Purification of Actin from Myxomycete Plasmodium and Induction of Antibody against Purified Actin

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ABSTRACT: Plasmodium actin was highly purified by gel filtration of the crude G-actin on Sephadex G-100 followed by ultracentrifugation of F-actin in the presence of 1 M urea and 1 mM ATP. The reduced viscosity of the purified plasmodium F-actin was 10 dl/g which was the same order as that of muscle F-actin. It activated plasmodium and muscle myosin ATPase by the same extents as muscle F-actin did. The purified plasmodium G-actin did not form Mg-polymer even when Mg^{++} was present. Thus, the physicochemical properties of purified plasmodium actin identical with those of muscle actin.

Antibody against the purified actin was induced in rabbits. The elicited antibody was immunologically monospecific for plasmodium actin, judging from the following results. (1) The addition of the antibody to a plasmodium F-actin solution increased the turbidity of the mixed solution, showing the formation of the antibody-actin complex. (2) In immunodiffusion and immunoelectrophoresis, the antibody formed single precipitin lines with the purified actin preparation and with the crude actin extract from the acetone-

dried powder of plasmodium. (3) The antibody inhibited polymerization of plasmodium G-actin. (4) Plasmodium F-actin filaments were decorated with antibody in electron micrographs. The antibody reacted not only with plasmodium F- and G-actin, but also reacted with sea urchin egg actin, but it did not react with actin from rabbit striated muscle.

Introduction

About ten years ago Hatano and Oosawa (1966) isolated actin from plasmodium of Physarum using its specific binding to muscle myosin and it was purified by salting out with ammonium sulfate. Hatano et al. (1967, 1972) reported that plasmodium G-actin polymerized to F-actin on the addition of 0.1 M KCl. However, it polymerized to another state of polymer (Mg-polymer) in the presence of Mg^{++}. The viscosity of Mg-polymer was one third or one fourth of that of F-actin and it showed the specific ATPase activity of the order of $1x10^{-3}$ μmole/mg actin /min. However, the sodium dodecyl sulfate (SDS) gel electrophoresis of plasmodium actin showed that some impurities were present in the actin preparation. Therefore, we further purified plasmodium actin by column chromatography on Sephadex G-100 followed by ultracentrifugation of F-actin in the presence of 1 M urea and 1 mM ATP and succeeded to obtain pure actin. Firstly we shall report in this paper that purified plasmodium F-actin shows the identical physicochemical properties with those of F-actin obtained from rabbit striated muscle. The

purified plasmodium G-actin did not polymerize to Mg-polymer, but polymerized to F-actin even when Mg^{++} was present. A factor involved in the formation of Mg-polymer was isolated from the initial actin preparation and fresh plasmodia and termed "plasmodium actinin" (Hatano and Owaribe, 1976). In the presence of plasmodium actinin the purified plasmodium G-actin polymerized to Mg-polymer on the addition of 2 mM $MgCl_2$.

The bundles of microfilaments, the diameter of which is around 60 Å, have been observed in living and glycerinated plasmodium of Physarum (Wohlfarth-Bottermann, 1962; Rhea, 1966; Nagai and Kamiya, 1966). Using the method developed by Ishikawa et al. (1969), Alléra et al. (1971) ascertained that microfilaments in question were F-actin filaments. Namely, these filaments were decorated with heavy meromyosin (HMM) from muscle to form arrowhead like structures. However, if actin exists as monomer or globular polymer such as Mg-polymer in plasmodium, it will be impossible or very difficult to decorate them with HMM from muscle. For example, we isolated actin from protoplasmic sol of plasmodium (endoplasm) where actin filaments have not been demonstrated (Wohlfarth-Bottermann, 1962).

We have tried to produce antibody to actin from plasmodium in order to examin the precise localization of actin in plasmodium immunohistochemically. Using the highly purified plasmodium actin as the antigen, we succeeded to induce antibody to actin. This was the first case of the induction of antibody to the native actin. We secondly, shall report in this paper the induction of the antibody against the highly purified actin from plasmodium and describe some properties of the antibody.

Materials and Methods

Preparation and Purification of Plasmodium Actin.

Plasmodium of myxomycete, Physarum polycephalum, was cultured in 15-l. buckets by supplying oatmeal every day (Camp, 1936). The initial G-actin was obtained from plasmodia after acetone treatment by the method of Hatano and Oosawa (1966). This crude actin was further purified by gel filtration (Rees and Young, 1967; Adelman and Taylor, 1969) followed by ultracentrifugation. About 7 ml of the actin solution of concentration 4 mg/ml was applied to a column of Sephadex G-100 of 2 cm in diameter

