosity of the solution was not changed during the measurement. The ATPase activity increased with the decrease of viscosity, as shown in the same figure. Thus, the activity is closely correlated with the formation of Mg-polymer.

Inhibition of the ATPase activity of Mg-polymer by ATP

Fig. 4 shows the rate of the ATP splitting by Mg-polymer at various concentrations of ATP. In the presence of 70 mM KCl, this ATPase activity was markedly

inhibited by high concentrations of ATP, whereas in the absence of KCl such inhibition by the substrate was not observed.

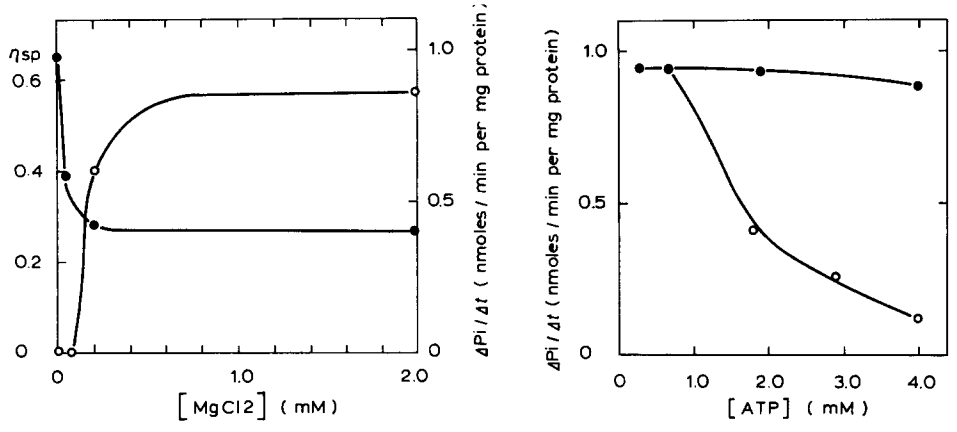

Fig. 3. Formation of Mg-polymer in the presence of various concentrations of $MgCl_2$. G-actin was preincubated with various concentrations of $MgCl_2$ indicated in the abscissa at 0° for 10 min and then polymerized by the addition of 70 mM KCl at 22°. Viscosity (●–●) and ATPase activity (○–○) were measured under the following conditions: protein concentration 1.1 mg/ml, 70 mM KCl, 1.5 mM $MgCl_2$, 1.3 mM ATP and 7 mM Tris–maleate buffer (pH 7.0), at 22°.

Fig. 4. Dependence of the ATPase activity of Mg-polymer on the ATP concentration in the presence or the absence of 70 mM KCl. G-actin was preincubated with 1.5 mM $MgCl_2$ and 50 μM ATP at 0° for 10 min and then polymerized to Mg-polymer at 22° on the addition of 70 mM KCl (○–○) or without KCl (●–●). ATPase activity was measured under the following conditions: protein concentration 1.45 mg/ml, 1.5 mM $MgCl_2$ and 7 mM Tris–maleate buffer (pH 7.0), at 22°.

Exchangeability of nucleotides bound to Mg-polymer

During polymerization of plasmodium actin to Mg-polymer, ATP bound to G-actin is split. Inorganic phosphate is liberated from actin, while ADP is tightly bound to the Mg-polymer. In connexion with the ATPase activity of Mg-polymer, the exchangeability of ADP bound to Mg-polymer with ATP in the solvent was investigated by the use of radioactive ADP.

G-actin labeled by [^{14}C]ATP was obtained by incubation in 40 μM [^{14}C]ATP. The labeled G-actin was polymerized to F-actin by the addition of KCl or to Mg-polymer by the addition of both $MgCl_2$ and KCl. Free nucleotides in the solutions of G-actin or polymers were removed by mixing one-seventh volume of Dowex 1 (Cl– type) (200–400 mesh) resin at 0° for 3 min. The resin was separated by centrifugation. The solutions of G-actin, F-actin and Mg-polymer were incubated in 0.2 mM unlabeled ATP, respectively. At intervals they were treated with the resin as above; the radioactivity of the solutions was then measured by a gas -flow counter.

