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PURIFICATION AND CHARACTERIZATION OF MYOSIN A FROM THE MYXOMYCETE PLASMODIUM

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SUMMARY

1. Methods have been developed for the isolation of myosin A-like protein (plasmodium myosin A) from a myxomycete plasmodium.

2. Plasmodium myosin A is soluble in 0.5 M KCl solution at pH 7.0. Its molecular characteristics are examined and compared with those of myosin A from rabbit striated muscle. The sedimentation coefficient ($s_{20,w}$) is 6.05 S which is the same order as that of muscle myosin A. However, the viscosity ($[\eta] = 1.60$ dl/g) is only about two-thirds of that of muscle myosin A.

3. Generally speaking, amino acid composition of plasmodium myosin A is very similar to that of muscle myosin A, but cysteine is not detected in plasmodium myosin A as far as we have examined.

4. ATPase activity of plasmodium myosin A is as high as that of muscle myosin A, and it is activated by Ca^{2+} and inhibited by Mg^{2+}. Plasmodium actin as well as muscle actin activates ATPase of plasmodium myosin A. ATPase activity of plasmodium myosin A is always inhibited by EDTA and *p*-chloromercuribenzoate, even at low concentrations, whereas ATPase of muscle myosin A is greatly activated at such low concentrations of EDTA and *p*-chloromercuribenzoate.

5. Plasmodium myosin A is completely soluble and forms only small aggregates with sedimentation coefficients which are about 9 S at low salt concentrations, including the physiological salt concentration of plasmodium (0.03 M KCl) at pH 7.0.

INTRODUCTION

HATANO AND TAZAWA[1] have isolated and purified myosin B (plasmodium myosin B) from the myxomycete plasmodium. From this myosin B they have also isolated myosin A-like protein (plasmodium myosin A) by ultracentrifugation of a 0.5 M KCl solution of myosin B in the presence of ATP and Mg^{2+}. Recently, ADELMAN *et al.*[2] reported that a similar myosin A-like protein has been isolated from the same myxomycete by column chromatography. On the other hand, HATANO AND OOSAWA[3,4] and HATANO *et al.*[5] have already isolated and purified an actin-like protein (plasmodium actin) from the same myxomycete plasmodium.

Plasmodium F-actin polymerized from purified G-actin could combine with

Abbreviation: PCMB, *p*-chloromercuribenzoate.

plasmodium myosin A to form actomyosin *in vitro*, which shows viscosity drop in 0.5 M KCl solution on the addition of ATP. Moreover, plasmodium F-actin and muscle myosin A (or *vice versa*) could combine with each other to form actomyosin. Thus plasmodium myosin B is not a single protein but a compound protein of actin and myosin A.

In this paper the method of purification of plasmodium myosin A is further developed and established, and some of its physicochemical properties are reported.

METHODS

Plasmodia

Plasmodia of the myxomycete, *Physarum polycephalum*, were cultured by the method described in the previous paper[4]. About 60–100 g of plasmodia were used at once for preparation of myosin B.

Preparation of myosin B

Myosin B was prepared by the method described in the previous paper[1], with a slight modification (see APPENDIX). From 100 g of plasmodia about 60–100 mg of myosin B were obtained and then were stored in an ice box.

Protein concentration

Protein concentration was measured by the biuret method with the stable modified reagent of GORNALL *et al.*[6] which was calibrated by determining the nitrogen content by the micro-Kjeldahl method. 0.066 was used as the extinction coefficient (per mg/ml) at 540 nm.

Inorganic phosphate

Inorganic phosphate was determined by the method of MARTIN AND DOTY[7].

Amino acid composition

The protein was hydrolyzed in 6 M HCl in sealed vials under low pressure for 22 h at 110°. The mixture of amino acids was freed of HCl and water by NaOH and P_2O_5 under low pressure in a desiccator. Amino acid composition of the hydrolyzed protein fraction was determined using a Spinco Model 120 automatic amino acid analyzer. Tryptophan was determined from the spectrum in 0.1 M NaOH (ref. 8).

Ultracentrifuge

Sedimentation coefficients were measured using a Spinco Model E analytical ultracentrifuge.

Viscosity

The viscosity was measured in a thermo-regulated water bath at 10.6° using Ostwald-type capillary viscometers of which volumes were 0.6 ml and flow times for the buffer solution were approx. 50 sec.