and 95 cm in hight, and eluted with a 4 mM Tris-HCl (pH 8.2) solution containing 0.1 mM ATP. The eluted solution was collected every 4 ml in small test tubes and the absorbance at 280 nm of each solution was measured. The absorbance showed two peaks, which were termed FI and FII (Figure 1). Only FII had polymerizability. The FII (32 ml total) were collected and concentrated to about 8 ml (2 mg/ml) by Diaflo ultrafiltration (Amicon, Lexington, Mass.). G-Actin in the concentrated FII was polymerized to F-actin by the addition of 0.1 M KCl. F-Actin, of which the concentration was about 2 mg/ml, was incubated in 1 M urea in the presence of 0.1 M KCl, 2 mM $MgCl_2$, 1 mM ATP, and 10 mM Tris-maleate buffer (pH 7.0) for 4 hr, at 4°. Then it was centrifuged at 100,000g for 90 min at 5°. The precipitated F-actin was dissolved into a solvent containing 0.5 mM ATP and 3 mM cysteine (pH 8.2) and dialyzed against a solution containing 0.05 mM ATP and 3 mM cysteine (pH 8.2) for 2 to 3 days. Pure G-actin was finally obtained by ultracentrifugation of the solution at 100,000g for 30 min. About 4 mg of purified actin was obtained from 8 g of acetone-dried powder of plasmodium or 100 g of fresh plasmodia.

In the latter half of the experiment the initial actin fraction was prepared from plasmodium myosin B directly, without acetone treatment. Crude actin was separated from myosin by heating myosin B at 55° for 15 min in the presence of 0.1 M KCl and 5 mM ATP at pH 7.0. This method, which is very simple and useful for obtaining actin in high yield, will be reported in another paper.

Production of Antibody.

Adult female white rabbits were used in this experiment. One-half milliliter of the purified plasmodium F-actin solution which contained 1 mg of actin was emulsified with an equal volume of Freund's complete adjuvant and then injected intradermally, subcutaneously, intramuscularly, and intraperitoneally at multiple sites of rabbits. The injections were carried out four times once a week and repeated further four or five times at intervals of 2 to 3 weeks. As an adjuvant, bacterial endotoxin was also injected. Namely, 1 mg of alum-precipitated plasmodium F-actin was suspended in 1 ml of phosphate buffered saline (PBS) containing 20 μg of Salmonella endotoxin and the suspension was injected intravenously 3 times at an interval of 2 days during the first 10 days of the course of immunization. Ten days

after the last injection the rabbits were bled. Blood was allowed to clot at room temperature and was kept overnight at 4°. Antiserum was obtained by centrifugation of blood at 1,500g for 15 min and further clarified at 10,000g for 30 min.

Antibody in the serum was fractionated into the γ-globulin fraction by salting out of serum with half-saturated ammonium sulfate. The salting out was repeated twice. The precipitated γ-globulin was then suspended in PBS and dialyzed against the same solution for 3 days and stored at -20° at a protein concentration of 35 mg/ml. This γ-globulin fraction was used as an "antibody fraction" in the following experiments. For electron microscopic observation the antibody fraction was further purified by DEAE-cellulose column chromatography (Sober and Peterson, 1958) and gel filtration on Sephadex G-200.

Preparation of Muscle Myosin, Muscle Actin, and Sea Urchin Egg Actin.

Muscle myosin was prepared from rabbit striated muscle by the method described by Perry (1955) and muscle G-actin was prepared by almost the same method as that of Straub (1943) except that tropomyosin and troponin were carefully removed before the acetone treatment of

myosin-extracted minced muscle (Ebashi and Ebashi, 1964). Sea urchin egg actin was prepared by the method of Hatano et al.(1969) from eggs of Hemicentrotus pulcherrimus.

Determination of Protein Concentration.

Protein concentration was measured by the biuret method (Gornall et al., 1949) using absorbances of 0.068 at 540 nm for plasmodium actin at 1 mg/ml, 0.070 for muscle actin, and 0.066 for muscle myosin, respectively.

Viscosity.

Viscosity was measured by Ostwald type capillary viscometers, of which the flow times were around 30 sec for the buffer solution.

ATPase Activity.

ATPase activity was determined by measuring liberated inorganic phosphates by the method of Taussky and Shorr (1953).

Sodium Dodecyl Sulfate (SDS) Gel Electrophoresis.

SDS gel electrophoresis was carried out by the method of Weber and Osborn (1969) with a slight modification. The 7.5 % polyacrylamide gels containing 0.1 % SDS were used. Smples were dissolved in 1 % SDS in 0.05 M phosphate buffer at pH 7.2 which contained 1 % mercaptoethanol and then boiled for 3 min before the electrophoresis.

Immunodiffusion and Immunoelectrophoresis.

These were carried out by the methods described by Clausen (1969). Agar plates for immunodiffusion were prepared with 1.3 % agar, 0.02 % sodium azide, and 0.02 M Tris-HCl buffer of pH 8.2, and those for immunoelectrophoresis were prepared with 1.3 % agar, 0.02 % sodium azide, and 0.022 M Veronal buffer of pH 8.6.

Electron Microscopy.

A drop of the plasmodium F-actin solution of about 0.07 mg/ml was placed on a Formvar-coated grid stabilized with a carbon film. After 1 or 2 min the solution was sucked up with a small piece of filter paper and a drop of the antibody solution of 2 mg/ml was transferred on the grid to let antibody react with F-actin on the surface of the grid for about 3 min. The complex of F-actin and antibody was observed with a Hitachi electron microscope at a magnification of 21,000 to 30,000 after negatively staining with 1 % uranyl acetate (Huxley, 1963).

Result

Purification of Plasmodium Actin and Purity of Antigen.

