MYR-1, a mammalian class I myosin, consisting of a heavy chain and 4–6 associated calmodulins, is represented by the 130-kDa myosin I (or MI130) from rat liver. MI130 translocates actin filaments in vitro in a Ca$^{2+}$-regulated manner. A decrease in motility observed at higher Ca$^{2+}$ concentrations has been attributed to calmodulin dissociation. To investigate mammalian myosin I regulation, we have coexpressed in baculovirus calmodulin and an epitope-tagged 85-kDa fragment representing the amino-terminal catalytic “motor” domain and the first calmodulin-binding IQ domain of rat myr-1; we refer to this truncated molecule here as MI1IQ. Association of calmodulin to MI1IQ is Ca$^{2+}$-insensitive. MI1IQ translocates actin filaments in vitro at a rate resembling MI130, but unlike MI130, does not exhibit sensitivity to 0.1–100 μM Ca$^{2+}$. In addition to demonstrating successful expression of a functional truncated mammalian myosin I in vitro, these results indicate that: 1) Ca$^{2+}$-induced calmodulin dissociation from MI130, but not MI1IQ, is not from the first IQ domain, 2) velocity is not affected by the length of the IQ region, and 3) the Ca$^{2+}$ sensitivity of actin translocation exhibited by MI130 involves 1 or more of the other 5 IQ domains and/or the carboxyl tail.

Class I myosins are small mechanochemical proteins able to couple nucleotide hydrolysis to the translocation of actin filaments. Multiple myosin I isoforms have been identified in organisms as diverse as yeast and humans. At least four class I myosins, designated by their genes as myr-1–4, are widely expressed in higher eukaryotic cells (see Ref. 1).

The myr-1 gene, which includes a motor domain followed by 6 calmodulin-binding, so-called IQ domains, and a carboxy-terminal tail region (2), codes for rat liver 130-kDa myosin I (3, 4) or MI130, as it is also known (5). Quantitation of the amount of calmodulin associated with the purified 130-kDa myosin I heavy chain has indicated that the heavy chain copurifies with 6 molecules of calmodulin (4), although isoforms corresponding to the 5 IQ and 4 IQ variants are also expressed in liver (2). myr-1 is most closely related in sequence to brush border myosin I (BBMI), which in microvilli cross-links the core bundle of actin filaments to the membrane (6, 7). Although expression of BBMI is essentially confined to intestine (8, 9), myr-1 is widely expressed (2). In NRK cells, MYR-1 is associated with the plasma membrane and in cell protrusions such as lamellipodia and membrane ruffles (10). Some 130-kDa myosin I is found in association with several subcellular fractions from rat liver, but the majority of this isoform in liver cells is most likely cytoskeleton-associated (11). These localization studies, together with recent kinetic analyses indicating among other things that the ATP-induced dissociation of actin-MI130 is slow, are consistent with a role for MYR-1 in maintenance of tension of the cytoskeleton (5).

The purified native 130-kDa myosin I translocates actin filaments in a Ca$^{2+}$-regulated manner. Motility is highest at pCa 7–8, and then decreases with increasing Ca$^{2+}$ concentrations. At free Ca$^{2+}$ concentrations above 0.1 μM, motility can be restored by the addition of exogenous calmodulin (12). One interpretation supported by in vitro actin binding assays is that calmodulin dissociates in the presence of Ca$^{2+}$ and that addition of excess calmodulin favors reassociation and therefore reestablishment of motility.

To assist in the molecular characterization of the 130-kDa myosin I, we have expressed in insect cells using the baculovirus expression system, a truncated myosin I heavy chain of 85 kDa representing the amino-terminal 728 amino acids of MYR-1, which includes the motor domain and the first of the 6 IQ domains that comprise the so-called neck region. This truncated myosin I heavy chain was co-expressed with calmodulin. The purified truncated myosin I heavy chain and its associated calmodulin are referred to here as MI1IQ. Biochemical analyses of this truncated myosin I have permitted evaluation of properties conferred on the parent 130-kDa myosin I molecule by the motor domain and the association of calmodulin to the first of 6 IQ domains. MI1IQ exhibits steady state ATPase activities resembling the parent molecule. Our results also indicate that binding of calmodulin to the first IQ domain in MYR-1 is insensitive to Ca$^{2+}$. Furthermore, we show that the truncated myosin I translocates actin filaments in vitro at a rate resembling that of the parent molecule, although unlike the parent molecule, the truncated form shows no sensitivity to Ca$^{2+}$ in the range of 0.1–100 μM. These results are discussed in view of the current lever arm model relating velocity to the length of the IQ region.

