The 110-kD Protein-Calmodulin Complex of the Intestinal Microvillus (Brush Border Myosin I) Is a Mechanoenzyme

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Abstract. The 110-kD protein-calmodulin complex (110K-CM) of the intestinal brush border serves to laterally tether microvillar actin filaments to the plasma membrane. Results from several laboratories have demonstrated that this complex shares many enzymatic and structural properties with myosin. The mechanochemical potential of purified avian 110K-CM was assessed using the *Nitella* bead motility assay (Sheetz, M. P., and J. A. Spudich. 1983. *Nature* (*Lond.*). 303:31-35). Under low Ca^{2+} conditions, 110K-CM-coated beads bound to actin cables, but no movement was observed. Using EGTA/calcium buffers (~5-10 μM free Ca^{2+}) movement of 110K-CM-coated beads along actin cables (average rate of ~8 nm/s) was observed. The movement was in the same direction as that for beads coated with skeletal muscle myosin. The motile preparations of 110K-CM were shown to be free of detectable contamination by conventional brush border myosin. Based on these and other observations demonstrating the myosin-like properties of 110K-CM, we propose that this complex be named "brush border myosin I."

Two general classes of the mechanoenzyme myosin have been characterized in eucaryotic cells (for reviews on myosin see Korn and Hammer, 1988; Warrick and Spudich, 1987). Conventional myosins, or myosin IIs, with their two globular heads and alpha helical coiled-coil tail, are ubiquitously expressed in both muscle and nonmuscle cells. The other class of myosins, termed mini-myosins or myosin Is, were first characterized in *Acanthamoeba* (Pollard and Korn, 1973) and later identified in *Dictyostelium* (Cote et al., 1985). These myosins, consisting of a single heavy chain and associated light chain(s), are tailless and consequently do not form filaments. Sequence analysis of *Acanthamoeba* myosin IB (Jung et al., 1987) indicates that this myosin is composed of a head domain homologous to the S_1 domain of conventional myosin, and a ~30-kD COOH-terminal domain that is not alpha helical (for review see Korn and Hammer, 1988; Korn et al., 1988). Functional studies indicate that the COOH-terminal end of both *Acanthamoeba* myosin IA and IB contain an ATP-insensitive actin binding site (see Korn and Hammer, 1988). In addition, *Acanthamoeba* myosin I promotes the movement of membrane vesicles in vitro (Adams and Pollard, 1986). The association of myosin I with membranes suggests that this class of myosins may play key roles in motile events involving the plasma membrane and/or membranous organelles.

Recent studies indicate that the expression of the tailless myosin I class of mechanoenzymes may not be restricted to amoeboid cells. The first evidence that vertebrates may express myosin Is was obtained by Collins and Borysenko (1984) who observed that the protein complex, 110-kD protein-calmodulin (110K-CM),[1] purified from avian intestinal brush borders exhibits myosin-like, K-EDTA ATPase activity. This complex, which consists of a 110-kD subunit bound to three to four molecules of calmodulin, serves to laterally tether actin bundles of the intestinal microvillus to the plasma membrane (for review see Mooseker, 1985). Subsequent studies from several laboratories have demonstrated that 110K-CM shares many properties with myosins. This includes ATP-dependent binding to and cross-linking of actin filaments (Howe and Mooseker, 1983; Collins and Borysenko, 1984; Verner and Bretscher, 1985; Conzelman and Mooseker, 1987; Coluccio and Bretscher, 1987) and myosin-like ATPase activity, including actin activation of its Mg-ATPase (Conzelman and Mooseker, 1987; Swanljung-Collins et al., 1987; Krizek et al., 1987). In addition, the 110K-CM complex decorates actin filaments in a fashion reminiscent of S_1 arrowhead complexes (Coluccio and Bretscher, 1987). Furthermore, immunochemical studies have revealed that the 110-kD subunit shares epitopes with skeletal muscle myosin S_1, which have been localized to the 50-kD actin-binding and 25-kD ATP-binding tryptic fragments of the myosin head (Carboni et al., 1988; myosin S_1 domain structure reviewed in Warrick and Spudich, 1987).

