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Purification and characterization of a mammalian myosin I

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ABSTRACT **Myosin I, an actin-dependent force-generating enzyme, has been purified from three mammalian sources: bovine adrenal medulla, adrenal cortex, and brain. The purification procedure includes extraction of tissue with ATP at low ionic strength and coprecipitation with actin, followed by gel filtration on Sepharose 4B, anion-exchange chromatography on Q Sepharose, and affinity chromatography on ATP-agarose. Mammalian myosin I molecules are composed of a heavy chain of 116 kDa and multiple low molecular weight polypeptides identified as calmodulin. The structural and enzymatic properties of adrenal medulla myosin I were further characterized. This enzyme exhibits high K^+,EDTA- and Ca^{2+}-ATPase specific activities (about 0.2 μmol·min^{-1} per mg of protein), whereas the Mg^{2+}-ATPase activity is very low (1–3 nmol·min^{-1}·mg^{-1}). The Mg^{2+}-ATPase of medulla myosin I is activated by F-actin in a Ca^{2+}-dependent manner: activity is stimulated 40-fold in the presence of EGTA and 90-fold in the presence of 10 μM Ca^{2+}. Two structural domains of the myosin I heavy chain were identified. A 74-kDa chymotryptic fragment contains the catalytic site, while a 36-kDa polypeptide contains the calmodulin-binding sites. These results indicate that mammalian myosin I is more closely related to myosin I from the avian intestinal brush border than to the enzymes isolated from the protozoans *Acanthamoeba* and *Dictyostelium*.**

Myosins I are actin-dependent force-generating proteins that differ in structure from conventional muscle-type myosins (myosins II) while sharing several of their enzymatic characteristics (for reviews, see refs. 1 and 2). The functions of myosins I have not been defined; however, their localization to the plasma membrane (3–5), their ability to interact directly with phospholipid bilayers (6, 7), and their mechanochemical activity have led to speculation that myosins I are the motors driving contractile activities at the cell membrane, including exocytosis, endocytosis, and changes in cell shape (6) (for recent reviews, see refs. 2 and 8).

Sequences of myosins I from lower eukaryotes and chicken brush border have revealed extensive homology to myosins II at the amino-terminal (head) domains, the regions involved in actin binding and ATP hydrolysis. However, the carboxyl-terminal (tail) domains of the two myosin types are distinct. Muscle and nonmuscle myosins II have elongated tail segments whose sequences induce a coiled-coil conformation and self-assembly into bipolar filaments (9). In contrast, myosin I tails are much shorter, do not promote coiled-coil interactions, and mediate the interaction of myosin I with membranes.

Despite molecular genetic evidence for several isoforms of myosin I (10–13), myosin I proteins have, until now, been purified only from the two lower eukaryotes *Acanthamoeba castellanii* (14) and *Dictyostelium discoideum* (15) and from avian intestinal brush border (16). Before discovery of its ATPase activity (17), the brush border enzyme was considered a structural protein, forming the radial links between the plasma membrane and the actin bundle of the microvillar core. The protozoan forms have heavy chains with molecular weights ranging from 125,000 to 140,000 that must be phosphorylated by specific myosin I kinases to allow the expression of actin-activated ATPase activity (18–20). Three *Acanthamoeba* myosin I isoenzymes (IA, IB, and IC), purified to apparent homogeneity, also contain light chains of molecular weights ranging from 14,000 to 27,000 (21). The role of these light chains in myosin I function is not known, since their removal does not affect enzymatic activity (19). The subunit composition of *Dictyostelium* myosin I is not yet known. Brush border myosin I is composed of a single heavy chain (molecular weight 119,000) and three or four associated calmodulin molecules, which may now be considered as light chains (22, 23). In contrast to protozoan myosins I, the light chains of brush border myosin I are reported to confer Ca^{2+} sensitivity to its actin-activated ATPase and motility-promoting activities (23–26).

