Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 6064–6068, June 1996
Neurobiology

An axoplasmic myosin with a calmodulin-like light chain

ELAINE L. BEARER*†‡, JOSEPH A. DEGIORGIS*†, HOWARD JAFFE§, NELSON A. MEDEIROS*†, AND THOMAS S. REESE†¶

*Department of Pathology and Laboratory Medicine, Brown University, Providence, RI 02912; †Marine Biological Laboratory, Woods Hole, MA 02543; §Laboratory of Neurochemistry/National Institute of Neurological Disorders and Stroke Protein/Peptide Sequencing Facility, and ¶Laboratory of Neurobiology, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892

Contributed by Thomas R. Reese, Janaury 3, 1996

ABSTRACT **Organelles in the axoplasm from the squid giant axon move along exogenous actin filaments toward their barbed ends. An ≈235-kDa protein, the only band recognized by a pan-myosin antibody in Western blots of isolated axoplasmic organelles, has been previously proposed to be a motor for these movements. Here, we purify this ≈235-kDa protein (p235) from axoplasm and demonstrate that it is a myosin, because it is recognized by a pan-myosin antibody and has an actin-activated Mg-ATPase activity per mg of protein 40-fold higher than that of axoplasm. By low-angle rotary shadowing, p235 differs from myosin II and it does not form bipolar filaments in low salt. The amino acid sequence of a 17-kDa protein that copurifies with p235 shows that it is a squid optic lobe calcium-binding protein, which is more similar by amino acid sequence to calmodulin (69% identity) than to the light chains of myosin II (33% identity). A polyclonal antibody to this light chain was raised by using a synthetic peptide representing the calcium binding domain least similar to calmodulin. We then cloned this light chain by reverse transcriptase-PCR and showed that this antibody recognizes the bacterially expressed protein but not brain calmodulin. In Western blots of sucrose gradient fractions, the 17-kDa protein is found in the organelle fraction, suggesting that it is a light chain of the p235 myosin that is also associated with organelles.**

Organelles from squid axoplasm move toward the barbed ends of actin filaments (1–3). The actin-based motor responsible for these movements is intrinsic to organelles, because KI-washed organelles separated from soluble axoplasmic proteins by sucrose density fractionation move on exogenous actin bundles in the absence of cytosol (4). This actin-based motility is presumably mediated by a myosin.

Although the myosin family has recently been expanded by means of molecular techniques to include at least 10 subclasses, the criteria for defining these classes rest primarily on their sequences (5). Existing information about these classes provides few clues as to which, if any, might be responsible for organelle movements. Thus, we set out to directly isolate the ≈235-kDa organelle-associated protein (p235) from axoplasm and confirm that it is a myosin.

Dissection of the axon and extrusion of its axoplasm provides a starting material enriched in the machinery of transport and devoid of cortical cytoplasm and nuclei (6, 7). The composition of myosins in the axon sheath differs from extruded axoplasm, which contains at least five myosins ranging from 240 kDa to 150 kDa (2). All of these axoplasmic myosins are present in the supernatant after sucrose density fractionation of KI-solubilized axoplasm; however, only one, an ~235-kDa band, cosediments with organelles through the gradient and appears to migrate at exactly the same molecular weight as the middle band of the three high molecular weight myosins present in the supernatant. Thus, like kinesin (8), a putative organelle myosin also appears in a soluble form present in high enough abundance to be purified.

Despite the difficulty of obtaining the large amounts typically needed for protein biochemistry, we chose extruded axoplasm as the source of p235 because it has a relatively simple complement of myosins (2, 9). We used cytosol after KI extraction and sucrose density fractionation as a source of soluble p235 because p235 ran at exactly the same apparent molecular weight as the band detected in the organelle fraction. Once p235 was purified from axoplasm, it was possible to demonstrate that it is a functional myosin, with characteristic actin-activated Mg-ATPase activity. Furthermore, its size and shape, as well as the characteristics of a putative light chain that copurified with p235, suggest that it is an unconventional myosin. Western blots using antibodies to the putative light chain show that it is also present in the organelle fraction.

MATERIALS AND METHODS

Squid (*Loligo pealeii*) and horseshoe crabs (*Limulus polyphemus*) were obtained through the Marine Resources Center at the Marine Biological Laboratories (Woods Hole, MA). Axons were dissected in Ca-free sea water and stored in liquid nitrogen until use.