Structural studies indicate that the 110-kD subunit, like the heavy chain of *Acanthamoeba* myosin Is, may be composed of two distinct domains: a myosin head domain of 80-90 kD at the presumed NH_2 terminus of the molecule, which con-

1. *Abbreviations used in this paper*: BB, brush border; 110K-CM, 110-kD protein-calmodulin.

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tains the ATPase and actin binding domains; and a 20–30 kD domain at the COOH-terminal end, which contains the calmodulin binding domains (Coluccio and Bretscher, 1988; Carboni et al., 1988). This model is consistent with results obtained from electron microscopy of the 110K-CM molecule (performed by J. Heuser, Washington University, St. Louis, MO), which reveals a tadpole-shaped molecule consisting of a head similar in size and shape to myosin S_1 and a ~12-nm tail (Conzelman and Mooseker, 1987). Together, these observations strongly suggest that 110K-CM is a vertebrate member of the monomeric, tailless myosin I family of mechanoenzymes. In fact, the presence of genes encoding myosin Is in vertebrates has been recently demonstrated by Hoshimaru and Nakanishi (1987) who have sequenced a bovine gene encoding a 119-kD protein structurally homologous to *Acanthamoeba* myosin I. This gene may well encode the bovine form of the 110-kD protein, as it is expressed in intestinal tissue (Hoshimaru and Nakanishi, 1987).

Although the studies summarized above firmly establish the myosin-like properties of avian 110K-CM, direct evidence that it is a mechanoenzyme has, until now, been lacking. In this study we have demonstrated that purified 110K-CM is a motor using the in vitro *Nitella* bead movement assay of Sheetz and Spudich (1983). Based on these observations we propose that the 110K-CM complex be named "brush border (BB) myosin I."

Materials and Methods

Purification and Enzymatic Characterization of BB Myosin I (110K-CM)

BBs were isolated from chicken intestines (Mooseker and Howe, 1982; Keller and Mooseker, 1982). BB myosin I was purified from ATP extracts of BBs by the method of Coluccio and Bretscher (1987). The ATPase activity of BB myosin I preparations used for motility studies was assayed by the method of Taussky and Shorr (1953). The Mg-ATPase of BB myosin I in the absence and presence of actin (0.9 mg/ml) was determined using assay conditions described in Conzelman and Mooseker (1987) with a BB myosin I concentration of 0.15 mg/ml. The effect of Ca^{2+} on the Mg-ATPase of BB myosin I in the presence and absence of actin was assayed by inclusion of 5 mM EGTA/$CaCl_2$ buffer (1:1 molar ratio to achieve a final free ion concentration of ~10 μM free Ca^{2+}; Portzehl et al. 1964) in the standard assay (Conzelman and Mooseker, 1987). The free Ca^{2+} in this buffer was sufficient to yield maximal calmodulin activation of gizzard myosin light chain kinase as assayed by the method in Keller et al. (1985). The actin used in these assays was purified by the method of Spudich and Watt (1971) from acetone powders of chicken breast muscle.

Assessment of BB Myosin I Preparation Contamination by Conventional BB Myosin

The level of BB myosin contamination in the BB myosin I preparations was determined by immunoblot analysis using a serial dilution of BB myosin (purified by the method of Keller and Mooseker, 1982) as a standard to determine the limits of detection. SDS-PAGE (Matsudaira and Burgess, 1978) of equal volume aliquots (6 μl) of BB myosin I at two to four times the final concentration used for motility assays (1 mg/ml) and BB myosin (0.3–9 μg/ml) was performed and the gel electrotransferred to nitrocellulose (Towbin et al., 1979). Immunostaining was performed as described in Shibayama et al. (1987) using a commercially available polyclonal antisera raised against human platelet myosin (Biomedical Technologies, Stoughton, MA). Immunoreactive bands were visualized by secondary staining with alkaline phosphatase–conjugated anti-rabbit IgG (Promega Biotec, Madison, WI) using procedures recommended by the supplier.