When the initial actin fraction which was prepared by the ordinary method of Hatano and Oosawa (1966) was used as the antigen, the immunoelectrophoretic pattern of antigen and antiserum from immunized rabbits showed a few bands, suggesting that antibodies against proteins other than actin were also induced. Actually, SDS gel electrophoresis revealed that the initial actin preparation contained small amounts of a few impurities (Figure 2a). Therefore, further purification of the initial G-actin was carried out by gel filtration on Sephadex G-100. The elution pattern of the initial G-actin had two peaks, FI and FII, as described previously (Figure 1). Polymerization activity localized only in the second fraction, FII. However, FII still contained small amounts of the other protein components (Figure 2c). One component, the molecular weight of which is a little smaller than that of actin, remarkably increased in FII after chromatography. This is not due to the concentration of this component in FII, but seems to be

a result of autolysis of G-actin during chromatography, although the reason for this is not known now.

To remove these components various procedures have been tried. Ultracentrifugation of F-actin at a low salt concentration(0.03 M KCl, pH 7.0; Laki et al., 1962; Martonosi, 1962), or at a high salt concentration (0.6 M KCl, pH 7.0; Spudich and Watt, 1971) in the presence or absence of 10 mM EDTA was not very effective. Finally, it was found that ultracentrifugation of F-actin in the presence of 1 M urea at low temperature was most useful for removing the impurities. As shown in Figure 2d and e, pure actin was obtained in high yield. As Szent-Györgyi and Joseph (1951) reported in the case of muscle F-actin, plasmodium F-actin did not show any denaturation by such a treatment.

Physicochemical Properties of Purified Plasmodium Actin.

Table 1 shows the yield of actin from 100g of fresh plasmodia and the reduced viscosities of the F-actin solution which were purified by the various methods. The reduced viscosity of plasmodium F-actin solutions which were prepared by the method described in "Materials and Methods" were around 10 dl/g which was the same as that of purified muscle F-actin. Purified plasmodium F-actin activated ATPase of plasmodium myosin 8 to 9 fold and

and that of muscle myosin 10 fold (Table 2). It can be said that the hydrodynamic properties of plasmodium and muscle F-actins and the activation of myosin ATPase by both F-actins were the same, when both F-actins were purified.

In the process of purification of plasmodium actin, the reduced viscosity of F-actin increased (Table 3). The reduced viscosity of F-actin of the initial actin preparation was 2.6 dl/g in this case, while that of purified F-actin was 10.1 dl/g. Similarly the viscosity of Mg-polymer increased to the same level of F-actin when actin was purified. Namely, the purified G-actin did not form Mg-polymer, even when 2 mM $MgCl_2$ was added to it. These results showed the initial actin preparation contained a factor in the presence of which G-actin polymerized to Mg-polymer on the addition of 2 mM $MgCl_2$. We isolated this factor from the initial actin preparation and termed plasmodium actinin. The natures of plasmodium actinin were reported in another paper (Hatano and Owaribe, 1976).

Specificity of Antibody.

Titers.

Antisera from immunized rabbits showed the precipitin reaction in the interfacial ring test. Titers which were expressed by the reciprocal of dilution were between 2^5 and 2^6.

Immunodiffusion and Immunoelectrophoresis.

As illustrated in the immunodiffusion pattern of Figure 3a, anti-actin antiserum formed single precipitin lines with purified actin and with water extract from the acetone-dried powder of plasmodium, but did not precipitate with muscle actin. Figure 3b shows an immunoelectrophoretic pattern of purified plasmodium actin and that of water extract from the acetone-dried powder of plasmodium. Both samples formed single precipitin arc lines against anti-actin antiserum. These experiments show that antiserum contains antibody which reacts with plasmodium actin and does not contain any other antibodies which react with the other proteins from plasmodium. Namely, antibody obtained here was immunologically of a single component specific to plasmodium actin.

Turbidimetry.

One-fifth volume of the antibody fraction of concentration 35 mg/ml was added to F-actin solution of 1 mg/ml and the change of the turbidity of the mixed solution was measured at 500 nm at 36.8°. The turbidity increased gradually and reached a constant level after 90 min as shown in Figure 4. The turbidity of the mixed solution of plasmodium F-actin and a normal γ-globulin fraction which was prepared from a nonimmunized rabbit did not increased. Moreover, the turbidity of the mixed solution of muscle F-actin and the antibody fraction did not show any increase. However, when F-actin from sea urchin egg was mixed with the antibody fraction, the turbidity of the solution increased in a similar way as in the case of the mixture of plasmodium F-actin and the antibody fraction. Thus, the antibody to plasmodium actin does not interact with F-actin from rabbit striated muscle, but interacts with F-actin from sea urchin eggs.

Inhibition of Polymerization of G-Actin by Antibody.

Plasmodium G-actin of 1 mg/ml was preincubated with the antibody fraction of 7 mg/ml in 3 mM cysteine, 0.05

mM ATP, and 10 mM Tris-maleate of pH 7.0 at 0° for 5 min. Then it was polymerized by the addition of 0.1 M KCl at 21.9°. After about 45 min, the viscosity of the mixed solution was measured. The viscosity was only about 30 % of that of F-actin polymerized without antibody. Polymerization of plasmodium G-actin was not inhibited by the normal γ-globulin fraction from a nonimmunized rabbit. Thus, the antibody interacted with plasmodium G-actin to inhibit its polymerization. On the other hand, polymerization of muscle G-actin was not inhibited by the antibody fraction.