Isolation of p235. Axoplasm was extruded from thawed axons and triturated in half strength motility buffer (½×) (10) containing 1 mM DTT and the protease inhibitors benzamidine, leupeptin, pepstatin, and aprotinin, all at 10 μg/ml. For isolation of p235 from cytosol, 400–800 μl of axoplasm from 400–600 cm of axon representing approximately 40 animals was used. To release proteins and organelles from the dense axoplasmic matrix, extruded axoplasm was triturated in 0.6 M KI in ½× motility buffer for 10 min followed by 1:1 dilution in ½× motilty buffer containing protease inhibitors (11, 12). KI-treated axoplasm layered on a three-step sucrose gradient (45%, 15%, 12%) was centrifuged at 180,000 × *g* for 1.5 h at 4°C in a Beckman 50.1 SW rotor. Fractions were removed by side puncture according to the markings on the tubes.

Cytosol from four sucrose density gradients was pooled and then separated into 100-μl aliquots. Each aliquot was diluted 15-fold with distilled water (1.5 ml) containing 5 mM DTT and protease inhibitors at 10 μg/ml each and incubated for 1 h on ice. Precipitates were collected by centrifugation at 15,000 × *g* for 15 min at 4°C. Proteins left in the low-salt supernatant were collected by precipitation in 10% trichloroacetic acid (TCA). Proteins present in each step of the isolation procedure were analyzed by SDS/PAGE and Western blotting a polyclonal pan-myosin antibody (2, 13).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. U57550).

‡To whom reprint requests should be addressed at: Department of Pathology and Laboratory Medicine, Division of Biology and Medicine, Box G, Brown University, Providence, RI 02912. e-mail: Elaine_Bearer@postoffice.brown.edu.

ATPase Assay. Pellets were resuspended in AB solution (4) or HNM solution (2), and ATPase was measured according to Bearer *et al.* (2). Soluble phosphate was measured colorimetrically (14).

Rotary Shadowing of p235. For low-salt preparations, either 10 μl of p235 at a concentration of 4 μg/20 μl of AB solution, or 2 μl of rabbit muscle myosin II at 20 μg/ml in myosin storage buffer (MS: 1 M KCl/2 mM $MgCl_2$/4 mM $NaHCO_3$/2 mM EGTA/1 mM DTT/3 mM NaN_3/50% glycerol) was mixed with 10 μl of 1 M ammonium acetate buffer, and distilled water was added to a final volume of 45 μl. Then 65 μl of glycerol was added and 50 μl of the mixture was sprayed onto freshly cleaved mica (15). For high-salt preparations, 1 μl of rabbit muscle myosin at 20 μg/ml in water was mixed with 125 μl of 2 M KCl, 225 μl of distilled water, and 650 μl of glycerol. Sprayed mica squares were rotary shadowed at 8–10° with a platinum-carbon mixture followed by carbon. Replicas were viewed at 120 kV in a JEOL 200-CX.

Amino Acid Sequencing. Sequences were obtained directly by digestion of the low-salt precipitate as well as from protein bands excised from SDS gels. For sequencing directly from the low-salt precipitate, ≈500 μl containing ≈15–20 μg of p235 squid myosin in 5 mM Tris, pH 7.4/0.5 mM DTT was dried in a Speed Vac (Savant). The dried residue was taken up in 50 μl of 8 M urea/0.4 M NH_4HCO_3 and subjected to proteolytic digestion with endoproteinase Lys-C (16). The resultant digest was separated on a narrow-bore (2.1 × 150 mm) Zorbax 300SB-C8 column (Mac-Mod Analytical, Chadds Ford, PA) at 35°C on a System Gold HPLC equipped with a model 507 autosampler, model 126 programmable solvent module, and model 168 diode array detector (Beckman). Samples were eluted by a gradient at 0.25 ml/min as described in ref. 17, except that solvent A was 0.13% hexafluorobutyric acid (HFBA) and solvent B was acetonitrile (0.13% HFBA). Absorbance pf the column effluent was monitored at 220 and 280 nm. Fractions were collected at 30-s intervals and stored at −70°C. Selected fractions containing peptide peaks were concentrated, taken up in 50 μl of 8 M urea/0.4 M NH_4HCO_3, warmed at 35°C, and further purified by RP-HPLC on a narrow-bore (2.1 × 250 mm) Vydac 218TP52 column and guard column (The Separations Group) at 35°C eluted at 0.25 ml/min utilizing the trifluoroacetic acid/acetonitrile gradient (17). Absorbance of the column effluent was monitored at 210 and 280 nm.