**EXPERIMENTAL PROCEDURES**

Cloning of myr-1 1 IQ Heavy Chain Construct and Calmodulin—cDNA encoding the entire open reading frame of myr-1b, the kind gift of Drs. Martin Bährer and Christian Ruppert (Adolf-Butenandt-Institut, Zellbiologie, Ludwig-Maximilians-Universitat, Munchen, Germany), was used as a template for polymerase chain reaction (PCR) with two
mutagenic oligonucleotide primers. The forward primer flanked the start codon and incorporated an XhoI restriction enzyme site (5'-TATGACTGATTCTTATAGGGCCAAGAAGGAG-3'). The reverse primer (5'-TACATGGCTGATCACTGCTGTCGCTGTTGCTGTATCTTCATGACGAAAG-3') was designed to incorporate a FLAG tag (aminoc acids DYKDDDDK) to aid in purification, followed by a stop codon and an XhoI site. The resulting construct coded for a truncated MYR-1 polypeptide chain terminating at lysine 728 followed by a COOH-terminal FLAG tag (Fig. 1).

Human calmodulin cDNA (13, 14) was amplified by PCR under standard conditions using a forward primer (5'-CAACTGACT-ACGCTCGAGATGCTGCTGACG-3') and a reverse primer (5'-TAAATACGATCATGATCTTTCGAGTT-3') to produce a product that included the entire open reading frame and a 5' XhoI cloning site and 3' NsiI cloning site. Both the myr-1 and calmodulin PCR products were purified using the Wizard PCR Preps DNA purification system (Promega, Madison, WI). Constructs were digested with their respective enzymes, repurified, and ligated into the pFastBacDUAL transfer vector (Life Technologies, Inc.). The construct representing the truncated MYR-1 heavy chain was inserted downstream of the polyhedrin promoter, and the calmodulin construct was inserted into the p10 promoter cloning site. For the truncated heavy chain construct, the vector was phosphorylated by using calf alkaline phosphatase to prevent self-ligation, gel-purified, and the insert orientation was checked by digestion with BamHI. Both inserts were sequenced entirely with internal and vector-specific oligonucleotides using automated sequencing (Beth Israel Deacness Medical Center, Boston, MA) or done manually using the Sequenase kit (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom). All oligonucleotides were synthesized on an Expedite vector-specific oligonucleotides using automated sequencing (Beth Israel Deacness Medical Center, Boston, MA) or done manually using the Sequenase kit (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom). All oligonucleotides were synthesized on an Expedite nucleic acid synthesizer (PerSpective Biosystems, Framingham, MA).

Recombinant Baculovirus Production and Infection of Sf9 Cells—The recombinant donor plasmid was transformed into DH10Bac Escherichia coli cells (Life Technologies, Inc.) for transposition into the bacmid. Recombinant bacmid DNA was isolated by potassium acetate precipitation followed by the Bac-to-Bac baculovirus expression systems instruction manual supplied by Life Technologies, Inc. Virus was produced by transfecting the recombinant bacmid DNA into Spodoptera frugiperda 9 (Sf9) insect cells with Cellfectin reagent (Life Technologies, Inc.) adapted from Toyoshima et al. (23). Coverslips were coated with 0.1% nitrocellulose in amyl acetate, and flow chambers were constructed by attaching the nitrocellulose-coated coverslips face down to a glass slide using double-sided sticky tape. Ten microliters of the myosin solution at ~150 µg/ml was infused and incubated for 2 min and then washed with 50 µl buffer M (10 mM imidazole, pH 7.2, 20 mM KCl, 5 mM MgCl₂, 0.1 mM EGTA, 1 mM DTT) containing 5 mg/ml bovine serum albumin for 2 min. Ten microliters of 0.024 µl rabbit skeletal muscle F-actin labeled with rhodamine phallolidin (prepared as suggested by Molecular Probes, Eugene, OR) was infused and allowed to bind for 1 min. The chamber was washed with 50 µl of buffer M containing 5 mg/ml BSA prior to initiation of motility with the addition of buffer M with 3 mM ATP, 0.5% methyl cellulose, 50 mM DTT, 3 mM glucose, 0.2 mg/ml glucose oxidase, 0.1 mg/ml catalase, and CaCl₂ and EGTA to effect final free Ca²⁺ concentrations ranging from 0.1 to 100 µM as previously determined (12). Slides were examined with a Nikon fluorescence microscope equipped with an oil immersion lens (Nikon Inc., Melville, NY) equipped with a heat stage to maintain temperature in the specimen chamber at 37 °C. Images were recorded digitally using a Scion LG-3 capture board (Scion Corp., Frederick, MD) and IPLab Scientific Image Processing software (Scanalytics, Inc., Fairfax, VA). The distance moved by numerous filaments over a fixed time was determined. Data are presented as average ± standard error (number).

