A low-molecular-weight myosin has been purified 1500-fold from extracts of Dictyostelium discoideum, based on the increase in K⁺-EDTA-ATPase specific activity. The purified enzyme resembles the single-headed, low-molecular-weight myosins IA and IB from Acanthamoeba castellanii, and differs from the conventional two-headed, high-molecular-weight myosin previously isolated from Dictyostelium, in several ways. It has higher K⁺-EDTA-ATPase activity than Ca⁺⁺-ATPase activity; it has a native molecular mass of about 150,000 and a single heavy chain of about 117,000; the 117,000-dalton heavy chain is phosphorylated by Acanthamoeba myosin IA heavy chain kinase; phosphorylation of its heavy chain enhances its actin-activated Mg⁺⁺-ATPase activity; and the 117,000-dalton heavy chain reacts with antibodies raised against the heavy chain of Acanthamoeba myosin IA. None of these properties is shared by the low-molecular-weight active fragment that can be produced by chymotryptic digestion of conventional Dictyostelium myosin. We conclude that Dictyostelium contains an enzyme of the myosin I type previously isolated only from Acanthamoeba.

Acanthamoeba castellanii contains two enzymes with the catalytic properties of a myosin but of much lower molecular weight than any other myosin described until now (1, 2). Acanthamoeba myosin IA contains a single heavy chain of 130,000 daltons and a single light chain of 17,000 daltons while myosin IB contains a heavy and light chain of 125,000 and 27,000 daltons, respectively (2). Some preparations (2) but not all (3), of both enzymes also contain a 14,000-dalton component. Both myosins IA and IB have high K⁺-EDTA-ATPase activity, lower Ca⁺⁺-ATPase activity, and very low Mg⁺⁺-ATPase activity that is 20-50-fold activated by F-actin as evidenced by their abilities to cross-link (10) and superprecipitate (11) actin filaments and to cause beads to which they are covalently attached to move unidirectionally along actin cables (12). Acanthamoeba contain about as much myosin IA and IB, together, as myosin II and it seems reasonable to assume that similar enzymes probably occur elsewhere.

Dictyostelium discoideum had previously been shown to contain only a conventional myosin consisting of a pair of 210,000-dalton heavy chains and two 18,000-dalton and two 16,000-dalton light chains (13). This myosin has a Ca⁺⁺-ATPase activity of about 0.7 μmol/min/mg (14), little, if any, K⁺-EDTA-ATPase activity (13), and actin-activated Mg⁺⁺-ATPase activity which is inhibited by phosphorylation of the rod portion of the heavy chain (14, 15). We now report the purification and partial characterization of a previously undetected low-molecular-weight Dictyostelium myosin which is similar to Acanthamoeba myosins IA and IB, and different from conventional high-molecular-weight Dictyostelium myosin, in its physicochemical and enzymatic properties.

**MATERIALS AND METHODS**

Dictyostelium discoideum strain Ax-3 (American Type Culture Collection, Rockville, MD) was grown in HL-5 medium (16) in the absence of antibiotics and with the addition of antifoam-A (Dow Corning; 60 μl/liter). For large-scale preparations, carboys containing 15 liters of medium were inoculated with 1 liter of culture containing about 5 × 10⁶ cells/ml. Cells were grown for 3 days at room temperature (22–25 °C) with continuous aeration, using a Challenger III air pump (Nasco, Fort Atkinson, WI) and a gas dispersion tube (Corning), and with magnetic stirring to keep the cells suspended. The cells were harvested by centrifugation at 2000 rpm and washed twice with ice-cold 50 mM NaCl, 10 mM imidazole-chloride, pH 7.5. The yield of packed cells from 1 carboy was 150–200 g.

Conventional Dictyostelium myosin was purified as described by Clarke and Spudich (13) and Mockrin and Spudich (17) and the soluble head-fragment was produced by chymotryptic digestion and isolated as described by Pelts et al. (15). Acanthamoeba myosin IB (3) and Acanthamoeba myosin I heavy chain kinase (4) were purified as described. The active, proteolyzed form of the kinase (4) was obtained. Rabbit skeletal muscle F-actin (18) was a gift of Dr. Lois Greene (Laboratory of Cell Biology, National Heart, Lung, and Blood Institute). The polyclonal antiserum to Acanthamoeba myosin IA heavy chain was raised in rabbits immunized with myosin IA. The antisem immunoprecipitated myosin IB as well as myosin IA.