For sequencing after SDS/PAGE, low-salt-precipitated protein from 800 μl of cytosol was pooled and electrophoresed through an 8.5% polyacrylamide curtain gel. Gels were stained with Coomassie blue, and the two low molecular mass bands, ≈17 and ≈10 kDa, were excised. Gel bands were subjected to *in situ* proteolytic digestion with endoproteinase Lys-C as described in ref. 18, modified as follows: after extraction of the proteolytic peptide fragments from the gel, SDS was precipitated by addition of an equal volume (≈200 μl) of 1 M guanidine hydrochloride and removed by filtration with a Millex-HV (Millipore) 0.45-μm microfilter unit (19). Filtered SDS-free digests were separated by RP/HPLC on the Vydac column (see above).

Selected fractions (125 μl) containing peptides were applied in 30-μl aliquots to a Biobrene (Applied Biosystems)-treated glass fiber filter and dried prior to amino acid sequencing on a model 477A pulsed-liquid protein sequencer equipped with a model 120A phenylthiohydantoin analyzer (Applied Biosystems) using methods and cycles supplied by the manufacturer. Data were collected and analyzed on a model 610A data analysis system (Applied Biosystems). Sequences were compared with those in the SwissProt database using the FASTA command of the GCG software package (20).

Cloning and Overexpression of the Calmodulin-Like Light Chain. Degenerate DNA primers were synthesized on the basis of the amino acid sequence at the amino and carboxyl ends of the light chain (21) [N**GGATCC**GCNAAAGAGTTNTC-NGAAAAGC and N**AAGCTT**NGGGTCATCAT(C/T)TT-NAC(A/G)AA] with the addition of a *Bam*HI site at the amino end and an *Hin*dIII site at the carboxyl ends and used by RT-PCR to amplify the cDNA encoding this protein from total RNA extracted from squid optic lobe (22). Resultant products were ligated into pCRII vector (Invitrogen) and sequenced in an automated sequencer (Keck Foundation Biotechnology Sequencing Resource, Yale University School of Medicine, New Haven, CT). Two identical clones were obtained and sequenced. The cDNA was excised from the pCRII vector with *Bam*HI and *Hin*dIII, ligated into pQE 30 (Qiagen, Chatsworth, CA), and overexpressed as a fusion protein with a six-histidine tag at the amino terminus. Overexpression in bacteria was induced with 1 mg/ml isopropyl β-D-thiogalactopyranoside at 30°C overnight. Bacteria were harvested, pelleted, and resuspended in gel sample buffer for Western blotting.

Anti-Peptide Antibodies and Western Blotting. A peptide designed according to the amino acid sequence of the second calcium- binding domain of the calmodulin-like protein (DT-DGNGTIQYA) was synthesized, coupled to ovalbumin, and used as an immunogen in rabbits (Babco, Oakland, CA). Resultant antisera had a titer against squid protein of 1/2000. Brain calmodulin (Sigma) in gel sample buffer was used as a negative control for crossreaction of the antisera with calmodulin by Western blotting, performed as described (2).

RESULTS

Isolation of a 235-kDa Myosin from Axoplasm. Axoplasm contains at least five proteins recognized by polyclonal antibodies raised to scallop muscle myosin II (Fig. 1, lane a).

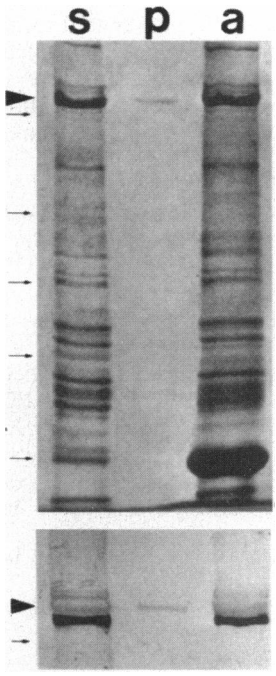

FIG. 1. Purification of the putative organelle myosin p235. Coomassie-stained 6% SDS gel (*top*) and corresponding immunoblot with the pan-myosin antibody (*bottom*) showing low-salt supernatant (lane s), low-salt pellet (lane p), and KI-treated axoplasm (lane a). Note that the pellet contains a single ≈235-kDa band recognized by the anti-myosin antibody (arrowhead indicates 235 kDa at left). In contrast, the low-salt supernatant displays a banding pattern similar to that of the starting material, solubilized axoplasm, including many myosins, one of which is a darker band than p235 in the blot. Molecular weight indicated by small arrows are 200, 116, 98, 68, and 45 kDa (*top*) and 200 kDa (*bottom*).