Northern blot (11, 27) and immunochemical (28) methods have failed to detect myosin I enzymes in tissue other than intestinal brush border. This paper describes the purification of myosin I from three mammalian tissues: bovine adrenal medulla, adrenal cortex, and brain. The adrenal medullary form of the enzyme has been characterized in terms of structure and enzymatic activity. The properties of this protein are consistent with its classification as a member of the myosin I family of molecular motors.

MATERIALS AND METHODS

Materials. Sepharose CL-4B and Q Sepharose were from Pharmacia LKB; ATP-agarose (attachment: N-6) and protease inhibitors were from Sigma; goat anti-rabbit IgG alkaline phosphatase immunoblot kit was from Bio-Rad; [γ-^{32}P]ATP was from DuPont/New England Nuclear; and [α-^{32}P]ATP was from ICN.

Purification of Proteins. Fresh bovine adrenals and bovine brains were obtained from a local slaughterhouse, cooled in ice, and processed within 2 hr. All subsequent purification steps were carried out at 4°C. All solutions used in the purification procedure contained 1 mM dithiothreitol, 1 mM sodium azide, and a range of protease inhibitors: 0.2 mM phenylmethylsulfonyl fluoride and 10 mg/liter each of N^{α}-benzoyl-L-arginine methyl ester, N^{α}-tosyl-L-arginine methyl ester, N^{α}-*p*-tosyl-L-lysine chloromethyl ketone, leupeptin, and pepstatin A.

The initial steps of myosin I purification were the same as those used for myosin II isolation from thymus (29) or brain (30). About 100 g of tissue was homogenized first in a Waring blender for 30 sec, then in a glass/Teflon homogenizer in 5 vol of buffer at pH 7.5 containing 0.34 M sucrose, 2 mM EDTA, 1 mM EGTA, 10 mM Tris·HCl, 10 mM NaH_2PO_4, 2 mM dithiothreitol, and 5 mM ATP. The pH of the homogenate was adjusted to 7.5 with 2 M Tris base (pH 11). After 20 min of extraction NaCl was added to a final concentration of 0.1 M and the homogenate was immediately centrifuged at 28,000 × *g* for 50 min. The supernatant was adjusted to 0.6 M NaCl with solid NaCl and to 7 mM $MgCl_2$ with a 1 M $MgCl_2$ solution and then subjected to ammonium sulfate fractionation. The

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35–65% saturated ammonium sulfate fraction was used for further purification. The precipitate was dissolved in 1 M NaCl/2 mM EDTA/0.5 mM EGTA/50 mM Tris·HCl/5 mM dithiothreitol, pH 7.5 (buffer A), then dialyzed against 50 mM NaCl/1 mM EDTA/0.2 mM EGTA/10 mM NaH_2PO_4/10 mM imidazole, pH 7.0, to precipitate the actomyosin complex. After overnight dialysis $MgCl_2$ was added to 5 mM, pH was adjusted to 6.35, and the sample was immediately centrifuged at 42,000 × g for 30 min. The pellet (crude actomyosin) was solubilized in buffer A containing 12 mM $MgCl_2$ and 10 mM ATP and ultracentrifuged at 300,000 × g for 1.5 hr. The supernatant was chromatographed on a Sepharose 4B column to separate myosin I from myosin II.

Fractions containing myosin I (the second peak of ATPase activity) were concentrated by dialysis against solid sucrose and then dialyzed against 15 mM Tris·HCl/0.1 M KCl/1 mM EGTA/1 mM dithiothreitol/7.5 mM sodium pyrophosphate, pH 7.5. The addition of pyrophosphate was necessary to prevent precipitation of myosin I. The samples were then chromatographed on a Q Sepharose column equilibrated with the dialysis buffer and eluted with a KCl gradient (0.1–0.5 M) in the same buffer. Pooled fractions of the major peak of ATPase activity were concentrated by dialysis against solid sucrose, dialyzed against a 10% sucrose/15 mM Tris·HCl, pH 7.5/0.05 M KCl/1 mM EGTA/1 mM dithiothreitol and loaded onto an ATP-agarose column equilibrated with the same solution but without sucrose. ATPase activity was eluted from the column with 1 M KCl. The peak was concentrated by dialysis against 50% glycerol/50 mM KCl/15 mM Tris·HCl, pH 7.5/1 mM dithiothreitol and stored at −20°C. Before experimentation, myosin I samples were dialyzed against appropriate buffers, then ultracentrifuged at 100,000 × g for 30 min to remove any aggregates formed during storage.