Muscle myosin A

Muscle myosin A was prepared from rabbit striated muscle by the method described by PERRY[9].

For salting out, recrystallized ammonium sulfate was used. Ice-cold deionized water was used throughout the experiment. All procedures were carried out at 4° in a cold room.

RESULTS

Purification of myosin A

Ultracentrifugation of myosin B. Myosin A was prepared from myosin B within at least 10 days after preparation of myosin B. A 0.5 M KCl solution of myosin B (about 100 mg, 5 mg/ml) was centrifuged at 100000 × *g* for 3 h in the presence of 4 mM ATP and 2 mM $MgCl_2$. In this case, we used capless tubes of which the volumes were approx. 8 ml. About 3 ml of the mixture of myosin B solution were put in each capless tube. The resultant supernatant was gathered in a test tube. This fraction of myosin A was termed crude myosin A preparation I. Sedimentation pattern of this preparation gave a single peak of about 6 S, but it was found to have a slight ATP sensitivity showing that it contained a small amount of F-actin still. As shown in the latter section this F-actin cannot be removed by ultracentrifugation because it consists of small fragments of F-actin, of which the sedimentation coefficient is only about 5 S.

Purification of myosin A by dialysis. To remove short fragments of F-actin from crude myosin A preparation I, it was dialyzed against 1 l of the 0.05 M KCl and 10 mM Tris–maleate buffer (pH 7.0) overnight. Even at such a low salt concentration, plasmodium myosin A is soluble and does not precipitate, while F-actin combined with myosin A to form precipitate of actomyosin which could easily be removed by centrifugation (10000 rev./min for 10 min). The amounts of precipitated protein were about 20–30% of total protein in the crude myosin A preparation I. These values seem to be too high considering the fact that F-actin was not observed in the sedimentation pattern of the crude myosin A fraction. This was due to coprecipitation of myosin A with mixed short F-actin as mentioned later. The precipitated protein was removed by centrifugation. This fraction (termed crude myosin A preparation II) showed no ATP response, showing that F-actin was removed completely. However, small components of 2–3 S existed in the sedimentation pattern of the crude myosin A preparation II.

Isoelectric precipitation of myosin A. To remove small components by isoelectric precipitation, myosin A was precipitated at pH 5.3 in the presence of 0.5 mM ATP. The pH was lowered very slowly by the addition of 0.1 M acetate buffer (pH 4.7) at 0°.

The resultant precipitates were gathered by centrifugation (5000 rev./min) and were dissolved again in about 5 ml of 0.5 M KCl and 30 mM Tris–maleate buffer (pH 7.0). After the solution was dialyzed against 0.5 M KCl and 10 mM Tris–maleate buffer (pH 7.0) solution overnight, it was ultracentrifuged at 100000 × *g* for 2 h; the purified myosin A solution was obtained. By these procedures about 5 ml of purified myosin A solution (1–2 mg/ml) were obtained. The sedimentation pattern of the purified myosin A solution is shown in Fig. 1, in which a single peak of about 6 S is seen.

A slightly heavier component than 6 S was sometimes seen in the sedimentation pattern of purified myosin A. It seems likely that it was a small aggregate of myosin A. From our experience, it was formed by the isoelectric precipitation at pH 5.3, specially

in summer. The same small aggregate has been reported to form in the case of cardiac myosin A (ref. 10).

The yield of myosin A is about 5 % of myosin B. Then, about 5 mg of purified myosin A were obtained from 100 mg myosin B or 100 g of fresh plasmodia.

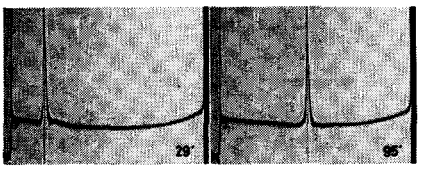

Fig. 1. Sedimentation pattern of plasmodium myosin A in 0.5 M KCl solution (pH 7.0). Ultracentrifugation was carried out at 59780 rev./min at 12.3°. Protein concentration, 3.1 mg/ml. Solvent conditions: 0.5 M KCl, 10 mM Tris–maleate buffer (pH 7.0); $s_{20,w}$ = 4.79 S. The time in min after reaching the maximum speed is shown in each photograph.