ATPase activities were measured, under conditions of linearity with respect to time and enzyme concentration, by the release of P³²P, from [γ-³²P]ATP (1). Ca⁺⁺-ATPase activities were assayed in 0.5 M KCl, 10 mM imidazole, pH 7.0, 10 mM CaCl₂, and 2 μM [γ-³²P]ATP for 10 min at 30 °C. K⁺-EDTA-ATPase activities were assayed in 0.5 M KCl, 2 mM EDTA, 15 mM imidazole, pH 7.5, and 2 μM [γ-³²P]ATP for 10 min at 30 °C. Protein concentrations were determined by the colorimetric assay of Bradford (19) using bovine serum albumin as standard. SDS-polyacrylamide gel electrophoresis was performed according to Laemmli (20) and proteins were stained with Coomassie Blue according to Fairbanks et al. (21). For autoradiography, the stained, dried gels were exposed at −76 °C to X-Omat XR5 film beneath an intensifier screen in an X-Omat cassette (Kodak). The demonstration that myosins IA, IB, and II (the latter a more conventional myosin isoenzyme that also occurs in Acanthamoeba (8, 9)) are separate and distinct gene products. Although neither myosin IA nor myosin IB is capable of forming filaments, both can support actin-dependent motile activity as evidenced by their abilities to cross-link (10) and superprecipitate (11) actin filaments and to cause beads to which they are covalently attached to move unidirectionally along actin cables (12). Acanthamoeba contain about as much myosin IA and IB, together, as myosin II and it seems reasonable to assume that similar enzymes probably occur elsewhere.

**COMMUNICATION**

Purification from Dictyostelium discoideum of a Low-molecular-
weight Myosin That Resembles Myosin I from Acanthamoeba castellanii*†

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The abbreviations used are: SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid.

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gels were stained at 500 nm using a Beckman DU-8 spectrophotometer. Immunoblot analysis was performed according to the methods of Towbin et al. (22) and Hawkes et al. (23).

Equilibrium ultracentrifugation was carried out at 12,000 rpm and 4 °C in a Beckman Model E analytical ultracentrifuge using a phototube-scanner with UV optics. A partial specific volume of 0.73 ml/g was assumed for the low-molecular-weight Dictyostelium myosin.

DE52 was obtained from Whatman and ADP-agarose from F-L Biochemicals. ATP, imidazole (grade III), pepstatin, leupeptin, diospropyl fluorophosphate, and PMSF were from Sigma. [γ-32P]ATP was from New England Nuclear. All other chemicals were reagent grade and deionized water was used throughout.

**Purification of Low-molecular-weight Dictyostelium Myosin—** All procedures were carried out at 4 °C. Cells (350 g) were suspended in 700 ml of extraction buffer containing 75 mM KC1, 12 mM sodium pyrophosphate, 5 mM dithiothreitol, 30 mM imidazole, and 10 mg of pepstatin, 5 mg of leupeptin, and 100 mg of PMSF/liter, pH 7.5, and homogenized with 20 strokes of a very tight-fitting 40 ml Dounce homogenizer. Immediately after homogenization, diospropyl fluorophosphate was added to a concentration of 1 mM and the extract was stirred for 30 min and centrifuged in a Beckman Type 19 rotor for 3 h at 50,000 rpm.

The pH of the supernatant solution (Extract, Table I) was adjusted to pH 8.0 and 1 ml Tris, pH 8.0, was added to the supernatant and the supernatant was applied to a column of DE52 (5 x 29 cm) equilibrated in buffer containing 10 mM KC1, 10 mM Tris, 1 mM dithiothreitol, and 0.1 mM PMSF, pH 8.0. The column was washed with equilibration buffer until the absorbance at 280 nm became constant and then a 4-liter gradient was applied to a final concentration of 500 mM KC1 in the same buffer. The column was assayed for K+,EDTA-ATPase activity and a single, sharp peak was detected at about 0.2 M KC1 (DEAE peak, Table I).