SDS Gel Electrophoresis of Antigen-Antibody Complex.

Protein components of the antigen-antibody precipitates were analyzed by SDS gel electrophoresis. After plasmodium F-actin and sea urchin egg F-actin were incubated with the antibody fraction in the weight ratio of 1 mg of F-actin to 7 mg of the antibody fraction at 36.8° for 60 min, the solution were kept at 4° for 2 days. The precipitates formed were collected by low-speed centrifugation and washed with 20 vol of PBS twice. Then SDS gel electrophoresis of the precipitates was carried

out. In the case of the precipitate of plasmodium F-actin the band of actin as well as the bands of γ-chain (heavy chain) and light chain if IgG were clearly seen in the electrophoretic pattern (Figure 5c). The band of actin was stronger than those of the subunits of the antibody, because only the definite ratio of the antibody fraction was added to the F-actin solution as mentioned above. It has been shown by turbidimetric examination that such a ratio of the antibody fraction was enough to precipitate F-actin to the maximum level in the solution. In the case of the precipitate of egg F-actin the band of actin as well as those of the μ-chain of IgM, the γ-chain of IgG (heavy chains), and the light chain were also observed as main bands (Figure 5e). It can be said that the precipitates formed by antigen-antibody reaction were actin and the anti-actin complex. When plasmodium G-actin was incubated with the antibody fraction in the same way as mentioned above, the electrophoretic pattern of the resultant precipitate was nearly the same as that of the precipitate of plasmodium F-actin and antibody fraction. These results also show that the antibody reacts not only with plasmodium F-actin, but also with

plasmodium G-actin.

Electron Microscopic Observation.

When the antibody fraction was added to F-actin on a grid for electron microscopy, the F-actin filaments were decorated with antibody, so that their surface became rough. Antibody molecules were observable on some parts of the electron micrograph (Figure 6b). The antibody strongly promoted the aggregation of actin filaments. These appearances are very similar to those of actin filaments treated with Acanthamoeba myosin (Pollard and Korn, 1973a,b) of which the molecular weight (about 180,000) and the shape are very similar to γ-globulin. On the contrary, the surface of plasmodium F-actin remained smooth when normal γ-globulin was added to F-actin. Similarly, no change of the appearance of F-actin filaments was observed when the antibody fraction was added to muscle F-actin.

Discussion

Actin has been isolated from various non-muscle cells (Hatano and Oosawa, 1966; Hatano et al., 1969;

Adelman and Taylor, 1969; Tatsumi et al., 1973; Weihing and Korn, 1971; Zucker-Franklin and Grusky, 1972; Yang and Perdue, 1972). It has been reported that the molecular weight (45,000) of plasmodium actin determined by SDS gel electrophoresis is the same as that of actin from rabbit striated muscle (Jockusch et al., 1971), and the amino acid composition of plasmodium actin appears to be very similar to that of muscle actin (Hatano and Oosawa, 1966). However, the reported physicochemical properties of F-actin obtained from non-muscle cells are not always identical with those of muscle F-actin. For example, the reduced viscosity of plasmodium F-actin which was prepared by the ordinary method of Hatano and Oosawa (1966) was 4-7 dl/g. The reduced viscosity of F-actin of star fish spermatozoa was less than 3 dl/g (Tilney et al., 1973). They are much lower than that of muscle F-actin (10 dl/g).

In this report we found that the hydrodynamic properties of plasmodium F-actin are the same as those of F-actin from rabbit striated muscle, when plasmodium actin was purified. Purified plasmodium F-actin acti-

vated plasmodium and muscle myosin ATPase by the same extents as muscle F-actin did. It can be said that the physicochemical properties of purified plasmodium F-actin are the same as those of rabbit muscle F-actin as far as the hydrodynamic properties and activation of myosin ATPase are concerned.

It is considered that plasmodium actin has a weak antigenicity to rabbit because of the similarity of its physicochemical natures to those of rabbit muscle actin. It is known in the case of such a weak immunogen that an appropriate dossage of the antigen is necessary to elicite antibody, because higher doses of the antigen produce high zone tolerance and lower doses of the antigen also produce low zone tolerance. We chose 1 mg of plasmodium actin once a week as the appropriate dose of the single administration. At the same time it is well known that to use adjuvants is effective to elicite antibody having a higher titer. F-Actin is thought to have many identical antigenic determinants considering its structure and F-actin is relatively resistant to hydrolytic enzymes such as trypsin. In these case endo-

toxin is effective as an adjuvant to stimulate bone marrow-derived cells. Salmonella endotoxin was used here in combination with Freund's complete adjuvant which stimulates thymus-derived cells.