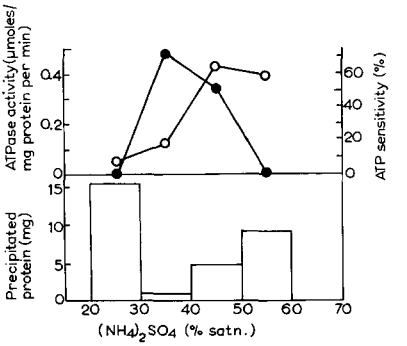

Fig. 2. Salting out of myosin B with ammonium sulfate in the presence of ATP and Mg^{2+}. ATPase activity was measured under the following conditions: 0.45 M KCl, 10 mM Tris–maleate buffer (pH 7.0), 1 mM $CaCl_2$, and 1 mM ATP at 21.9°. ATP sensitivity was expressed as the following formula[11]: ATP sensitivity (%) = $100\,(Z_\eta - Z_{\eta,\mathrm{ATP}})/Z_{\eta,\mathrm{ATP}}$; $Z_\eta = \ln \eta_{\mathrm{rel.}}/c$; $Z_{\eta,\mathrm{ATP}} = \ln \eta_{\mathrm{rel.,ATP}}/c_{\mathrm{ATP}}$; c and c_{ATP} are the respective concentrations of protein (g/l) before and after addition of ATP, $\eta_{\mathrm{rel.}}$ and $\eta_{\mathrm{rel.,ATP}}$ are the relative viscosities of protein solutions before and after the addition of ATP. Lower: amount of protein precipitated; upper: O—O, ATPase activity; ●—●, ATP sensitivity.

Salting out of myosin B with ammonium sulfate

Myosin B (1–2 mg/ml) was fractionated by salting out with ammonium sulfate in the presence of 2 mM ATP and 1 mM $MgCl_2$ (Fig. 2). Precipitated proteins in each fraction were gathered by centrifugation and were dissolved in a 0.5 M KCl solution containing 10 mM Tris–maleate buffer (pH 7.0). The solutions were dialyzed overnight in a cold room against 0.5 M KCl and 10 mM Tris–maleate buffer (pH 7.0) to remove ammonium sulfate. Then, ATP sensitivity[11] and ATPase activity of each protein solution were determined.

Since plasmodium actin has been known to be precipitating from 20 to 35 % saturation of ammonium sulfate[3], the protein fraction precipitated at from 20 to 35 % saturation was considered to be mostly actin (natural F-actin). On the other hand, the protein fraction precipitating from 35 to 60 % saturation showed ATPase activity, and its sedimentation pattern gave the main peak of about 6 S, showing that the proteins of this fraction were mostly myosin A. However, it was not pure myosin since it showed about 15 % of ATP sensitivity, whereas the sensitivity of the original myosin B was 100–130 % (ref. 1). Moreover, a very small peak of about 20 S of F-actin was seen in the sedimentation pattern of the solution. To obtain pure myosin A from this preparation of myosin A, the further purifications by ultracentrifugation, dialysis, and isoelectric precipitation are necessary.

Solubility of plasmodium myosin A

As shown in the previous paper[1] and in the earlier section, plasmodium myosin A is soluble and forms only small aggregates in the solution of low salt concentration, including the physiological salt solution of plasmodium (0.03 M KCl) at pH 7.0 (Fig. 3).

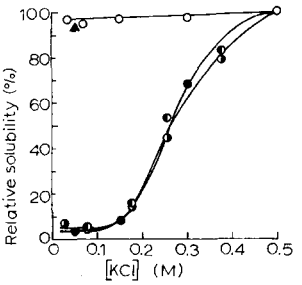

Fig. 3. Relative solubility of plasmodium myosin B, myosin A and muscle myosin A at varied KCl concentrations (pH 7.0). Plasmodium myosin B was centrifuged at 15000 rev./min for 15 min at varied KCl concentrations. Plasmodium myosin A and muscle myosin A were dialyzed overnight against solutions of varied KCl concentrations containing 10 mM Tris–maleate buffer (pH 7.0). Then they were centrifuged at 30000 rev./min for 30 min. Solubility was determined by measuring protein content of the supernatant, which was expressed as percent when the protein content of the supernatant of 0.5 M KCl solution of each myosin A or myosin B was 100%. ◐—◐ (a), plasmodium myosin B prepared after a 2-h extraction; ◑—◑ (b), plasmodium myosin B prepared immediately after homogenization; ○—○ (c), plasmodium myosin A prepared from (a); ▲—▲ (d), plasmodium myosin A prepared from (b); ●—● (e), muscle myosin A which was treated with the extract of plasmodium.