Calmodulin was isolated from brain as described by Watterson et al. (31). Actin was isolated from rabbit skeletal muscle according to the procedure of Strzelecka-Golaszewska et al. (32).

Gel Overlay with ^{125}I-Labeled Calmodulin. Calmodulin (2 mg) was labeled with ^{125}I by using Iodo-Beads as outlined by the manufacturer (Pierce). The unreacted reagent was separated from ^{125}I-labeled protein by extensive dialysis against 20 mM imidazole·HCl, pH 7.0. The specific radioactivity for ^{125}I-calmodulin was 2 × 10^8 cpm/mmol. A gel overlay procedure with ^{125}I-calmodulin was based on a method described by Glenney and Weber (33).

Labeling with [α-^{32}P]ATP. Myosin was photoaffinity labeled at the ATP-binding site with [α-^{32}P]ATP by using the method described by Maruta and Korn (34). Myosin (2 μg) in 0.1 M KCl/15 mM Tris·HCl, pH 7.5/5 mM dithiothreitol/50% glycerol was incubated with 0.26 μM [α-^{32}P]ATP (1000–3000 Ci/mmol; 1 Ci = 37 GBq) for 30 min at room temperature under ultraviolet light (254 nm). The labeled myosin was used for chymotryptic digestion.

Digestion with Chymotrypsin. Myosin I was digested with chymotrypsin [1:50 (wt/wt) enzyme-to-myosin ratio] in the presence of 0.1 mM $CaCl_2$ or 1 mM EGTA for 20 min at room temperature. The reaction was terminated by adding phenylmethylsulfonyl fluoride to a final concentration of 2 mM. The digests were analyzed by SDS/PAGE.

Immunoblotting. Samples were run on an SDS gel, blotted onto Immobilon-P transfer membrane (Millipore), and allowed to react with antibodies raised against either bovine tracheal whole myosin II (gift from Kristine Kamm, University of Texas Southwestern) or brush border myosin I (gift from Jimmy Collins, Eastern Virginia Medical School). To identify calmodulin, samples were electrophoresed on a urea/glycerol/polyacrylamide gel (23), transferred onto Immobilon, and allowed to react with anti-calmodulin antibodies (monoclonal affinity-purified IgG) (gift of Malu Tansey, University of Texas Southwestern). Binding of antibodies was detected by second antibodies (Bio-Rad) coupled to alkaline phosphatase.

Other Procedures. ATPase activities were measured by the release of $^{32}P_i$ from [γ-^{32}P]ATP (35) after incubation at 37°C. K^+,EDTA-ATPase was assayed in 0.5 M KCl/2 mM EDTA/1 mM ATP/15 mM imidazole·HCl, pH 7.5. Ca^{2+}-ATPase was assayed in 2 mM $CaCl_2$/15 mM imidazole·HCl, pH 7.5/1 mM ATP and either 10 mM KCl or 90 mM NaCl (to assay Sepharose 4B fractions). NH_4^+, EDTA-ATPase was assayed in 0.4 M NH_4Cl/35 mM EDTA/25 mM Tris·HCl, pH 7.5/1 mM ATP. Actin-activated ATPase was assayed in 2% (vol/vol) glycerol/20 mM KCl/2 mM $MgCl_2$/1 mM ATP/20 mM imidazole·HCl, pH 7.5. Protein concentration was determined as described by Bradford (36), using bovine serum albumin as a standard. SDS/PAGE was carried out on microslab gels according to the method of Matsudaira and Burgess (37).