The DEAE peak (350 ml) was pooled and concentrated by precipitation with 2 M ammonium sulfate. The precipitate was redissolved completely in buffer containing 10% sucrose, 15 mM imidazole, 1 mM EGTA, and 1 mM dithiothreitol, pH 7.5, and was dialyzed overnight against 4 liters of this buffer. The flocculent precipitate that formed during dialysis against this low ionic strength buffer was collected by centrifugation in a Sorvall GSA rotor at 8000 rpm for 10 min. This precipitate contained essentially all of the K+,EDTA-ATPase activity. Because actin was a major component detected by SDS-polyacrylamide gel electrophoresis (not shown), this precipitate was assumed to be crude actomyosin (Actomyosin, Table I).

The actomyosin precipitate was homogenized in 20 ml of the previous dialysis buffer with the addition of 0.1 M KC1 and 5 mM Mg2+-ATP to dissociate the actomyosin and solubilize the myosin. The precipitate was resuspended and the activity in the supernatant was taken as the precipitate as F-actin. The homogenate was immediately centrifuged in a Beckman Type 65 rotor at 60,000 rpm for 1 h. Most of the K+,EDTA-ATPase activity was released into the supernatant (Mg2+-ATP extract, Table I) while the pellet consisted of a lower, brown layer and an upper, clear layer of F-actin. The major component detected by SDS-polyacrylamide gel electrophoresis was residual actin and a 117,000-dalton polypeptide (not shown).

The Mg2+-ATP extract was dialyzed overnight against the same dialysis buffer as previously used to form the actomyosin precipitate. This time, no precipitate formed. The dialyzed solution was applied to an ADP-agarose column (1.5 x 20 cm) equilibrated in buffer containing 50 mM KC1, 15 mM Tris, and 1 mM dithiothreitol, pH 7.5. The protein was eluted with a 200-ml gradient from 50 mM to 1 M KC1 in the same buffer. The major peak of K+,EDTA-ATPase activity was eluted at about 400 mM KC1 (ADP-agarose peak, Table I).

**RESULTS**

Clarke and Spudich (13) found that the conventional Dictyostelium myosin was normally insoluble at low ionic strength but that it could be extracted in buffer containing 30% sucrose. We confirmed that about 80% of the Ca2+-ATPase activity detectable in whole homogenates of Dictyostelium was recovered in high-speed supernatants when the extracting buffer contained 30% sucrose and 40 mM sodium pyrophosphate. In addition, we found that the whole homogenate also contained K+,EDTA-ATPase activity, equal to about 10% of the Ca2+-ATPase activity, 80% of which was also extracted by the sucrose/pyrophosphate buffer. However, with buffer containing 12 mM sodium pyrophosphate and 75 mM KC1 but no sucrose, 100% of the K+,EDTA-ATPase activity was still recovered in the high-speed supernatant, but only about 10-20% of the Ca2+-ATPase activity. These were the first observations of K+,EDTA-ATPase activity in Dictyostelium. As only myosins (but not all myosins) have been reported to possess ATPase activity in the absence of divalent cations and the presence of EDTA, these results suggested that Dictyostelium might contain a myosin different from the conventional myosin that had been described previously; one that might be similar to Acanthamoeba myosins IA and IB. Therefore, we purified the K+,EDTA-ATPase activity by a procedure similar to that previously used for the purification of myosins IA and IB from Acanthamoeba.

In four steps, including DEAE-cellulose chromatography, precipitation of an actomyosin complex, solubilization of the myosin, and chromatography on ADP-agarose (see "Materials and Methods"), the K+,EDTA-ATPase activity was purified about 1500-fold in about 7% yield from the starting extract (Table I). The most highly purified material had a ratio of K+,EDTA-ATPase to Ca2+-ATPase of 20 compared to a ratio of 2 for the original extract and less than 0.5 for the whole homogenate.

By SDS-polyacrylamide gel electrophoresis, the major component of the most highly purified fraction (ADP-agarose peak) had a molecular weight of 117,000 (Fig. 1, Lane A). This is similar to the sizes of the single heavy chains of Acanthamoeba myosins IA and IB (125,000 and 130,000) and much smaller than the two heavy chains of conventional Dictyostelium myosin (215,000). Only one component was detected by sedimentation equilibrium analysis of the ADP-agarose fractions. It had a molecular weight of 150,000 indicating that it contained only one heavy chain, again suggesting a strong similarity to the single-headed Acanthamoeba myosins IA and IB. The low-molecular-weight Dictyostelium myosin was insufficiently pure to determine the composition of its light chains, if any, unequivocally.