**Before use in the motility assays described below, MI1IQ molecules unable to bind actin in an ATP-removable manner were removed by incubation for 30 min with 1.2 µM F-actin in the presence of 10 mM ATP, followed by centrifugation at 75,000 rpm for 20 min using a TLA100 rotor in a Beckman tabletop ultracentrifuge; the supernatant was retained.**

**SDS Polyacrylamide Gel Electrophoresis (PAGE) and Immunoblotting—**SDS polyacrylamide gel electrophoresis was performed according to Laemmli (15) on 7.5%/15% polyacrylamide split mini-gels (16). Immunoblotting was performed according to Towbin et al. (17). Secondary antibodies coupled to hors eradish peroxidase were obtained from Sigma. Detection was performed using the ECL chemiluminescence system (Amersham Pharmacia Biotech). Molecular weight markers included myosin II heavy chain, 200,000 Da; phosphorylase b, 93,000 Da; bovine serum albumin, 63,000 Da; ovalbumin, 43,000 Da; carbonic anhydrase, 31,000 Da; and soybean trypsin inhibitor, 21,000 Da.

**Electron Microscopy—**MI1IQ on the parent molecule, MI1, was applied to mica and rotary-shadowed as described previously (18). Electron micrographs were taken with a Phillips 300 electron microscope at an accelerating voltage of 60 kV.

**ATPase Assays—**ATPase assays were performed as described by Pollard (19). The Ca²⁺-ATPase activity of myosin I was measured in 10 mM Tris, pH 7.0, 1 mM DTT, 5 mM CaCl₂, and KCl concentrations as noted. The K⁺-ATPase activity was measured in 10 mM Tris, pH 7.0, 100 mM KCl, 1 mM MgCl₂, and 2 mM EDTA. The actin-activated Mg²⁺-ATPase activity was performed in 10 mM Tris, pH 7.0, 1 mM DTT, 1 mM MgCl₂, and the proper ratio of 2 mM EGTA and 2 mM CaEGTA to effect the indicated pCa (20, 21). The concentration of F-actin was varied from 0 to 300 µM. All reactions were done at 37 °C. Samples lacking either MI1IQ or actin served as controls. Standard curves were generated with known amounts of phosphate. For actin-activated ATPases, the reported values were corrected for activity due to the presence of actin.

**Actin-binding Assays—**Rabbit skeletal muscle actin filaments (22) at 2 µM were incubated on ice in 10 mM Tris, pH 7.0, 0.5 mM DTT, 100 mM KCl, 1 mM MgCl₂, and either 1 mM EGTA or CaCl₂ from 0.1 to 1.0 mM with 0.3 µM MI1IQ for 30 min. The samples were centrifuged at 100,000 × g for 20 min in a Beckman TLA-100 ultra centrifuge. Supernatants were separated from pellets. Pellets were resuspended in 1 mM Tris base and prepared for SDS polyacrylamide gel electrophoresis. After precipitation in 10% trichloroacetic acid, supernatant proteins were collected by microcentrifugation (15,000 × g for 2 min) and resuspended in 1 mM Tris base followed by preparation for SDS-PAGE. Equivalent amounts of supernatants and pellets were analyzed by SDS polyacrylamide gel electrophoresis.