RESULTS

Purification of Mammalian Myosin I. The first steps for the purification of mammalian myosin I are similar to those found most suitable for the preparation of nonmuscle myosin II (29, 30). They include tissue extraction with low ionic strength/ATP solution, fractionation with ammonium sulfate, and precipitation of both myosins, myosin II and myosin I, in a complex with actin by dialysis against a low ionic strength solution. The crude actomyosin precipitate is solubilized in a high ionic strength solution containing MgATP and clarified by ultracentrifugation. These purification steps remove a large amount of protein as well as considerable Mg^{2+}- and Ca^{2+}-activated ATPase. Fractionation of the solubilized actomyosin on a Sepharose 4B column yields only two peaks of K^+,EDTA- and Ca^{2+}-ATPase activities (Fig. 1). The first peak represents pure myosin II (Fig. 1 *Inset*, lane 1), whereas the second peak of ATPase activity, eluting before the major peak of protein, contains myosin I (Fig. 1 *Inset*, lane 2).

The next step of the purification is ion-exchange chromatography on a Q Sepharose column (Fig. 2). The major peak of ATPase activity elutes at 0.33 M KCl. Due to the strong binding of myosin I to this medium, an extensive purification of the enzyme is achieved (Fig. 2 *Inset*). To ascertain that the

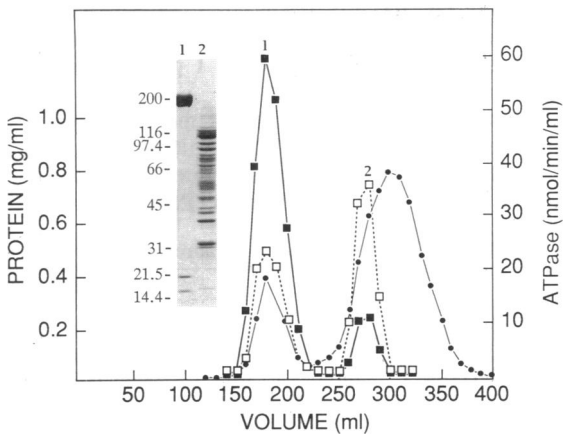

FIG. 1. Gel filtration of crude actomyosin on Sepharose 4B. A Sepharose CL-4B column (90 × 2.6 cm) was preequilibrated with 0.6 M KI/5 mM $Na_2S_2O_3$/50 mM Tris·HCl, pH 7.5/1 mM EGTA/5 mM dithiothreitol/10 mM MgATP and then eluted with 0.9 M NaCl/2 mM EDTA/0.25 mM EGTA/25 mM Tris·HCl, pH 7.5/0.25 mM dithiothreitol and protease inhibitors at 40 ml/hr. Fractions were assayed for protein (●) and for K^+,EDTA-ATPase (■) or Ca^{2+}-ATPase (□). (*Inset*) Protein patterns of two peaks of ATPase activity in SDS/PAGE. Numbers on left are positions of markers (kDa).