However, since muscle myosin A becomes soluble at low salt concentrations (*e.g.* 0.1 M KCl) when it was digested to H-meromyosin by trypsin[12], the following possibility arose in the case of plasmodium myosin A. Namely, plasmodium myosin A might be digested by some proteinases in the homogenate of plasmodium and made soluble at low salt concentrations like H-meromyosin of muscle myosin A. Plasmodium myosin B itself, from which myosin A is prepared, completely precipitates at low salt concentrations (Fig. 3), and it does show the typical superprecipitation[1], while acto-H-meromyosin is soluble at low salt concentrations and does not show superprecipitation. Accordingly, the high solubility of plasmodium myosin A is not considered to be the result of digestion by proteinases in the homogenate. We have carried out the following experiment to deny such a possibility.

Yield of myosin B as well as that of myosin A increased with the extraction time until 2 h and then remained constant at least for 5 h. We prepared myosin B as soon as possible after the homogenization of plasmodium. It took 20 min from homogenization of plasmodium until dilution of supernatant of the homogenate. Although yield of myosin B was a little smaller, solubility of the myosin B against varied KCl concentrations was completely the same as that of myosin B prepared after a 2-h extraction (Fig. 3). Moreover, from this myosin B myosin A was prepared. The purified myosin A was soluble at low salt concentrations of KCl (Fig. 3), and its yield (6.2%) from myosin B was also as the same order (about 5%) with that of myosin A which was prepared from myosin B from the 2-h extraction.

Next, striated muscle myosin A was incubated in the extract of plasmodium for 5 h at 4°, which had been dialyzed against 0.05 M KCl and 10 mM Tris–maleate

buffer (pH 7.0) to remove plasmodium myosin B. Then, myosin A was recovered by ultracentrifugation of the solution in the presence of ATP and Mg^{2+} to remove plasmodium F-actin which had remained in the extract to combine with myosin A. After the supernatant was dialyzed against 0.5 M KCl and 10 mM Tris–maleate buffer (pH 7.0), its solubility was compared with that of muscle myosin A untreated with the extract. As shown in Fig. 3 solubility of muscle myosin A was not changed by such a treatment. It can be concluded from these facts that the high solubility of plasmodium myosin A in monovalent salt solutions is not an artifact due to digestion by proteinases in the extract or to adsorption of some materials which might make myosin A soluble, but it is a characteristic nature of plasmodium myosin A.

A possibility of the existence of myosin A which is insoluble at low salt concentrations

In the purification procedure of myosin A, the insoluble proteins at low salt concentration were removed after dialysis of the supernatant of ultracentrifugation of myosin B (crude myosin A preparation I) against 0.05 M KCl solution. A possibility arises that all of myosin A might not be soluble, but the insoluble myosin A was lost in the course of preparation. Actually, as described before, a relatively large amount of protein was precipitated by dialysis. This precipitate was dissolved in 0.5 M KCl solution. Sedimentation pattern of the solution gave a main peak of myosin A ($s_{20,w} = 5.51$ S). If this solution was ultracentrifuged again in the presence of ATP and Mg^{2+}, almost all amounts of protein (90 %) in the supernatant were precipitated again by dialysis against 0.05 M KCl solution.

However, plasmodium F-actin polymerized from purified G-actin is partly destroyed to short fragments by ultracentrifugation. Due to higher acceleration and higher pH during ultracentrifugation, more F-actin are fragmented to short pieces. The sedimentation coefficient of short F-actin is about 5 S, which is of the same order as that of myosin A (S. HATANO, unpublished results). This experiment suggests that (natural) F-actin was partly destroyed to short fragments during ultracentrifugation of myosin B and that short F-actin combined with plasmodium myosin A to make an insoluble complex in 0.05 M KCl solution. To remove such short F-actin, the precipitate was fractionated by salting out with ammonium sulfate. A small amount of protein was precipitated from 20 to 35 % saturation, while a great part was precipitated from 35 to 60 % saturation. After fractionation, when the 0.5 M KCl solution of the protein which precipitated at from 35 to 60 % saturation was dialyzed against 0.05 M KCl solution (pH 7.0), only a small amount of precipitate (results of two experiments were 30 % and 33 % of total protein) was formed. (As mentioned earlier, F-actin cannot be removed completely by this procedure.) Namely, by removing short fragments of F-actin, a great part of the protein (myosin A) in the precipitate became soluble. It is reasonable to consider that all pure myosin A of plasmodium is soluble at low salt concentrations at pH 7.0, although small aggregates are formed as shown in the next section.