As shown in Fig. 5, [^{14}C]ATP bound to G-actin was immediately released, showing that ATP bound to G-actin can be easily exchanged with free ATP in the solution. On the other hand, [^{14}C]ADP bound to F-actin was not released for at least 30 min at 20° even when unlabeled ATP was added to the solvent. ADP in F-actin is not exchangeable with free ATP. Bound [^{14}C]ADP was released from Mg-polymer exponentially. The time required for the release of half the nucleotides was about 10 min. In parallel with the release of radioactive nucleotides, Mg-polymer

split ATP. Inorganic phosphates equimolar to actin were liberated in about 15 min at the same solvent condition. The two events, the exchange of nucleotides and the splitting of ATP, are closely correlated.

The ATPase activity of copolymers of plasmodium and muscle actins

It was already reported that plasmodium and muscle actins can copolymerize on the addition of salts[3]. Here, the ATPase activity of copolymers formed at various ratios of the two actins was investigated.

Fig. 6 shows the viscosity and the ATPase activity of copolymers formed by the addition of 2 mM $MgCl_2$ and 0.1 M KCl. Pure muscle F-actin showed high viscosity but no ATPase activity. Pure plasmodium Mg-polymer showed low viscosity but high ATPase activity. With increasing content of muscle actin in the copolymer, the ATPase activity rapidly decreased, while the viscosity showed no appreciable change until the content of muscle actin exceeded about 60%. When a small amount of plasmodium actin was incorporated into the polymer of muscle actin, the viscosity decreased very much, the ATPase activity remaining very low until the content of plasmodium actin exceeded about 30 %.

For the comparison with copolymers, the viscosity and the ATPase activity of

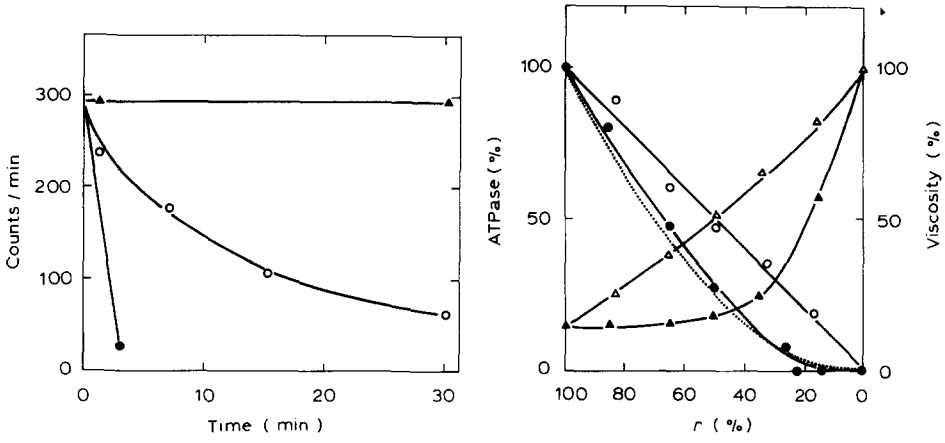

Fig. 5. Exchange of bound nucleotides of Mg-polymer and F-actin with free nucleotides in the solution. To obtain polymers labeled by [^{14}C]ADP, G-actin was preincubated with [^{14}C]ATP, and then polymerized to F-actin by the addition of 70 mM KCl, or to Mg-polymer by the addition of 2 mM $MgCl_2$ at 20°. After incubation with 0.2 mM unlabeled ATP for a certain period indicated in the abscissa at 20°, amounts of bound nucleotides of G-actin (●–●), F-actin (▲–▲) or Mg-polymer (○–○) were determined. Protein concentration 1 mg/ml, and 5 mM Tris–maleate buffer (pH 7.0).