A few reports have recently appeared on the induction of antibodies to actins isolated from skeletal muscle of several species of animals (Pepe, 1968; Wilson and Finck, 1971; Hirabayashi and Hayashi,1972). In a special case, an auto-antibody to actin has been found in the serum of a patient with chronic aggressive hepatitis (Gabbiani et al., 1973; Trenchev et al., 1974). These antibody preparations, however, contained more or less antibodies which interacted with proteins other than actin. At the beginning of this study, actin prepared by the ordinary method of Hatano and Oosawa (1966) was used as the antigen. Then the immunoelectrophoretic pattern showed a few bands suggesting that antibodies against proteins other than actin in the initial preparation were also induced as mentioned above.

Lazarides and Weber (1974) purified actin from mouse fibroblasts by gel electrophoresis in the presence

of SDS. Then they obtained antibody by using this actin as the antigen. We have tried to induce the antibody against plasmodium actin by a similar method. After the SDS gel electrophoresis of plasmodium actin, actin was extracted from a band containing actin with an 8 M urea solution (pH 8.2). After dialysis against PBS to remove urea and SDS, it was used as the antigen. The elicited antibody clearly showed passive hemagglutination, when it was mixed with rabbit red blood cells which had been coated with plasmodium actin. But this antibody did not precipitate with plasmodium actin showing that titer of the antibody was very low.

In this study the further purification of plasmodium actin was essential to successfully obtain pure antibody specific to this actin. That is, the antibody fraction (γ-globulin fraction) from rabbits which were immunized with this purified actin reacted with pure plasmodium actin to form the precipitate of actin and antibody. Therefore, this antibody will be useful for the immunological histochemistry of actin in plasmodium. Considering the fact that the antibody interacts with sea urchin egg actin, it will also be useful in various nonmuscle cells or tissues.

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Table 1. Viscosity of Plasmodium and Muscle F-Actin

	plasmodium F-actin						muscle F-actin
method of purification	1	2	3	4			see text
yield (mg) from 100g plasmodia	0.4	1.1	2.6	4.0			-
ηsp/C (dl/g)							
0.1 M KCl added	8.6	9.2	10.8	10.1	10.0	10.9	9.8
2 mM $MgCl_2$ added	7.6	8.7	10.0	9.5	7.3	10.4	10.0

Plasmodium actin was prepared from the initial actin preparation (fraction of 15 to 35 % saturation) by 1) repeated chromatography (3 times) on Sephadex G-100 column. 2) the same chromatography as 1) (one time) followed by ultracentrifugation of F-actin in 0.03 M KCl solution. 3) ultracentrifugation of F-actin in 0.6 M KCl solution. and 4) the standard method described in Materials and Methods. The viscosities of all F-actin solutions were measured by the same viscometer under the same conditions (10 mM Tris-maleate buffer; pH 7.0, protein concentration 1 mg/ml, 21.9°).

Table 2. Activation of Plasmodium and Muscle Myosin ATPase by Plasmodium and Muscle F-Actin.

	plasmodium myosin			muscle myosin		
	myosin only	plasmodium F-actin added	muscle F-actin added	myosin only	plasmodium F-actin added	muscle F-actin added
	(μmole ATP/mg of myosin/min)					
no Me^{++} added	0.020	-	-	0.010	-	-
$MgCl_2$ added	0.008	0.065	0.078	0.008	0.083	0.082
$CaCl_2$ added	0.13	-	-	0.50	-	-

The reaction solution contained 0.018 M KCl, 0.74 mM ATP and 9 mM Tris-maleate buffer (pH 7.0). Protein; plasmodium myosin 0.29 mg, plasmodium F-actin 0.29 mg, muscle myosin 0.30 mg, muscle F-actin 0.29 mg. $MgCl_2$ added 2 mM, $CaCl_2$ added 1.8 mM. 21.9°.

Table 3. Reduced Viscosity of F-Actin and Mg-Polymer of Each Step of Actin Fraction in Purification Procedure.

	extract from dry actomyosin	initial actin	FII obtained by chromatography	purified actin
		(ηsp/C (dl/g))		
0.1 M KCl added	0.0	2.6	8.7	10.1
2 mM $MgCl_2$ added	0.0	1.1	4.7	9.5
ηsp-Mg/ηsp-K (%)	-	33	54	94
yield from 100 g plasmodia (mg)	20	7	5	4

The viscosity was measured in a solution containing 10 mM Tris-maleate buffer (pH 7.0) at 21.9°.