**Fluorescence Microscopy—**Binding of calmodulin to the truncated heavy chain was examined by incubating 200 µl of MI1IQ at 50 µg/ml in 1 mM EGTA or 0.2 mM Ca²⁺ with 30 µl of anti-FLAG-Sepharose beads. After 30 min on ice, the beads were collected by microcentrifugation and heated in SDS-PAGE sample buffer. The supernatants were collected, precipitated with 10% trichloroacetic acid, dissolved in 1 M Tris base, then heated in SDS-PAGE sample buffer. Equivalent amounts of supernatants and pellets were analyzed by SDS polyacrylamide gel electrophoresis, followed by densitometric analyses with NIH Image software. **Motility Assays—**The ability of MI1IQ and MI1IQ to translocate actin filaments was determined in vitro using the sliding filament assay adapted from Toyoshima et al. (23). Coverslips were coated with 0.1% nitrocellulose in amyl acetate, and flow chambers were constructed by attaching the nitrocellulose-coated coverslips face down to a glass slide using double-sided sticky tape. Ten microliters of the myosin solution at ~150 µg/ml was infused and incubated for 2 min and then washed with 50 µl buffer M (10 mM imidazole, pH 7.2, 20 mM KCl, 5 mM MgCl₂, 0.1 mM EGTA, 1 mM DTT) containing 5 mg/ml bovine serum albumin for 2 min. Ten microliters of 0.024 µl rabbit skeletal muscle F-actin labeled with rhodamine phallolidin (prepared as suggested by Molecular Probes, Eugene, OR) was infused and allowed to bind for 1 min. The chamber was washed with 50 µl of buffer M containing 5 mg/ml BSA prior to initiation of motility with the addition of buffer M with 3 mM ATP, 0.5% methyl cellulose, 50 mM DTT, 3 mM glucose, 0.2 mg/ml glucose oxidase, 0.1 mg/ml catalase, and CaCl₂ and EGTA to effect final free Ca²⁺ concentrations ranging from 0.1 to 100 µM as previously determined (12). Slides were examined with a Nikon fluorescence microscope equipped with an oil immersion lens (Nikon Inc., Melville, NY) equipped with a heat stage to maintain temperature in the specimen chamber at 37 °C. Images were recorded digitally using a Scion LG-3 capture board (Scion Corp., Frederick, MD) and IPLab Scientific Image Processing software (Scanalytics, Inc., Fairfax, VA). The distance moved by numerous filaments over a fixed time was determined. Data are presented as average ± standard error (number).
RESULTS

Characterization of Expressed Truncated Myosin I—Addition of increasing amounts of FLAG peptide from 40 to 120 μg/ml resulted in elution of polypeptides of 85 and 17 kDa from an anti-FLAG affinity column loaded with extracts of SF9 cells designed to co-express both a truncated myosin I and calmodulin (Fig. 2). The smaller polypeptide comigrated with calmodulin in samples containing native rat liver 130-kDa myosin I (Fig. 2, lane 9). The 85 kDa polypeptide was recognized in immunoblots with anti-myr-1 (Fig. 2, lane 9, 10, and 12 (arrowhead)).

FIG. 2. Purification of MI130 from baculovirus extracts. Lane 1, total cell lysate; lane 2, cell lysate supernatant; lane 3, cell lysate pellet; lane 4, 20% ammonium sulfate pellet; lane 5, 100,000 × g pellet; lane 6, 100,000 × g supernatant; lane 7, flow-through from FLAG affinity column; lane 8, eluate at 40 μg/ml FLAG peptide; lane 9, eluate at 80 μg/ml FLAG peptide; lane 10, eluate at 120 μg/ml FLAG peptide; lane 11, molecular weight markers; lane 12, MI130 after concentration, 0.6 μg/ml FLAG peptide; lane 13, molecular size markers. Sizes × 10^{-2} are indicated at right.

Image Analysis—Images of blots and polyacrylamide gels were captured with a U-Max Supervista X-12 scanner and prepared for publication with Adobe Photoshop.

Other Procedures—Native MI130 was prepared from rat liver by gel filtration, cation, and anion exchange chromatography as described previously (3). Actin was prepared according to Ref. 22.