Fig. 6. ATPase activity and viscosity of copolymers of plasmodium and muscle actins at various ratios. ATPase activity (●—●) and viscosity (▲—▲) of copolymers, and ATPase activity (○—○) and viscosity (△—△) of simple mixtures of plasmodium Mg-polymer and muscle F-actin were measured under the following conditions: 2 mM $MgCl_2$, 0.1 M KCl, 0.15 mM ATP and 8 mM Tris–maleate buffer (pH 7.0), at 22°. Protein concentration, 1.2 mg/ml; where the abscissa, r = (plasmodium actin)/(plasmodium and muscle actins) × 100 (the wt. %). Copolymers were formed from mixtures of plasmodium and muscle G-actins on the addition of 2 mM $MgCl_2$ and 0.1 M KCl. For simple mixtures, two kinds of G-actin were polymerized independently on the addition of 2 mM $MgCl_2$ and 0.1 M KCl and then mixed. The dotted line was calculated assuming that the ATPase activity of copolymer is proportional to the square of the fraction r of plasmodium actin to the total actin.

simple mixtures of two polymers, muscle F-actin and plasmodium Mg-polymer, were measured at various ratios. These properties changed linearly with the change of the fraction of the two polymers.

DISCUSSION

Electronmicrographs in the previous paper showed that plasmodium F-actin had a two-stranded helical structure similar to muscle F-actin; on the other hand, Mg-polymer was seen as amorphous or globular particles, whose diameters were 100–600 Å. These observations were made on specimens stained by phosphotungstate of pH 7.0 (ref. 3). However, electronmicrographs taken shortly after negatively staining with 1 % uranyl acetate (pH 4.3) showed that Mg-polymer is a fibrous and flexible polymer, of which the thickness is about 80Å; whereas F-actin is a two-stranded rigid polymer as observed previously. Taking into consideration this latest information, the possible mechanism of the ATPase activity of Mg-polymer is discussed.

Among various states of plasmodium actin, only Mg-polymer exhibits steady ATPase activity. Plasmodium G-actin does not show ATPase activity. During polymerization of G-actin to F-actin or to Mg-polymer, ATP bound to G-actin is split. Therefore, it is reasonable to consider that the splitting of ATP is made possible by interaction between actin molecules.

F-actin solutions from muscle actin show high ATPase activity under sonic vibration or at high temperature. Two mechanisms have been proposed to explain this ATPase. In one, the splitting of ATP was assumed to be associated with binding of G-actin having ATP at the end of the actin polymer. The high ATPase activity is due to the cyclic reaction of binding and detachment of actin molecules at the ends of polymers, the rate of which is increased by an increase in the number of polymers by, for example, sonic vibration[15]. The other mechanism is a cycle of some intrapolymer conformational change of actin polymers, for example, the cycle of interruption and repair of bonds between actin molecules without fragmentation of polymers. Sonic vibration[16–18] or high temperature[19] was assumed to loosen the bond.

For the ATPase of Mg-polymer the same two mechanisms can also be assumed. To distinguish which mechanism is actually working, the G–F cycle at the end of polymers or the intrapolymer cycle everywhere in the polymers, we measured the exchange of ADP bound to actin polymers with ATP in the solvent. For the steady splitting of ATP, the latter must be incorporated into actin from the solvent. With muscle actin during the splitting of ATP, bound ADP was found to be replaced by ATP in the solvent. The ATP splitting and the exchange of ADP are associated with the same cyclic process. If the release of radioactive ADP bound to the polymer is followed in a unlabeled ATP solution, the above two mechanisms are expected to give two different relations between the release of radioactive ADP and the splitting of ATP.