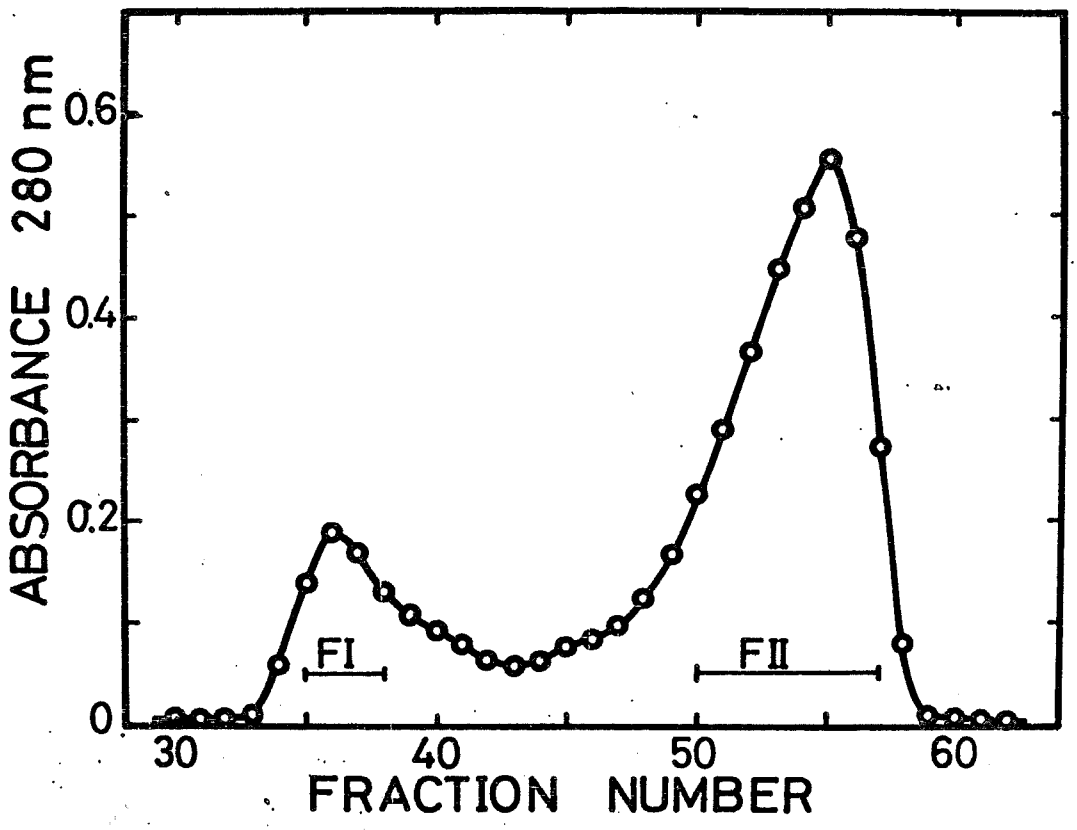

FIGURE 1: Gel filtration of initial plasmodium actin on Sephadex G-100. Actin (28 mg) was applied to a 2 x 90 cm column and was eluted with 4 mM Tris-HCl buffer (pH 8.2) containing 0.1 mM ATP. Fractions of 4 ml were collected at a flow rate of 12 ml/h. FI and FII were pooled for the further purification.

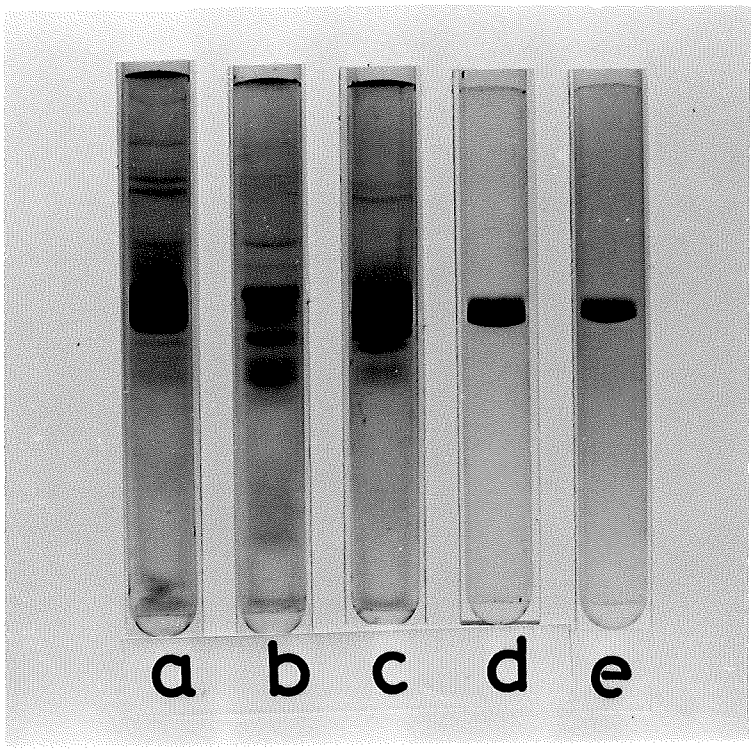

FIGURE 2: Purification of plasmodium actin as monitored by SDS gel electrophoresis: (a) initial plasmodium actin; (b) FI; (c) FII; (d) purified plasmodium actin (40 μg); (e) the same as d (20 μg).