Physical Characteristics—The parent molecule and the expressed truncated myosin I were rotary-shadowed and viewed by electron microscopy to learn the overall shape and surface features of the molecules. Molecules of native rat liver MI130 were shaped like tadpoles, i.e. they have a globular head and elongated tail (Fig. 4, top panel). On the other hand, the expressed truncated myosin I molecules appeared globular (Fig. 4, bottom panel). This is consistent with the expected morphology since the expressed myosin I has an abbreviated calmodulin-binding region and lacks altogether the carboxy-terminal tail.

ATPase Activity—The ATPase activity of the expressed truncated myosin I was assessed beginning with the dependence on KCl in the presence of EDTA and compared with the native parent molecule (Fig. 5A). The ATPase activity of both the parent molecule and the truncated fragment decreased with increasing amounts of KCl from 30 to 500 mM. These results are in contrast to those observed in 5 mM CaCl2 conditions in which the ATPase activity of both the parent molecule (0.44 s^{-1}) and the expressed truncated form (0.40 s^{-1}) were relatively unaffected by changes in KCl concentration (Fig. 5B).

In the absence of actin, the Mg2+-ATPase activity was low (0.015 s^{-1}) and independent of the calcium ion concentration. The ATPase activity was activated by increasing amounts of F-actin as a function of the free Ca2+ concentration (Fig. 6A). At 20 μM actin, the ATPase was activated 3.5-fold at pCa 8.9 and 12.5-fold at pCa 4.6. Similar results were observed for the native molecule (Fig. 6B) with a small decrease in the [Ca2+] required for activation. Note that, for both proteins, Ca2+ acts as a modulator of actin activation and not as an on/off switch. Some reduction in actin activation was apparent at pCa > 4.6, and this may explain the variability in the Ca2+ dependence of the actin-activated ATPases reported previously.

Actin-binding Assays—Co-sedimentation assays indicated that myosin I fragment sediments in an actin-dependent manner (Fig. 7). More MI130 consistently pelleted with actin in buffers containing Ca2+ ranging from 0.1 to 1.0 mM than in 1 mM EGTA. Quantitation of the amount of MI130 binding to 2 μM actin indicates that 70% of the heavy chain pelleted with EGTA versus 90% in 0.1 mM or 100% in 1 mM CaCl2. The behavior of calmodulin in regard to its association with actin mimics the heavy chain. Little or no change in the amount of calmodulin associating with the heavy chain was observed as a consequence of increasing CaCl2 concentration from 0.1 to 1.0 mM.

Calmodulin Binding—The effect of CaCl2 on the association of calmodulin with MI130 was examined using immunoadsorption on anti-FLAG-Sepharose beads followed by separation of
supernatants and pellets (Fig. 8). In buffer containing 10 mM Tris, pH 8.0, 100 mM KCl, 1 mM MgCl$_2$, and either 1 mM EGTA (±Ca$^{2+}$) or 0.2 mM CaCl$_2$ (±Ca$^{2+}$), all of the myosin I fragment (10 μg) bound to the Sepharose beads. Similarly, the majority of the calmodulin in either 1 mM EGTA or 0.2 mM Ca$^{2+}$ remained associated with the heavy chain and appeared in the pellets. Although some calmodulin appears in both supernatants, presumably as a consequence of the treatment, no difference in the percentage is observed (22% versus 23% as determined by densitometry).

Motility Assays—As a control, we first confirmed that purified rat liver 130-kDa myosin I translocates actin filaments in a Ca$^{2+}$-regulated manner over a pCa range of 4–7. Conditions were chosen so that no movement of the actin filaments was observed without the addition of ATP. With ATP and at pCa 7, the average rate of translocation by MI 130 was 0.077 ± 0.007 μm/s. At pCa 4, in the presence of ATP, very few filaments moved, and the rate of those that did move was considerably slower (data not shown).

Next, we investigated whether MI1IQ translocates actin filaments in vitro. Our results indicate that rhodamine phalloidin-labeled actin filaments bind to a surface to which MI1IQ is attached. No movement was observed in the absence of ATP. Upon addition of ATP, most of the actin filaments in each field moved. At 0.1 μM Ca$^{2+}$, the average velocity was 0.048 ± 0.002 μm/s (Fig. 9). With increasing free Ca$^{2+}$ concentration to 100 μM, little change in the percentage of filaments moving or their average velocity was observed (Fig. 9). To duplicate samples, bacterially expressed calmodulin was added to a final concentration of 1 μM. An approximately 20% increase in the rate was observed at each calcium ion condition.