The purified low-molecular-weight Dictyostelium myosin had K+,EDTA-ATPase and Ca2+-ATPase activities of 4.2 and 0.24 µmol/min/mg, respectively (Table I), which are similar to the activities of Acanthamoeba myosins IA and IB (2). These activities are very different from the values reported for the conventional Dictyostelium myosin (13, 14), and confirmed by us, which has higher Ca2+-ATPase activity and essentially no K+,EDTA-ATPase activity.

The low-molecular-weight Dictyostelium myosin had a Mg2+-ATPase activity of about 0.5 µmol/min/mg which was activated only slightly by skeletal muscle F-actin (Fig. 2). However, the 117,000-dalton polypeptide of the Dictyostelium myosin was phosphorylated by Acanthamoeba myosin I heavy chain kinase (Fig. 1, Lanes B and b), although less than 20% as much 32P was incorporated as into an approximately equivalent concentration of Acanthamoeba myosin IB heavy chain kinase (Fig. 1, Lanes C and c), and the Mg2+-ATPase activity of the phosphorylated Dictyostelium enzyme was stimulated more than 6-fold by F-actin (Fig. 2). This is less than the actin-activation observed for the phosphorylated Acanthamoeba myosins IA and IB (4) but that may be due to incomplete phosphorylation of the Dictyostelium myosin by the Acanthamoeba kinase (compare Lanes b and c, Fig. 1). It is important to note that the Acanthamoeba myosin I heavy chain kinase did not phosphorylate the heavy chains of conventional Dictyostelium.
tryptic-cleavage product of conventional kinase in 50 μl of buffer containing 2 mM MgCl₂, 2 mM [γ-32P]ATP, kinase.

Passage through DEAE-Sepharose and acetylated myosin  

The entire samples were applied to an SDS-polyacrylamide gel.

Actomyosin

The low-molecular-weight protein from Dictyostelium differs from conventional Dictyostelium myosin and its low-molecular-weight chymotryptic cleavage product both of which 1) have very much higher Ca²⁺-ATPase activity than K⁺-EDTA-ATPase activity; 2) do not cross-react with the antibodies to Acanthamoeba myosin I heavy chain; 3) are not phosphorylated by Acanthamoeba myosin I kinase; 4) require light chain phosphorylation and not heavy chain phosphorylation for expression of their actin-activated Me-ATPase activities. In addition, immunoblots of whole extracts of Dictyostelium failed to detect any polypeptide larger than 117,000 daltons that reacted with the antibody to Acanthamoeba myosin I (data not shown). Therefore, there is no reason to suppose that the low-molecular-weight myosin is a degradation product of conventional Dictyostelium myosin or of any other high-molecular-weight myosin.

**DISCUSSION**

In summary, the low-molecular-weight ATPase purified from *D. discoideum* resembled myosins IA and IB from *A. castellani*. In the following ways: 1) similar molecular weights of the heavy chains (117,000 versus 125,000 and 130,000) and of the native enzymes (all about 150,000); 2) high K⁺,EDTA-ATPase and low Ca²⁺-ATPase activities; 3) ATP-dissociable binding to F-actin; 4) cross-reactivity with antibodies to Acanthamoeba myosin IA; 5) phosphorylation of the heavy chain by a highly purified, highly specific heavy chain kinase from Acanthamoeba; and 6) actin-activated Mg²⁺-ATPase activity that is enhanced by phosphorylation of the heavy chain.

**TABLE I**

| Fraction             | Volume | Protein | Ca²⁺-ATPase | K⁺,EDTA-ATPase |
|----------------------|--------|---------|-------------|----------------|
| Extract              | 730    | 14,600  | 23.4        | 44.5           |
| DEAE peak            | 350    | 630     | 1           | 19.3           |
| Acetylated myosin    | 30     | 26      | 0.86        | 10.6           |
| Mg²⁺-ATP extract     | 20     | 3.4     | 0.5         | 9              |
| ADP-agarose peak     | 35     | 0.69    | 0.17        | 3.2            |