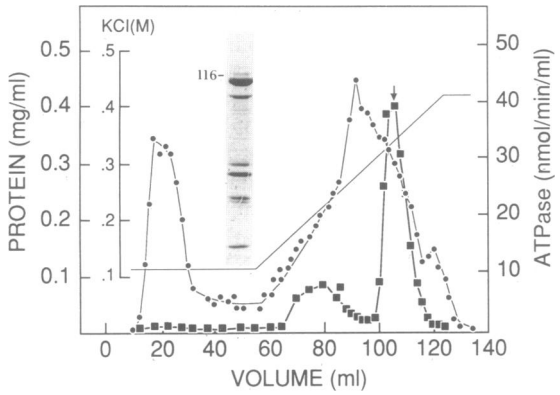

FIG. 2. Q Sepharose chromatography of the second peak of ATPase activity from Sepharose 4B column. A 15-ml column was equilibrated with 15 mM Tris·HCl/0.1 M KCl/1 mM EGTA/7.5 mM sodium pyrophosphate/1 mM dithiothreitol, pH 7.5, and eluted at 20 ml/hr. Bound proteins were eluted with a linear gradient (100 ml) of 0.1–0.5 M KCl. ●, Protein; ■, K^+,EDTA-ATPase activity. (*Inset*) Protein pattern of the major peak of ATPase activity.

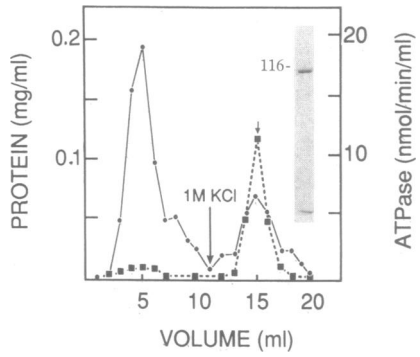

FIG. 3. Chromatography on ATP-agarose. Fractions from the Q Sepharose column were loaded on a 2-ml ATP-agarose column, equilibrated with 15 mM Tris·HCl, pH 7.5/50 mM KCl/1 mM EGTA/1 mM dithiothreitol at a flow rate about 5 ml/hr, then eluted with 1 M KCl at 20 ml/hr. ●, Protein; ■, K^+,EDTA-ATPase activity. (*Inset*) Protein pattern of the ATPase activity peak.

peaks containing myosin I from Sepharose 4B and Q Sepharose columns were not contaminated by adrenal myosin II or its proteolytic fragments, the immunoreactivity of these fractions with anti-myosin II antibodies was examined. Whereas anti-myosin II antibodies recognize adrenal myosin II from the Sepharose 4B column, no polypeptide in the myosin I-containing fractions reacted with these antibodies (data not shown).

Consistently, a minor peak of ATPase activity at 0.2 M KCl also is eluted from the Q Sepharose column. However, since this peak exhibits very low activity and contains many polypeptides as revealed by SDS/PAGE, the further purification of the ATPase(s) from these fractions was not undertaken in the present study. Final purification of myosin I is achieved by affinity chromatography on an ATP-agarose column (Fig. 3). ATPase activity is eluted from the column with either 1 M KCl or 10 mM ATP.

When the procedure outlined above is used, between 0.2 and 0.5 mg of pure myosin I per 100 g of adrenal cortex or medulla is obtained. Although this procedure provides an extensive purification of myosin I from brain, both the yield (about 50 μg per 100 g of tissue) and the purity are significantly lower than those achieved from adrenal tissues. Therefore, the method for brain myosin I purification must be improved.

The results of one purification of medulla myosin I are summarized in Table 1. Since at the initial steps of purification the ATPase activity is mostly due to the presence of myosin II, the recovery of myosin I ATPase activity is related to the activity of the second peak from the Sepharose 4B column.

Structure of Adrenal Medulla Myosin I. Myosin I is composed of high (116-kDa) and low (about 16-kDa) molecular mass polypeptides (Fig. 4*A*). The electrophoretic mobility of the low molecular mass polypeptide is dependent on the Ca^{2+} concentration: at 0.1 mM Ca^{2+} it migrates as a 16-kDa polypeptide, whereas at 2 mM EGTA its molecular mass corresponds to 19 kDa (Fig. 4*B*). The molecular mass of the small subunit and its shift in electrophoretic mobility are characteristic of calmodulin. Indeed, this polypeptide was recognized by anti-calmodulin antibodies (Fig. 4*C*). Moreover, the ability of the 116-kDa myosin I heavy chain to bind purified calmodulin was confirmed by using a gel overlay method with ^{125}I-calmodulin (Fig. 5*B*). Therefore, by several criteria, the low molecular mass subunit may be identified as calmodulin. The molar ratio of the 116-kDa heavy chain to calmodulin was in the range of 1:2.6 to 1:3.3 as estimated by scanning of Coomassie-blue-stained gels of three purified myosin I preparations. Purified brush border myosin I, with a known stoichiometry of four calmodulins per 119-kDa heavy chains (23), was used as a standard (chicken brush border myosin I was a gift of Jimmy Collins, Eastern Virginia Medical School). To determine its native structure, myosin I was subjected to gel filtration on a calibrated Sephacryl S-300 column and sedimentation on a 5–20% sucrose gradient (data not shown). The Stokes radius (6.9 nm) and sedimentation coefficient (7.5 S) suggest that myosin I is an asymmetric protein composed of a single heavy chain and four calmodulin light chains.