Some characteristics of plasmodium myosin A

Sedimentation coefficient. The sedimentation coefficient of plasmodium myosin A in 0.5 M KCl solution slightly depended on the protein concentration, and extrapolation to infinite dilution gave the value 6.05 for $s_{20,w}$ (Fig. 4). This value was almost the same as that of striated and cardiac muscle myosin A ($s_{20,w}$: 6.16 ± 0.30 S (ref. 13)).

Fig. 5 shows the sedimentation pattern of plasmodium myosin A in 0.05 M KCl solution (pH 7.0), in which there are four peaks. $s_{20,w}$ of the main peak was about 9 S (results of three experiment were 8.83 S, 9.04 S, 9.47 S). If the 6-S component in 0.5 M KCl solution is assumed to be a monomer of plasmodium myosin A, these small values of the sedimentation coefficient of myosin A aggregates suggest that plasmodium myosin A exists as oligomers (dimer, trimer, *etc.*) in 0.05 M KCl solution (pH 7.0). In the case of striated muscle myosin A, it has been reported to form large aggregates ($s_{20,w}$: 1100, 330, 180 S (ref. 14)) in the solution in such low salt concentrations.

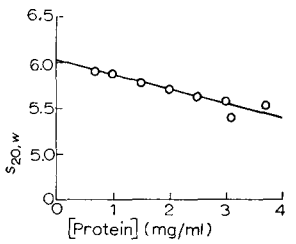

Fig. 4. Concentration dependence of sedimentation coefficient of plasmodium myosin A. Solvent conditions: 0.5 M KCl, 10 mM Tris–maleate buffer (pH 7.0). Ultracentrifugation was carried out at 59780 rev./min at 11.2°.

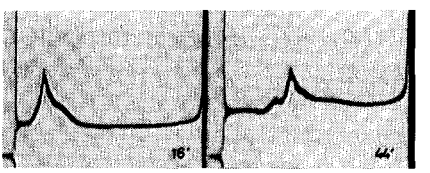

Fig. 5. Sedimentation pattern of plasmodium myosin A in 0.05 M KCl solution (pH 7.0). Ultracentrifugation was carried out at 50740 rev./min at 13.6°. Protein concentration, 2.3 mg/ml. Solvent conditions: 0.05 M KCl, 10 mM Tris–maleate buffer (pH 7.0). $s_{20,w}$ of the main peak = 8.83 S. Time in min after reaching the maximum speed is shown in each photograph.

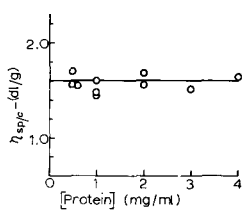

Fig. 6. Concentration dependence of specific viscosity of plasmodium myosin A. Solvent conditions: 0.5 M KCl, 10 mM Tris–maleate buffer (pH 7.0).

Viscosity. Specific viscosity did not depend on the protein concentration in the range from 0.5 to 4 mg/ml (Fig. 6). Extrapolation to infinite dilution gave the value 1.60 dl/g, which is about only two-thirds of that of skeletal myosin A of rabbit (2.17–2.35 dl/g (refs. 15, 16)). This is one of the characteristics of plasmodium myosin A.

Amino acid composition. As in the case of plasmodium actin[4], the amino acid composition of plasmodium myosin A is surprisingly similar to striated muscle myosin A of rabbit[17] in spite of the long distance between taxonogical positions of the two organisms (Table I).

However, there is an important difference between them. Namely, cysteine could not be detected in plasmodium myosin A using an automatic amino acid analyzer; the analysis has been repeated three times, but a peak of cysteine was never

formed, whereas cysteine residues in muscle myosin A were clearly determined using the same procedure. We also carried out the spectrophotometric titration of cysteine residues of plasmodium myosin A with *p*-chloromercuribenzoate[18] (PCMB), but so far we could not detect any cysteine residue. This will be discussed later.