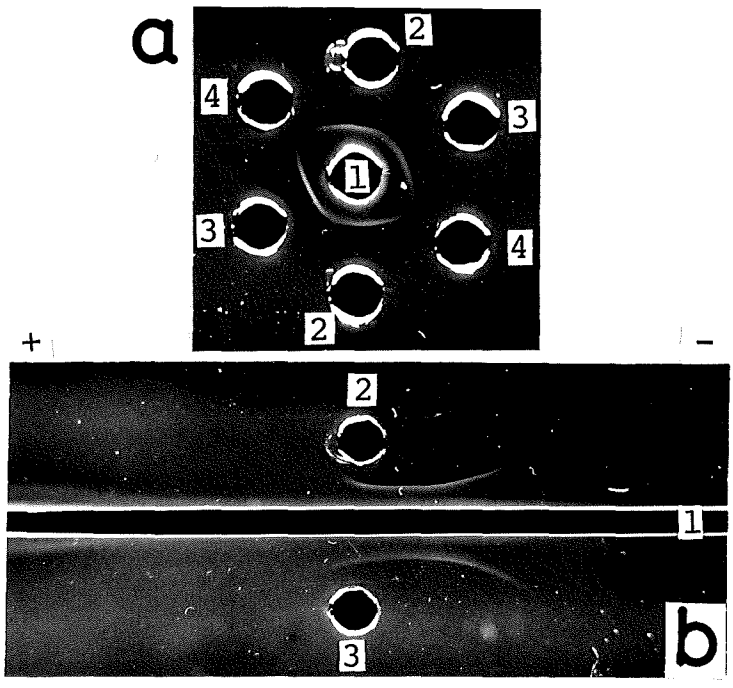

FIGURE 3: Comparison of precipitation patterns produced in immunodiffusion (a) and immunoelectrophoresis (b): (1) antibody fraction to plasmodium actin; (2) purified plasmodium actin; (3) water extract from acetone-dried powder of plasmodium; (4) muscle actin. Each well contained approximately the same amount of actin. Single precipitin line appeared in every case except in the case of muscle actin.

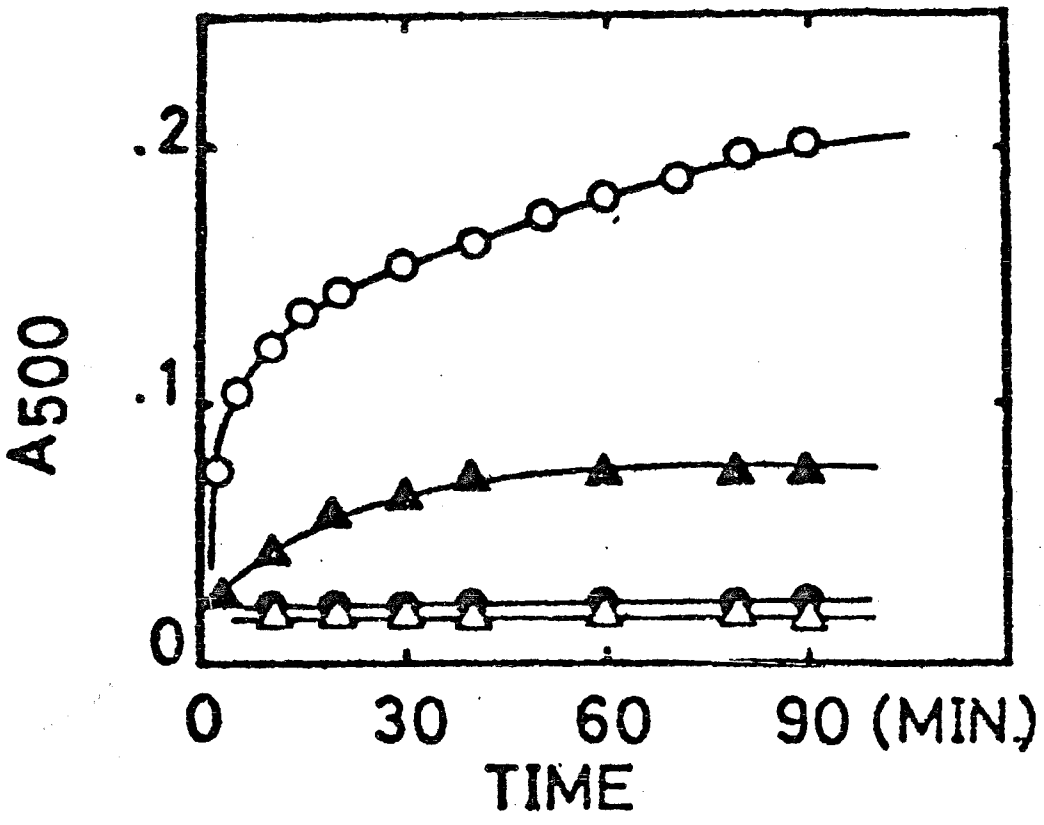

FIGURE 4: Turbidity change of mixture of actin and antibody fraction. Antibody was added to each actin solution at 0 min and tubidity of each solution at 500 nm was measured at 36.8°. (o) plasmodium actin and antibody; (▲) sea urchin egg actin and antibody; (●) plasmodium actin and normal γ-globulin fraction; (△) muscle actin and antibody.