Fifteen-fold dilution of cytosol from a sucrose density gradient separation of axoplasm precipitated a single band migrating with an apparent molecular weight of ~235 kDa (Fig. 1, lane p). The pan-myosin antibody identified this protein as the middle of the three high molecular weight myosins in axoplasm and demonstrated that this species is enriched in the low-salt precipitate compared to other myosins. Approximately one-half of the ~235-kDa myosin, which we shall refer to as p235, remained in the low-salt supernatant (Fig. 1, lane s). Typically, 4 μg of p235 were obtained from 50 cm of axon (squid have two giant axons each ~0.6 mm in diameter and 7–10 cm long), not enough for further purification by column chromatography.

ATPase Activity of p235. In contrast to cytosol (Table 1), where baseline specific activity ATP hydrolysis was high and increased only 2-fold with added actin, the p235-enriched low-salt pellet had a barely detectable baseline activity that increased 40-fold in the presence of actin, releasing 250 nmol of phosphate per mg of protein per minute (Table 1). This rate of ATP hydrolysis per mg of protein is in the same range as nonmuscle myosin IIs measured under similar conditions: 69–420 nmol of phosphate released per mg of protein per minute (23).

Structure of p235 by Glycerol-Spray, Low-Angle Rotary Shadowing. Myosin II and p235 appeared distinctly different by electron microscopy of low angle, rotary shadowed preparations (Fig. 2). Myosin II has two heads 18 nm in diameter and a tail 150 nm long (24). Purified myosin II from rabbit skeletal muscle sprayed onto the mica in buffer containing 125 mM of salt appeared as typical thick filaments (data not shown). With 250 mM of salt in the spray buffer, myosin molecules had two heads averaging 17 nm in length (Fig. 2*a*). In contrast, replicas of p235 resuspended in 102 mM of salt revealed some two-headed molecules as well as many single heads and other fragments, but no bipolar filaments (Fig. 2 *b–f*). The majority of the heads varied from between 20 to 25 nm in diameter. Larger heads, up to ≈35 nm in diameter, appear to be superimpositions of multiple heads. The slender, long neck between the head and tail of p235 also differentiated it from myosin II. Finally, the tails of p235 appeared twice as thick as those of myosin II, but they are only 75–120 nm long as compared with 150 nm for myosin II.

Calmodulin-Like Protein Associated with p235. In SDS/8.5% PAGE a prominent low-molecular weight band, or sometimes two bands, copurified with p235 through extraction in 0.6 M KI, sucrose density fractionation, and precipitation in low salt. Like the light chains of other myosins these low molecular weight bands migrated near 17 kDa (Fig. 3*A*). Edman degradation of the low-salt pellet containing p235 yielded four sequences: (*i*) QIAEIKDAFDMFDIDGDGQI, (*ii*) XMGPTDPEKEMREAFXV, (*iii*) XXXXTPXDAELEEM, and (*iv*) XLGRTPXXAELEE. Exact matches to the squid optic lobe calcium-binding protein, cabo_lolpe (21), for each sequence respectively were: (*i*) amino acids 8–27, (*ii*) amino acids 75–91, (*iii*) amino acids 38–51, and (*iv*) amino acids 38–50 (Fig. 3*B*).

Table 1. Actin-activated Mg-ATPase activity in cytosol and purified p235

	Pi, μmol released/h	Protein,* μg	Specific activity, nmol Pi/mg/min
KI cytosol			
− actin	18	25	12
+ actin	27	25	18
p235			
− actin	0.3	0.8	6
+ actin	12	0.8	250

Samples were incubated for 1 h with (+) or without (−) 50 μg of actin in 30 mM Hepes (pH 7.6), 20 mM $NaCl_2$, 4 mM EGTA, and 10 mM Mg_2-ATP. Measurements were zeroed against blanks containing 10 mM ATP and 50 μM of actin without addition of cytosol or p235. *Protein concentrations determined by Bradford assay.