To identify functional domains on the myosin I heavy chain, the protein was subjected to limited proteolysis with

Table 1. Purification of myosin I from adrenal medullae

Fraction	Volume, ml	Protein, mg	K^+,EDTA-ATPase activity: Specific activity, μmol·min^{-1}·mg^{-1}	K^+,EDTA-ATPase activity: Total activity, μmol·min^{-1}	K^+,EDTA-ATPase activity: Recovery, %	Ca^{2+}-ATPase activity: Specific activity, μmol·min^{-1}·mg^{-1}	Ca^{2+}-ATPase activity: Total activity, μmol·min^{-1}	Ca^{2+}-ATPase activity: Recovery, %
Extract	515	5560	0.005	27.8		0.023	128.0	
Actomyosin pellet	18	240	0.060	14.4		0.058	13.9	
Supernatant before Sepharose 4B	15	126	0.110	13.86		0.020	2.52	
Sepharose 4B								
Myosin II	4	9.5	0.270	2.56		0.066	0.627	
Myosin I	23	30.0	0.025	0.75	100	0.028	0.840	100
Q Sepharose	4	2.5	0.092	0.23	31	0.111	0.280	33.3
ATP-agarose	1.8	0.5	0.190	0.09	12	0.200	0.100	12.0

The purification procedure is described under *Materials and Methods*.

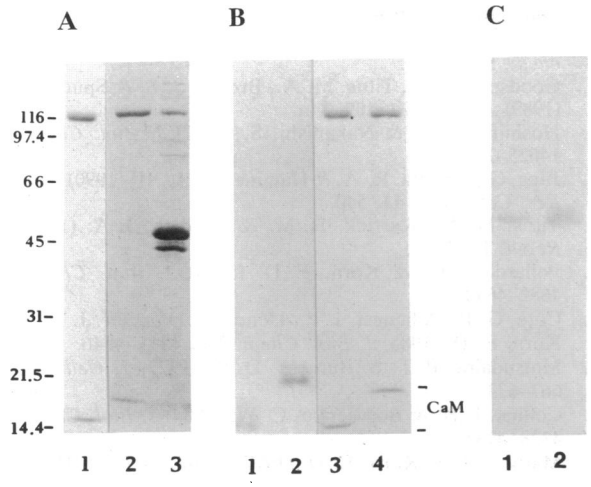

FIG. 4. Purified myosin I. (*A*) SDS/PAGE of myosin I preparations from adrenal medulla (lane 1), adrenal cortex (lane 2), and brain (lane 3). Molecular weight markers are shown to the left of the gel. (*B* and *C*) Identification of calmodulin in myosin I preparations. (*B*) Samples of medullar myosin I were separated by SDS/PAGE in the presence of 0.1 mM $CaCl_2$ or 2 mM EGTA (lanes 3 and 4, respectively). Samples of pure calmodulin (CaM) (lanes 1 and 2) were run as a standard. The 16-kDa band migrated faster in the presence of Ca^{2+} (lanes 1 and 3) than in the presence of EGTA (lanes 2 and 4). (*C*) Immunoblot of pure calmodulin (lane 1) and medullary myosin I (lane 2) with anti-calmodulin antibodies. Samples were separated by urea/glycerol/PAGE in the presence of 2 mM EGTA.