DISCUSSION

These studies indicate that coexpression in baculovirus of a truncated myosin I representing the motor domain and a partial neck region consisting of one calmodulin-binding domain together with calmodulin results in a functional molecule in terms of its ability to bind and translocate actin filaments. Furthermore, baculovirus expression of this two-subunit enzyme does not result in detectable denaturation or proteolysis. Other reports of successful expression in baculovirus of any of the many known myosins include truncated forms of non-muscle (24) and smooth muscle myosin II (25) and the mammalian myosin I, MIMIb (26, 27).

As observed with the intact 130-kDa myosin I$^2$ and BBMI (16), binding of the truncated myosin I to actin is sensitive to the Ca$^{2+}$ concentration with more heavy chain binding in the presence of Ca$^{2+}$ than in its absence. Unlike most calmodulin-dependent enzymes, binding of calmodulin to myosin I does not require Ca$^{2+}$. Accordingly, isolation of MI1IQ is performed in the presence of EGTA without loss of calmodulin. Furthermore, we have demonstrated by immunoabsorption with anti-FLAG that calmodulin binding to the truncated MYR-1 is insensitive to Ca$^{2+}$. Some calmodulin is released into the supernatant in

L. Coluccio, unpublished results.

FIG. 5. The EDTA (A) and Ca$^{2+}$ (B) ATPase activity of MI$^{130}$ and MI$^{113Q}$ as a function of KCl concentration. The activity, measured as described under “Experimental Procedures,” is represented as mean ± standard error (n = 3).

FIG. 6. Mg$^{2+}$-ATPase activity of MI$^{113Q}$ (A) and MI$^{130}$ (B) as a function of actin concentration at different pCa. The base-line activity in the presence of actin only was subtracted from each point. Each value represents the average of two points.

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the presence of Ca$^{2+}$ and actin when sedimentation assays are done with the parent molecule, indicating that calmodulin binding to at least one of the IQ domains in the parent molecule is Ca$^{2+}$-sensitive. Similar assays done with BBMI also result in release of some of the calmodulin from the complex in the presence of actin in CaCl$_2$-containing buffers (16). We have observed that in the presence of actin filaments, calmodulin remains associated with the MI1IQ heavy chain in buffers containing Ca$^{2+}$, indicating that binding of calmodulin to the first IQ domain is not Ca$^{2+}$-sensitive. These results illustrate the disparity in behavior among IQ domains.

We have determined that MI1IQ translocates actin filaments in vitro. The overall rate of movement was 0.048 ± 0.002 µm/s versus 0.077 ± 0.007 µm/s observed in this study and 0.05–0.05 µm/s observed previously for the parent molecule under the same conditions of 0.1 µM free Ca$^{2+}$ (12). The present study benefits from advancements made in the analysis of the motility data, which might be responsible for the small discrepancy between the two values obtained for the parent molecule.

The velocity of Dictyostelium myosin II mutants was found to be linearly related to the length of the neck region (28), which supports the hypothesis that the neck region of myosin functions as a lever arm, which can amplify small changes originating in the motor domain in order to produce larger conformational changes resulting in movement (i.e. steps; see Refs. 29 and 30). Our results do not support this model for myosin I since the velocity observed for the truncated molecule with 1 IQ domain is only slightly lower than that observed for the purified parent molecule, which contains at least 4 and up to 6 calmodulins (2, 4). Velocity in the motility assay is related to the duty cycle and step size (31). Recent studies with an optical laser trap have indicated that MI130 produces movement in two distinct steps for an overall motion of 11 nm (32). Future laser trap studies will reveal whether the step size exhibited by MI1IQ by virtue of its truncated neck region is smaller.

No change in the ability of truncated MYR-1 to translocate actin filaments is observed in buffers containing increasing amounts of free Ca$^{2+}$ from 0.1 to 100 µM. This is in direct contrast to results obtained with either the parent molecule or BBMI, where increasing Ca$^{2+}$ concentrations inhibit the ability of MI130 (12) and BBMI (33) to support motility. Since addition of exogenous calmodulin permits actin filament translocation to occur in the presence of Ca$^{2+}$, it seems likely that the inability of truncated myosin I to move is due to the absence of one or more IQ domains that bind calmodulin. Thus, it appears that the IQ domain that is not Ca$^{2+}$-sensitive is required for motility.