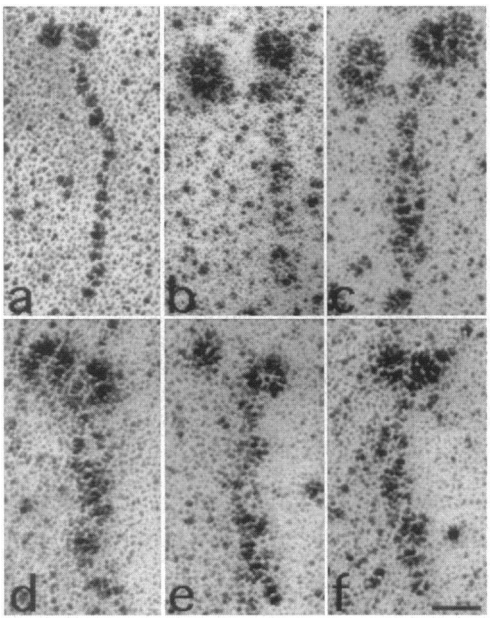

FIG. 2. Rotary shadowing of myosin II (*a*) and p235 (*b-f*). Myosin II resuspended in 100 mM ammonium acetate shows typical two headed form (*a*). p235 heads are larger and the tail is shorter and thicker than those of myosin II tail (*b–f*). Some larger heads are present (*c* and *d*), which may represent aggregates of the two-headed form. Whereas the variability makes it difficult to further define the structure to this molecule, these images clearly differentiate it from myosin II. (Bar = 50 nm.)

To determine whether these sequences arose from the prominent low molecular weight bands migrating at 17 and 10 kDa, these bands were excised from Coomassie-stained SDS gels, and both bands digested *in situ*, yielding digests in which many of the peptide peaks eluted at identical retention times. Amino acid sequencing of a peptide from the 17-kDa band eluting at 75.2 min gave XXXEEISEMIREAXIXG. Amino acid sequencing of a peptide from the 10-kDa band also eluting at 75.2 min gave LTSEEISEMIREADIDGDGM. Both of these sequences matched cabo_lolpe at amino acid 119–135, with the expected cleavage by endoproteinase Lys-C at K amino acid 118. The observed migration time on SDS/PAGE of the upper band is consistent with the calculated molecular weight of cabo_lolpe (17 kDa), whereas the lower band is presumed to be a 10-kDa cleavage product of cabo_lolpe. Since 8.5% gels do not separate low-molecular weight proteins well, the stoichiometry between the ≈235-kDa heavy chain and this calmodulin-like protein was not determined.

The p235 associated light chain is 69% identical to calmodulins of related species by amino acid sequence, but only 33% identical to the regulatory light chain of squid mantle muscle myosin II (Fig. 3*B*). The calmodulins are 98% identical to each other, but like the p235 light chain are 33% identical to RLC. This comparison also disclosed four conserved calcium-binding sites between the p235 light chain and the calmodulins, one of which is also present in the regulatory light chain. Furthermore, three of the four known serine/threonine phosphorylation sites identified in calmodulins (25) are present in the p235 light chain. A fifth potential serine phosphorylation sequence, SLG, is highly conserved in all four proteins.

Because the p235 light chain is very similar to calmodulin, antibodies were raised against a synthetic peptide from the second calcium-binding site, which was designed to maximize differences between the two species. Antisera raised against the synthetic peptide recognized bacterially expressed light chain but failed to detect purified brain calmodulin, demon-