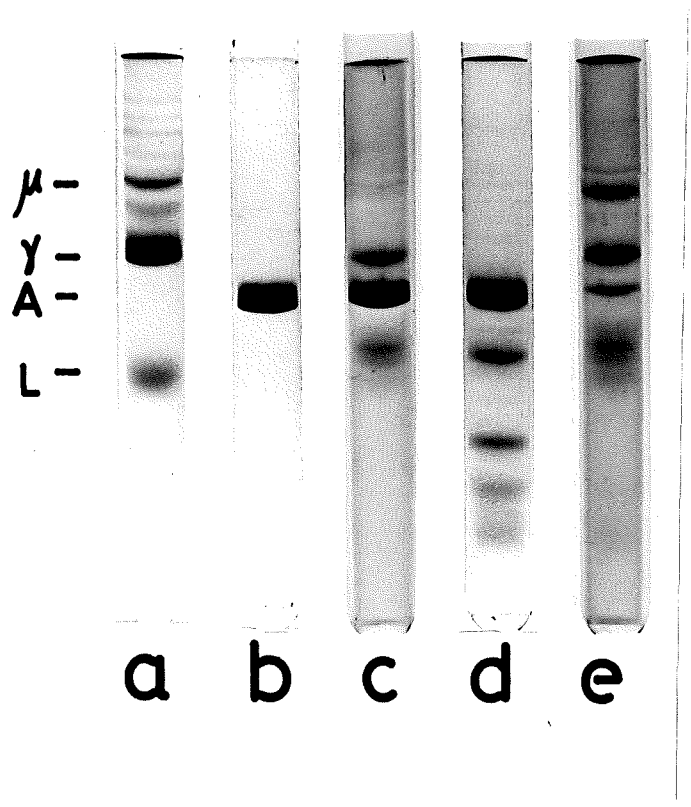

FIGURE 5: SDS gel electrophoresis of actin-antibody complex: (a) antibody fraction; (b) purified plasmodium actin; (c) precipitate of plasmodium actin with antibody; (d) sea urchin egg actin; (e) precipitate of egg actin with antibody; (γ) γ-chain; (μ) μ-chain; (A) actin; (L) light chain.

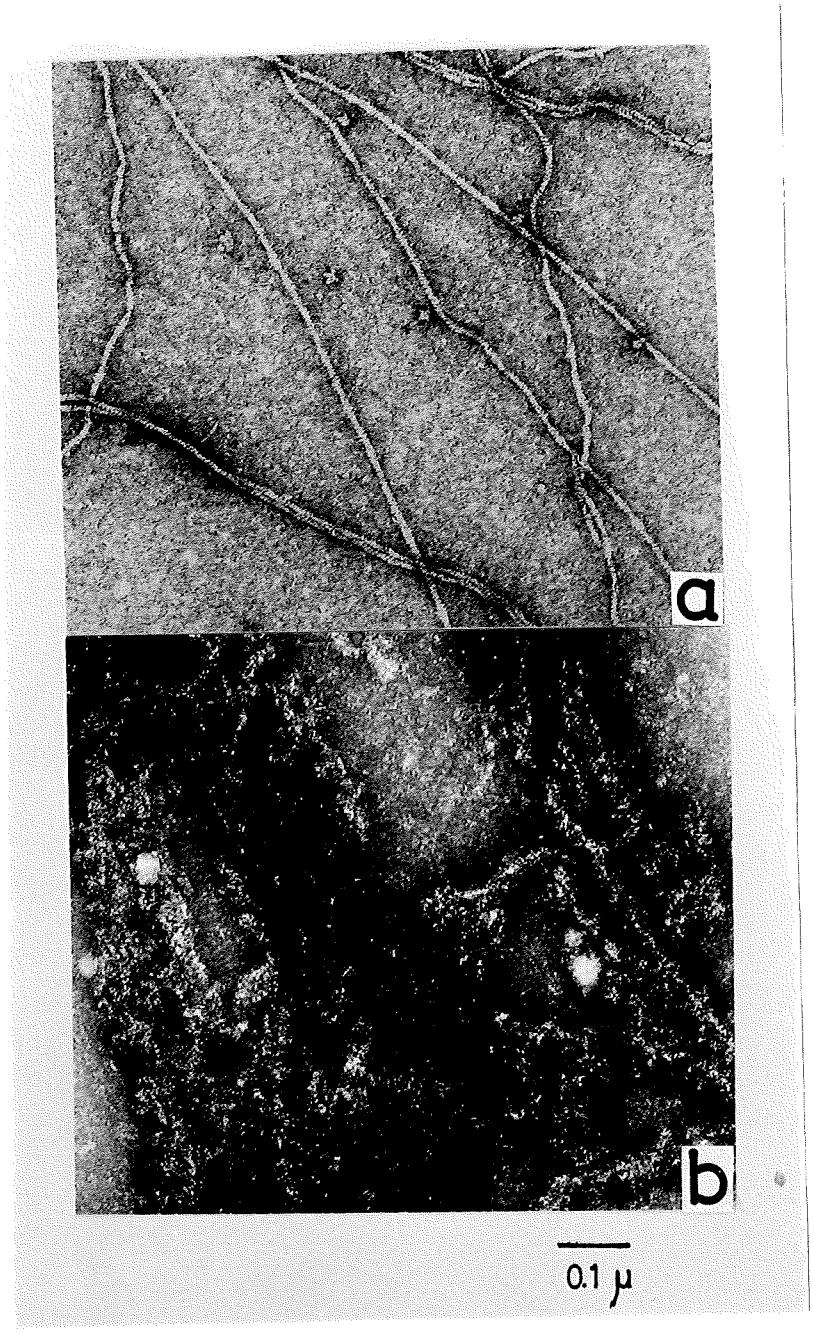

FIGURE 6: Electron micrographs of plasmodium F-actin treated with antibody: (a) plasmodium F-actin; (b) plasmodium F-actin treated with antibody.