chymotrypsin (Fig. 5*A*). Digestion in the presence of 100 μM Ca^{2+} yields two major peptide fragments, 74 and 36 kDa. A gel overlay method with ^{125}I-calmodulin shows that calmodulin binds to the intact heavy chain and to the 36-kDa fragment (Fig. 5*B*). Photoaffinity labeling with [α-^{32}P]ATP shows that the intact heavy chain and the 74-kDa fragment contain the nucleotide-binding site (Fig. 5*C*). Chymotryptic digestion in the presence of 1 mM EGTA produces a single major polypeptide of 100 kDa, which contains both calmodulin- and ATP-binding sites (data not shown).

Actin-Activated ATPase Activity. All known myosins interact with F-actin in an ATP-sensitive manner and exhibit actin-activated Mg^{2+}-ATPase activity. A cosedimentation assay was used to confirm that medullary myosin I has a higher affinity for actin in the absence than in the presence of

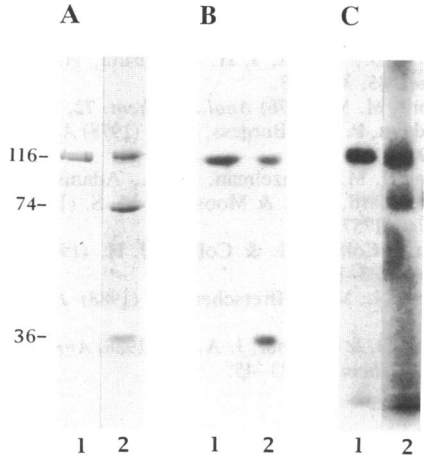

FIG. 5. Identification of functional domains of medullary myosin I. Myosin I was digested with chymotrypsin in the presence of 0.1 mM Ca^{2+}. Lanes 1, intact myosin, lanes 2, chymotryptic digest. (*A*) Gel stained with Coomassie blue. (*B*) Autoradiogram of the gel overlaid with ^{125}I-calmodulin. (*C*) Autoradiogram of the gel of [α-^{32}P]ATP-labeled myosin digest.

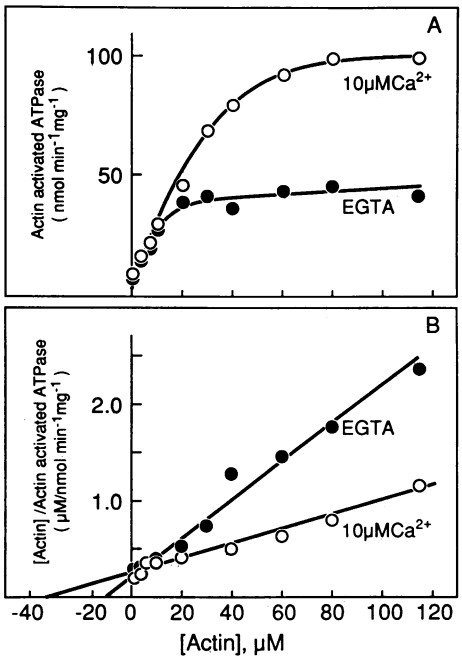

FIG. 6. Actin-activated ATPase activity. (*A*) The Mg^{2+}-ATPase activity was assayed as function of actin concentration in the presence of either 1 mM EGTA (●) or 10 μM Ca^{2+} (○). (*B*) Hanes plot of the data shown in *A*. The slopes of this plot give $1/V_{max}$ and the intercepts on the *x*-axis give $-K_{ATPase}$ (actin concentration required for half-maximal activation).