In the presence of a sufficient amount of ATP in the solvent, the total amount of ATP split per actin monomer increases with t and is written as αt. If the exchange and the splitting are due to a G–F cycle at the ends of polymers, radioactive ADP's are released only in the peripheral region of the polymers. Those in the middle part

are not released in a short period. Simple calculation by KASAI AND OOSAWA[20] showed that the probability of the release is given approximately by

$$1-\exp\left(-(b\alpha t)^{1/2}\right) \tag{1}$$

where b is the reciprocal of the average degree of polymerization. On the other hand, if the exchange and the splitting are due to an intrapolymer cycle taking place everywhere in the polymer at random, the probability of the release of radioactive ADP is given by:

$$1-\exp\left(-\alpha t\right) \tag{2}$$

When ATP equimolar to actin was split, that is, at time $t = 1/\alpha$, the probability of release is about 0.13 according to (1) if $b = 1/100$, and it attains 0.63 according to (2). The experimental result in Fig. 5, where the fraction of release was about 0.7 at the time of splitting of equimolar ATP, supports the idea of the intrapolymer cycle.

On the basis of the above analysis and the recent electronmicroscopic observations, the following speculation can be made on the mechanism of the ATP splitting by Mg-polymer. Mg-polymer is assumed to have a basic structure similar to that of F-actin, in which, however, there are many interrupted points, *i.e.* loosened or broken bonds as in F-actin postulated by ASAKURA *et al.*[18] to explain its ATPase activity under and after sonic vibration. Bound ADP's buried in the two-stranded polymer structure of F-actin are unmasked at interrupted points and become exchangeable with free ATP in the solvent. The bound ATP's are readily split into ADP and inorganic phosphate during the recovery of the bond. Thus, the repetition of the partial interruption and repair of the bonds in many places on the polymer results in the steady ATP splitting and ADP exchange. The sequence of events is expressed as

$$\underset{\text{(masked)}}{\text{Mg-polymer–ADP}} \leftrightharpoons \underset{\text{(unmasked)}}{\text{Mg-polymer–ADP}} \tag{a}$$

$$\underset{\text{(unmasked)}}{\text{Mg-polymer–ADP}} + \text{ATP} \leftrightharpoons \underset{\text{(unmasked)}}{\text{Mg-polymer–ATP}} + \text{ADP} \tag{b}$$

$$\underset{\text{(unmasked)}}{\text{Mg-polymer–ATP}} \longrightarrow \underset{\text{(masked)}}{\text{Mg-polymer–ADP}} + P_i \tag{c}$$

The special character of Mg-polymer as the ATPase is due to the fact that Reaction (a) is not always shifted to the left .In Reaction (c) the interaction between actin molecules in the polymer is indispensable to the ATP splitting.

In relation to the above scheme, it must be remarked that in Fig. 3, polymers formed at very low concentrations of Mg^{2+}, *e.g.* 0.05 mM, show no appreciable ATPase activity in spite of the decrease of viscosity. In these polymers actin molecules with and without bound Mg^{2+} are contained. If the interaction between actin molecules both having Mg^{2+} is needed for the interruption–repair cycle accompanied by the ATP splitting, the chance of such interaction is very small at a small fraction of actin molecules having Mg^{2+} in the polymer. A very small number of interruption points can produce a large decrease of viscosity but cannot produce an appreciable amount of ATPase.

A similar situation is found for copolymers of plasmodium and muscle actins. The ATPase activity of copolymers is very much smaller than that of the simple mixtures of two polymers, plasmodium Mg-polymer and muscle F-actin. As shown in Fig. 6, the activity is approximately proportional to the square of the fraction of plasmodium actin in the copolymer. This is understandable if the ATP splitting is made possible by the interaction of two neighboring plasmodium actin molecules in the polymer.

ACKNOWLEDGEMENTS

We thank Professor F. Oosawa for many helpful discussions and suggestions and Dr S. Asakura for his interest. We are indebted to Dr M. Kasai for providing facilities for the isotope treatments.

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