**FIG. 1.** Phosphorylation of low-molecular-weight myosin from *Dictyostelium* by *Acanthamoeba* myosin I heavy chain kinase. About 5 μg of each myosin preparation was incubated for 5 min at 30 °C with 0.5 μg of *Acanthamoeba* myosin I heavy chain kinase in 50 μl of buffer containing 2 mM MgCl₂, 2 mM [γ-32P]ATP, 10 mM imidazole, pH 7.0, and 1 mM EGTA. Under these conditions, 1 mol of P is incorporated/mol of *Acanthamoeba* myosin I heavy chain. The entire samples were applied to an SDS-polyacrylamide gel. Lanes A-G, Coomassie Blue-stained gel; Lanes a-f, autoradiogram. Lanes A and a, low-molecular-weight *Dictyostelium* myosin; Lanes B and b, low-molecular-weight *Dictyostelium* myosin + kinase; Lanes C and c, *Acanthamoeba* myosin IB + kinase; Lanes D and d, conventional *Dictyostelium* myosin + kinase; Lanes E and e, chymotryptic-cleavage product of conventional *Dictyostelium* myosin + kinase; Lanes F and f, kinase alone; and Lane G, standards, myosin heavy chain (200,000), β-galactosidase (116,250), phosphorylase b (97,300), bovine serum albumin (66,200), and ovalbumin (45,000).

myosin (Fig. 1, Lanes D and d) nor the soluble, active head-fragment that can be derived from it by chymotryptic digestion (Fig. 1, Lanes E and e).

The similarities between the low-molecular-weight *Dictyostelium* myosin and *Acanthamoeba* myosins IA and IB were confirmed and extended by immunoblot analysis; the 117,000-dalton component of the *Dictyostelium* myosin reacted with antiserum raised against *Acanthamoeba* myosin I (Fig. 3, Lanes A and a) at least as well as did the 125,000-dalton heavy chain of *Acanthamoeba* myosin IB (Fig. 3, Lanes D and d). Neither the intact heavy chain of conventional *Dictyostelium* myosin (Fig. 3, Lanes B and b) nor its chymotryptic head-fragment (Fig. 3, Lanes C and c) showed any reaction with the *Acanthamoeba* myosin I antibody.

**FIG. 2.** Actin activation of the Mg²⁺-ATPase activity of the low-molecular-weight myosin from *Dictyostelium* before and after phosphorylation by *Acanthamoeba* myosin I kinase. The *Dictyostelium* myosin (55 μg/ml) was incubated for 5 min at 30 °C with and without *Acanthamoeba* myosin I heavy chain kinase (1.1 μg/ml) in the buffer described in Fig. 1, but with nonradioactive ATP. This is sufficient kinase to phosphorylate *Acanthamoeba* myosin I maximally. Aliquots of 20 μl were incubated at 30 °C in a total volume of 500 μl of the same buffer but containing [γ-32P]ATP and F-actin as indicated in the figure. Aliquots were removed at 0 and 10 min to measure 32P incorporation.
A Low-molecular-weight Myosin from Dictyostelium

It seems reasonable at this time to refer to the two distinct myosins of Dictyostelium as myosin I and myosin II, by analogy to the Acanthamoeba isoenzymes. Moreover, preliminary evidence suggests that there may be two forms of Dictyostelium myosin I as there are for Acanthamoeba myosin I. A protein eluting slightly earlier from ADP-agarose, the final purification step, had similar K+-EDTA-ATPase and Ca2+-ATPase activities to the enzyme described in this paper. It had a slightly larger heavy chain (about 125,000 daltons) which was also phosphorylated (but less well) by Acanthamoeba myosin I heavy chain kinase with a small enhancement of actin-activated Mg2+-ATPase activity.

The discovery in Dictyostelium of a previously unrecognized myosin that resembles Acanthamoeba myosins IA and IB raises the possibility that similar low-molecular-weight, single-headed myosins might occur generally. A review of the literature suggests two possibilities: a myosin of apparently low molecular weight, and with a heavy chain of about 100,000 daltons, has been isolated from tomato (24), but it has yet to be proved that it was not a degradation product of a larger molecule; and Collins and Borysenko (25) have shown that the 110,000-dalton calmodulin- and F-actin-binding protein of intestinal microvilli has Ca2+-ATPase activity, but it has not yet been found to have actin-activated Mg2+-ATPase activity.

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