FIG. 7. Association of MI1IQ with actin as a function of Ca$^{2+}$ concentration. A, actin at 2 µM (lane 1) was incubated with 0.3 µM MI1IQ (lane 2) in buffer containing 1 mM EGTA (lanes 1–3) or CaCl$_2$ at 0.1 mM (lane 4), 0.2 mM (lane 5), 0.5 mM (lane 6), or 1.0 mM (lane 7) for 30 min before centrifugation and separation of supernatants (s) and pellets (p) by SDS-PAGE. B, densitometric analyses of the amount of calmodulin and myosin I heavy chain in the supernatants and pellets. Representative results from one of three experiments is shown.

FIG. 8. Association of calmodulin with truncated myosin I. 10 µg of MI1IQ were incubated with anti-FLAG-coupled beads in 1 mM EGTA (lanes 1 and 2) or 0.2 mM CaCl$_2$ (lanes 3 and 4) for 60 min, then centrifuged to separate the beads from the soluble proteins. Equivalent amounts of supernatants (lanes 1 and 3) and pellets (lanes 2 and 4) were analyzed by SDS-PAGE. Bands migrating at 55 and 25 kDa correspond to antibody chains. Cam, calmodulin; 85-kDa MI refers to the truncated myosin I heavy chain. Migration of molecular size standards (×10$^{-3}$) is indicated at left.

FIG. 9. Translocation of fluorescently labeled phalloidin-stabilized actin filaments by MI1IQ as a function of Ca$^{2+}$ concentration from 0.1 to 100 µM with exogenous calmodulin. Means are expressed ± standard error. N refers to the number of filaments.
location by these myosins I at the higher Ca$^{2+}$ concentrations, it has been supposed that calmodulin dissociation is in part responsible for the attenuation of activity seen at higher Ca$^{2+}$ concentrations. Since we do not see a change in the rate of actin translocation by MI1IQ as a function of Ca$^{2+}$ concentration, our results indicate that the decrease in motor activity observed with MI130 in buffers containing Ca$^{2+}$ cannot be due to a Ca$^{2+}$-induced change in or dissociation of calmodulin from the first IQ domain. In the presence of exogenous calmodulin, there is an approximate 20% increase in the rate of movement of actin filaments by MI1IQ at all the pCa conditions tested. We attribute this to the possibility that some of the calmodulin associated with the expressed protein is dissociating at the very low protein concentrations at which the motility assays are performed, but this effect is not a function of Ca$^{2+}$ concentration. Recently, Zhu et al. (27) coexpressed the myr-2/MMIβ heavy chain along with calmodulins mutant in their ability to bind Ca$^{2+}$ and concluded that the decrease in motility of MMI130 observed with increasing Ca$^{2+}$ concentration is due to Ca$^{2+}$ binding to calmodulin, which occurs at lower Ca$^{2+}$ concentrations than required for calmodulin dissociation.

The actin-activated Mg$^{2+}$-ATPase activities of MI1IQ and MI130 exhibit a Ca$^{2+}$-sensitivity; however, translocation of actin filaments in vitro by either MI1IQ or MI130 does not increase in buffers containing increasing amounts of Ca$^{2+}$. These results indicate that the Ca$^{2+}$ sensitivity of the actin-activated Mg$^{2+}$-ATPase activity is not directly coupled to motility. The reason for this discrepancy is unclear; however, it is understood that translating motor activity as measured by ATPase activity into movement as measured by the ability to translocate F-actin relies on a number of factors, not the least of which is the orientation which the motor molecule assumes on the substrate (23). As described in the accompanying article (34), we have also observed that MI1IQ exhibits transient kinetic properties indistinguishable from the parent molecule. In addition, our results indicate that in equilibrium, actin-myosin I exists in two forms, one of which is unable to bind ATP.

The ability to express myosin I constructs in baculovirus enables the design of future studies using mutational analyses to investigate the importance of specific regions of the molecules in various biochemical properties including motility.

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