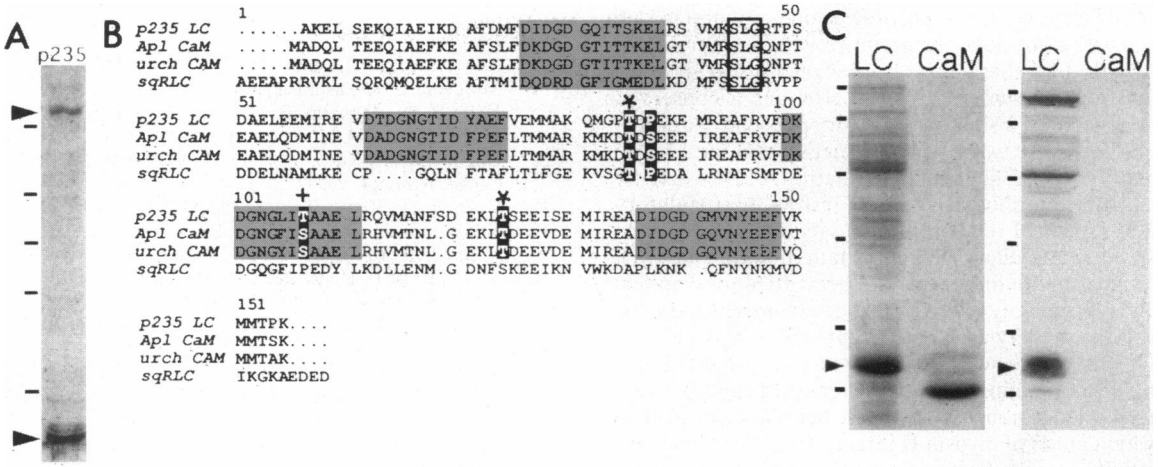

FIG. 3. Calmodulin-like light chain associated with p235. (*A*) Coomassie-stained 8.5% SDS gel of p235 purified by low-salt precipitation. The arrowhead indicates a prominant band migrating below the 34 kDa standard. Molecular weight standards, at left, are: 200 kDa, 98 kDa, 68 kDa, 45 kDa, and 34 kDa. (*B*) The amino acid sequence of five peptides obtained from the low-molecular weight band were identical to that of squid optic lobe calcium binding protein (see *Results*). Comparison of this amino acid sequence (p235 LC) with sequences of calmodulin from *Aplysia californica* (Apl CaM), *Arabacia punctulata* (sea urchin, urch CaM), and squid muscle myosin regulatory light chain (sqRLC) reveal highly conserved sequences in calcium-binding domains (gray shadding). Comparison with the calmodulins also identifies potential serine/threonine phosphorylation sites (white letters on black baclground), with conserved Ts indicated with an (*), and semiconservative S/T substitutions with a (+). Another highly conserved short sequence, SLG, which might serve as an additional phosphorylation site, is indicated by a box. (*C*) Coomassie-stained gel (left two lanes) and corresponding Western blot (right two lanes) of bacterial pellet containing expressed p235 light chain and bacterial proteins (LC) and purified bovine brain calmodulin (CaM). The blot was probed with a polyclonal anti-peptide antibody raised against the second calcium-binding motif of p235 LC, DTDGNGTIQYA. Position of migration of the LC is indicated by arrowhead. The polyclonal antibody reacts against other bacterial proteins but not with purified brain calmodulin. Molecular weight standards indicated to the left of gel and blot: 99, 68, 45, 31, 21, and 14 kDa.

strating that this strategy produced an antiserum specific for this light chain (Fig. 3*C*).

Calmodulin-Like Protein Cosediments with Axoplasmic Organelles. The calmodulin-like putative p235 light chain was detected in Western blotting of the 15% fraction of a sucrose gradient loaded with KI-solubilized axoplasm and probed with the specific anti-light chain polyclonal antibody (Fig. 4, lane G) and Coomassie-stained gel (Fig. 4, lane B). Under the conditions of this fractionation, a protein of 17 kDa would not penetrate the 15% sucrose layer. Hence, the presence of this protein at this level of the gradient indicates that it must be associated with some heavier component of axoplasm, such as organelles, which are highly enriched in the 15% fraction.

DISCUSSION

We have isolated from squid axoplasm a protein complex, p235, which appears to be a myosin because its heavy chain is recognized by an anti-myosin antibody, and it has actin-dependent ATPase activity. By low-angle rotary shadowing, p235 has a structure consistent with myosins in general, but distinctly different from conventional myosin II. Also, p235 copurifies with a 17-kDa calmodulin-like protein with features similar to the light chains of other myosins (26). This putative p235 light chain also cosediments with organelles.

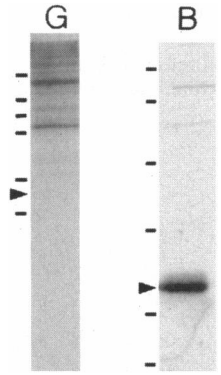

FIG. 4. Calmodulin-like light chain cosediments with organelles. Coomassie-stained SDS/15% PAGE (lane G) and corresponding Western blot (lane B) of the 15% fraction of a sucrose density gradient probed with anti-light chain antibody. Arrowhead indicates 17-kDa light chain; molecular weight standards are indicated at 98, 68, 45, 31, 21, and 14 kDa.