ATPase activity. We compared the ATPase activity of plasmodium myosin A (EC 3.6.1.3) with that of striated muscle myosin A of rabbit under the same conditions

TABLE I

AMINO ACID COMPOSITION OF PLASMODIUM MYOSIN A AND RABBIT STRIATED-MUSCLE MYOSIN A

Amino acid	*Plasmodium myosin A (moles/10⁵ g protein)*	*Muscle myosin A (ref. 16) (moles/10⁵ g protein)*
Lys	89	85
His	18	15
Arg	51	41
Asp	91	85
Thr	37	41
Ser	34	41
Glu	145	155
Pro	41	22
Gly	43	39
Ala	72	78
Cys	<1	8.6
Val	38	42
Met	16	22
Ile	31	42
Leu	76	79
Tyr	15	18
Phe	23	27
Trp*	8.7	7.2**

* Determined from the spectrum in 0.1 M NaOH.
** Determined by the authors.

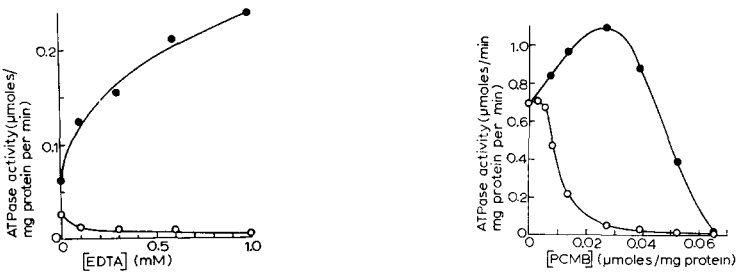

Fig. 7. Effect of EDTA on ATPase activity of plasmodium myosin A and muscle myosin A. Myosin A was preincubated with varied concentrations of EDTA for 5 min at 25°. ATPase activity was measured under the following conditions: 0.45 M KCl, 10 mM Tris–maleate buffer (pH 7.0), 1 mM ATP, at 25°. Protein concentration: plasmodium myosin A, 0.23 mg/ml; muscle myosin A, 0.26 mg/ml. O—O, plasmodium myosin A; ●—●, muscle myosin A.

Fig. 8. Effect of PCMB on ATPase activity of plasmodium myosin A and muscle myosin A. Myosin A was preincubated with varied concentrations of PCMB for 10 min at 0°. ATPase activity was measured under the following conditions: 0.4 M KCl, 10 mM Tris–maleate buffer (pH 7.0), 5 mM $CaCl_2$, 5 mM ATP, at 25.0°. O—O, plasmodium myosin A; ●—●, muscle myosin A.

TABLE II

ATPase ACTIVITY OF PLASMODIUM MYOSIN A AND MUSCLE MYOSIN A

ATPase activity was measured under the following conditions: 10 mM Tris–maleate buffer (pH 7.0) with or without 1 mM $MgCl_2$, $CaCl_2$ or EDTA, 1 mM ATP at 25.0°.

	Split-P (μmole/min per mg protein)	
	Plasmodium myosin A	*Muscle myosin A*
	0.03 M KCl	*0.135 M KCl*
No metal ions added	0.030	0.028
$MgCl_2$	0.027	0.019
$CaCl_2$	0.182	0.306
EDTA	0.019	0.028
Plasmodium actin	0.047	—
Muscle actin	0.057	—
Plasmodium actin *plus* $MgCl_2$	0.054	—
	0.45 M KCl	*0.45 M KCl*
No metal ions added	0.046	0.061
$MgCl_2$	0.030	0.016
$CaCl_2$	0.537	0.232
EDTA	0.007	0.239

(0.5 M KCl, 5 mM $CaCl_2$, 5 mM ATP, 10 mM Tris–maleate buffer (pH 7.0)). ATPase activity of the former (0.680 μmole split-P/mg protein per min) was found to be as high as that of the latter (0.686 μmole split-P/mg protein per min) (also see Table II).