ATP (data not shown). The Mg^{2+}-ATPase activity of medullary myosin I is very low (1–3 nmol·min^{-1}·mg^{-1}) but is strongly activated by actin (Fig. 6*A*). Moreover, the extent of actin activation depends on the presence or absence of Ca^{2+}. In 1 mM EGTA, the ATPase activity is stimulated 40-fold to a V_{max} of 50 nmol·min^{-1}·mg^{-1}, while in 10 μM Ca^{2+}, a 90-fold stimulation to 128 nmol·min^{-1}·mg^{-1} is observed (Fig. 6*B*). Ca^{2+} increases the V_{max} of the ATPase reaction but also increases the K_m. Therefore, the enhanced activity in the presence of Ca^{2+} cannot be explained by an increase in the affinity of adrenal myosin I for F-actin. This enhancement of actin-activated ATPase activity occurs over a Ca^{2+} concentration range of 1–100 μM. However, at 100 μM Ca^{2+}, an appreciable non-actin-dependent Mg^{2+} ATPase activity (about 10 nmol·min^{-1}·mg^{-1}) was observed.

DISCUSSION

This paper describes the first purification and characterization of a mammalian myosin I. Until now the only available information about myosin I from higher eukaryotes was derived from one tissue, the intestinal brush border: the avian microvillar protein has been purified and characterized and the bovine brush border gene has been sequenced. Several recent reports have raised doubts whether myosin I is present in tissues other than the intestinal epithelium. For example, Northern blot analyses using nucleotide probes derived from the avian (27) or bovine (11) brush border sequences detected myosin I mRNA only in intestinal tissue. Significantly, this method did not reveal the presence of myosin I mRNA in bovine adrenal medulla or brain. Immunoblotting and immunolocalization studies using antibodies against the brush border myosin I heavy chain also showed that in the chicken its distribution is restricted to intestinal epithelial tissue. The failure to detect myosin I mRNA or protein in nonintestinal tissues suggests that intestinal and nonintestinal forms of the enzyme have significantly different primary structures. Partial protein sequence analysis of adrenal medullary myosin I

supports this suggestion. Although the mammalian protein contains the characteristic HYAGE sequence common to all currently known forms of myosin I, three other fragments (APLGGGRVPGWIW, ANLXYAGGVXW, and LTVISFTEXEVE) are not identical to any known myosin I sequence, including that of the bovine intestinal brush border. Also, immunoblots (39) of purified adrenal medullary myosin I (4 μg loading) showed no reactivity with polyclonal antibodies raised against native avian brush border myosin I (data not shown).

Despite the apparent differences between intestinal epithelial myosin I and the brain and adrenal enzymes, they are clearly more similar to each other than to their protozoan counterparts. Among their shared characteristics are heavy chain molecular weight, the presence of multiple calmodulin light chains, and similar chymotryptic digestion patterns (38, 40). In addition, the actin-activated ATPase activities of brush border (23, 25) and adrenal medullary myosins I are Ca^{2+} sensitive: In 10 μM Ca^{2+} the activation is 2- to 3-fold higher than in the presence of EGTA. Although the exact mechanism of Ca^{2+} stimulation is not yet known, steady-state kinetic experiments (Fig. 6) indicate that the enhanced activation is due to an increase in the V_{max} of the reaction rather than to a change in the affinity of myosin I for F-actin. In view of the relatively small effect of Ca^{2+}, it is reasonable to assume that another level of regulation occurs in the cell. Phosphorylation is a likely regulatory mechanism since this modification affects the actin-activated ATPase activities of most forms of myosin (41). For example, protozoan myosins I are strictly dependent on heavy chain phosphorylation by a specific myosin I kinase for expression of their actin-activated ATPase activities. In contrast, heavy chain phosphorylation of protozoan myosins II inhibits their enzymatic activities.

The results described in this paper suggest that myosin I may be a ubiquitous actin-dependent force-generating enzyme. Its relative abundance in well-characterized secretory cells affords the possibility of future investigations aimed at determining the function of myosin I in various aspects of intracellular motility.

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