Purification and Properties of p235. Since p235 was identified in axoplasm, which can only be obtained in small amounts, purification was of necessity a simple procedure depending on low-salt precipitation, a strategy used to purify both conventional (27–29) and unconventional myosins (30). This simple purification scheme could not be expected to produce highly purified protein in abundance. However, Western blotting shows significant enrichment for the 235-kDa myosin compared to the other myosins detected in axoplasm. Furthermore, the low-salt-precipitated protein comigrated with the middle of the three high molecular weight myosins, the same migration as that of the myosin copurifying with organelles (2).

Because actin-activated ATPase activity suggests that p235 is a myosin, the structure of p235 frequently seen in glycerol spray preparations—two heads larger than those of myosin II and a thick tail shorter than that of myosin II—suggests that it is more similar to unconventional myosin V than to conventional myosin II (31). That the head of p235 is larger than that of myosin II could be due to a longer amino acid sequence in the head domain (32) or to the presence of a large number of light chains (31). Its failure to form bipolar filaments as well as the characteristics of an associated light chain (see below) further indicate that it is an unconventional myosin. One subclass of unconventional myosins, myosin IX, has members with molecular weights as large as 235 kDa (refs. 33 and 34; Myosin IX is primarily defined by the sequence between its ATPase and actin-binding domains though only rat and human sequences have been reported. No myosins of any class from the squid have yet been sequenced.

Calmodulin-Like Light Chain. The copurification of the calmodulin-like 17- and ≈235-kDa proteins suggests that they

are associated because the 17-kDa protein does not precipitate in low salt on its own (35). Furthermore, it has a number of similarities with the light chains of other myosins: (*i*) it binds four calcium ions *in vitro* (35); (*ii*) it contains a conserved threonine known to be a site for regulatory phosphorylation of calmodulin (25). These two properties suggest that the 17-kDa protein might be a p235 light chain regulating ATPase activity through calcium and phosphorylation, just as the regulatory light chain of myosin II regulates its activity (26). The 17-kDa protein is a fairly abundant soluble protein in optic lobe (21), and hence, like calmodulin, it may interact with more than one protein. Such promiscuity would provide a mechanism for coordinating the activity of the different target myosins.

Although other unconventional myosins have light chains that resemble calmodulin in their biochemical properties (31, 36), to our knowledge none of these have been sequenced. The regulatory light chain of myosin II inhibits its actin-dependent ATPase activity unless phosphorylated by myosin light chain kinase (26), but it is still not known whether calmodulin-like light chains perform similar functions for unconventional myosins. Another calmodulin-like protein in yeast, cdc4, co-purifies with a 200-kDa protein, and phenotypic defects in cdc4 mutants suggest that it regulates a myosin (37).

The fact that organelles move along actin filaments in axoplasmic spreads (1, 2) suggests that organelles have a myosin on their surfaces. We have recently shown that organelles move on actin filaments even after organelles are separated from soluble myosins by sucrose density fractionation in the presence of KI (4). This finding confirms that actin-based motility is intrinsic to organelles, probably mediated by a myosin tightly associated with their surfaces. Because the ≈235-kDa myosin is the only band detected by this pan-myosin antibody in KI-washed and sucrose-density fractionated organelles, it could well be the motor for these organelle movements. The association of the 17-kDa putative light chain with KI-washed organelles is further evidence for this notion.

We thank John Chludzinski for help with the photography, and Bonnie Reese and Kasia Hammar for help in the laboratory. We are particularly grateful to Sven Beushausen for the gift of squid optic lobe RNA and advice on RT-PCR, to Ben Greenfield for his help with cloning the light chain, and to Andrew Szent-Györgyi for his polyclonal antibody to scallop myosin II as well as his advice. This work was supported in part by National Institutes of Health Grant GM47368 and Council for Tobacco Research Grant 3192.