The ATPase activity depended on the KCl concentration, and it was activated by Ca^{2+} and inhibited by Mg^{2+}. Plasmodium actin and muscle actin activated the ATPase activity of plasmodium myosin A at low salt concentrations. However, the activation by both actins was not so remarkable as in the case of muscle myosin A (Table II). EDTA always inhibited the ATPase activity of plasmodium myosin A in the range of concentration from 0.1 to 1.0 mM (Fig. 7), whereas this range of concentration of EDTA very much activates ATPase activity of muscle myosin A (ref. 19). As in the case of EDTA, PCMB always inhibited plasmodium myosin A ATPase while it activated muscle myosin A ATPase at low concentrations[20] (Fig. 8).

DISCUSSION

Methods for purification of myosin A from plasmodium have been developed in this paper. Purified myosin A had an ATPase activity as high as that of striated muscle myosin A. The sedimentation coefficient of plasmodium myosin A in 0.5 M KCl solution was also the same as that of striated muscle myosin A. The molecular weight of plasmodium myosin A is supposed to be of the same order as that of striated muscle myosin A ($4.7–5.1 \cdot 10^5$ (refs. 21–23)).

However, the intrinsic viscosity is much lower (about two-thirds) than that of striated muscle myosin A (refs. 14, 15). If plasmodium myosin A is a rod-like protein like muscle myosin A, it is presumed to be more flexible or globular than muscle myosin A.

Generally speaking, the amino acid composition of plasmodium myosin A is

very similar to muscle myosin A as in the case of plasmodium actin and muscle actin[3]. This probably means that contractile proteins such as actin and myosin A already existed in antiquity before primitive organisms evolved to myxomycete or vertebrate. However, there was an important difference between them. As far as we examined, we could not detect any cysteine residue in plasmodium myosin A. However, PCMB clearly inhibited the ATPase of plasmodium myosin A. Taking into consideration the resolving power of the automatic amino acid analyzer and of the spectrophotometric analysis of cysteine residue, it may be stated that the cysteine content in plasmodium myosin A, if present, must be less than 1 mole per 10^5 g of myosin A.

The ATPase of plasmodium myosin A behaved simply for EDTA and PCMB, namely, it was always inhibited by EDTA and PCMB even when their concentrations were low, when the ATPase of muscle myosin A was activated. BLUM[24] proposed that NTPase of muscle myosin A was classified into two groups: one is ATPase and CTPase, and the other is ITPase and GTPase. The latter NTPases behave more simply to SH reagent and EDTA, namely, PCMB and EDTA always inhibited ITPase and GTPase just as in the case of ATPase of plasmodium myosin A.

The most characteristic nature of plasmodium myosin A is that it is soluble and makes only small aggregates (about 9 S) in the solution of low salt concentrations which corresponds to the physiological condition of plasmodium at pH 7.0. It is assumed that such small aggregates of myosin A are oligomers (dimers, trimers, *etc.*) of myosin A molecules. As already pointed out in the previous paper[1], small aggregates of myosin A exist in plasmodium, probably binding to or detaching from microfilaments (F-actin) according to the condition in the plasmodium.

If pH of myosin A solution was lowered, myosin A was increasingly precipitated and almost all myosin A was precipitated at pH 5.3 (ref. 1). Shape and size of myosin A aggregates or precipitates at varied pH's below 7.0 in the physiological salt concentration of plasmodium (0.03 M KCl) were under investigation.

APPENDIX

Preparation of plasmodium myosin B

100 g of plasmodia were put into 3 vol. of 0.53 M KCl and 20 mM EDTA (pH 7.0) and were homogenized in a Potter–Elvehjem-type glass homogenizer as soon as possible (within 30 sec). The pH of the homogenate was adjusted to 8.2, and the solution was gently stirred with magnetic stirrer in a cold room for 2 h. The homogenate was centrifuged at 20000 rev./min for 30 min. A clear but yellowish supernatant was filtrated through gauze, and 4 vol. of cold water were added. After the pH of the solution was lowered to 6.5 by the addition of 0.1 M acetate buffer (pH 4.7), it was allowed to stand for 15 min. The solution was centrifuged at 4000 rev./min for 10 min, and the precipitate of myosin B was collected. The precipitate was washed twice with about 10 vol. of 0.05 M KCl and 10 mM Tris–maleate buffer (pH 7.0), and it was dissolved in about 20 ml of 0.5 M KCl solution containing 10 mM Tris–maleate buffer (pH 7.0). This gave the solution of myosin B, of which the concentration was about 5 mg/ml. Myosin B solution could be stored in an ice box at 0°. (It became a gel after about 12 h.)

ACKNOWLEDGMENTS

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