1. Kuznetsov, S. A., Langford, G. M. & Weiss, D. G. (1992) *Nature (London)* **356,** 722–725.
2. Bearer, E. L., DeGiorgis, J. A., Bodner, R. A., Kao, A. W. & Reese, T. S. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11252–11256.
3. Langford, G. M., Kuznetsov, S. A., Johnson, D., Cohen, D. L. & Weiss, D. G. (1994) *J. Cell Sci.* **107,** 2291–2298.
4. Bearer, E. L., DeGiorgis, J. A., Medeiros, N. A. & Reese, T. S. (1996) *Cell Motil. Cytoskeleton* **33,** 106–114.
5. Goodson, H. V. & Spudich, J. A. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 659–663.
6. Morris, J. R. & Lasek, R. J. (1982) *J. Cell Biol.* **92,** 192–198.
7. Metuzals, J. & Tasaki, I. (1978) *J. Cell Biol.* **78,** 597–621.
8. Vale, R. D., Schnapp, B. J., Reese, T. S. & Sheetz, M. P. (1985) *Cell* **40,** 449–454.
9. Cheney, R. E., Riley, M. A. & Mooseker, M. S. (1993) *Cell Motil. Cytoskeleton* **24,** 215–223.
10. Vale, R. D., Reese, T. S. & Sheetz, M. P. (1985) *Cell* **42,** 39–50.
11. Schroer, T. A., Schnapp, B. J., Reese T. S. & Sheetz, M. P. (1988) *J. Cell Biol.* **107,** 1785–1792.
12. Schnapp, B. J., Reese, T. S. & Bechtold, R. (1992) *J. Cell Biol.* **119,** 389–399.
13. Wallimann, T. & Szent-Györgyi, A. G. (1981) *Biochemistry* **20,** 1176–1187.
14. Taussky, H. H. & Shorr, E. (1953) *J. Biol. Chem.* **202,** 675–685.
15. Tyler, J. M. & Branton, D. (1980) *J. Ultrastruct. Res.* **71,** 95–102.
16. Stone, K. L., LoPresti, M. B. & Williams, K. R. (1990) in *Laboratory Methodology in Biochemistry*, ed. Fini, C. F. (CRC, Boca Ratan, FL), pp. 181–205.
17. Fernandez, J., DeMott, M., Atherton, D. & Mische, S. M. (1992) *Anal. Biochem.* **201,** 255–264.
18. Kawasaki, H., Emori, Y. & Suzuki, K. (1990) *Anal. Biochem.* **191,** 332–336.
19. Riviere, L. R., Fleming, M., Elicone, C. & Tempst, P. (1991) in *Techniques in Protein Chemistry II*, ed. Villafranca, J. J. (Academic, San Diego), pp. 171–179.
20. Devereux, H., Haeberli, L. & Smithies, J. (1984) *Nucleic Acids Res.* **12,** 387–395.
21. Head, J. F. (1989) *J. Biol. Chem* **264,** 7202–7209.
22. Beushausen, S., Kladakis, A. & Jaffe, H. (1993) *DNA Cell Biol.* **12,** 901–909.
23. Kelley, C. A., Takahashi, M., Yu, J. H. & Adelstein, R. S. (1993) *J. Biol. Chem.* **268,** 12848–12854.
24. Elliott, A., Offer, G. & Burridge, K. (1975) *Proc. R. Soc. London B* **193,** 45–53.
25. Quadroni, M., James, P. & Carafoli, E. (1992) *J. Biol. Chem.* **269,** 16116–16122.
26. Tan, J. L., Ravid, S. & Spudich, J. A. (1992) *Annu. Rev. Biochem.* **61,** 721–759.
27. Pollard, T. D. (1982) *Methods Enzymol.* **85,** 331–356.
28. Burridge, K. & Bray, D. (1975) *J. Mol. Biol.* **99,** 1–14.
29. See, Y. S. & Metuzals, J. (1976) *J. Biol. Chem.* **251,** 7682–7689.
30. Bearer, E. L. (1991) *J. Cell Biol.* **115,** 1629–1638.
31. Cheney, R. E., O'Shea, M. K., Heuser, J. E., Coelho, M. V., Wolenski, J. S., Espreafico, E. M., Forscher, P., Larson, R. E. & Mooseker, M. S. (1993) *Cell* **75,** 13–23.
32. Takahashi, M., Kawamoto, S. & Adelstein, R. S. (1992) *J. Biol. Chem.* **267,** 17864–17871.
33. Reinhard, J., Scheel, A. A., Diekmann, D., Hall, A., Ruppert, C. & Bahler, M. (1995) *EMBO J.* **14,** 697–704.
34. Bement, W. M., Hasson, T., Wirth, J. A., Cheney, R. E. & Mooseker, M. S. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 6549–6553.
35. Sheldon, A. & Head, J. F. (1988) *J. Biol. Chem.* **263,** 14384–14389.
36. Collins, K., Sellers, J. R. & Matsudaira, P. (1990) *J. Cell Biol.* **110,** 1137–1147.
37. McCollum, D., Balasubramanian, M. K., Pelcher, L. E., Hemmingsen, S. M. & Gould, K. L. (1995) *J. Cell Biol.* **130,** 651–660.