In Vitro Motility Assay

The mechanochemical potential of BB myosin I was assessed using the *Nitella* bead movement assay of Sheetz and Spudich (1983) using methods exactly as described in Sheetz et al. (1986). Latex beads coated with BB myosin I were prepared by incubating 9 vol of BB myosin I (0.25–0.5 mg/ml in 10 mM imidazole-Cl, pH 7.2, 2 mM $MgCl_2$, 75 mM KCl, 1.0 mM EGTA, 0.5 mM DTT) with 1 vol of Covasphere bead (1 μm diam) suspension (Covalent Technologies, Ann Arbor, MI). The BB myosin I-coated beads were stored on ice and remained motile for at least 24 h after preparation. As recommended by Sheetz et al. (1986), phalloidin (50 μg/ml) was added to the *Nitella* dissection buffer to stabilize the actin cables. The dissection/motility buffer contained 2 mM ATP and either 1 mM EGTA or 1 mM Ca^{2+} buffer (1 mM $CaCl_2$, 1 mM EGTA, ~5–10 μM free Ca^{2+}). Our preparations of dissected *Nitella* generally sustained movement of BB myosin I or skeletal muscle myosin–coated beads for up to 12 h. Movements of BB myosin I-coated beads along *Nitella* actin cables were documented by time-lapse video light microscopy using a 40× water immersion lens and bright-field illumination. The time lapse rate was 60 times real time using an RCA time-lapse video recorder (1/2 in. VHS format). Rates of bead movement were determined from video copy images taken at 3–5-min intervals using a video copy processor (model P60U; Mitsubishi, Cypress, CA). Since it was noted that bead movement slowed down with time, rates were determined for each 3–5-min interval. For the results presented here, over 50 individual bead (or bead cluster) rates were measured. Photographs of individual time-lapse frames stabilized by passage through a time base corrector were made directly from a video monitor using a 35-mm camera.

Results

Motile Preparations of BB Myosin I are Free of Conventional BB Myosin Contamination and Exhibit Ca^{2+}-sensitive, Actin-activated Mg-ATPase

Because BB myosin I is purified from ATP extracts of BBs that also contain high concentrations of BB myosin, it was

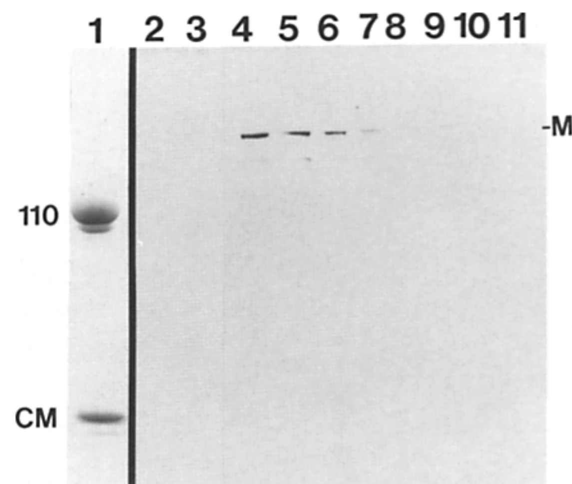


Figure 1. Assessment of BB myosin contamination in motile preparations of BB myosin I. Lane *1*, Coomassie blue-stained SDS-PAGE of BB myosin I preparation. The two predominant bands are the 110-kD subunit (*110*) and calmodulin (*CM*). The band directly below the 110K is a proteolytic fragment of 110K as determined by immunoblot analysis with 110-kD protein–specific antibodies (not shown). Lanes *2–11*, immunoblot of purified BB myosin I stained with antimyosin heavy chain (lanes *2* and *3*; gel loaded with 6 μl of BB myosin I at 1 and 0.5 mg/ml, respectively) and a serial dilution of purified BB myosin (lanes *4–11*). The BB myosin lanes (lanes *4–11*) were loaded with 6 μl of myosin at 9, 4.5, 2.3, 1.7, 1, 0.8, 0.5, or 0.25 μg/ml, respectively. Immunostaining of BB myosin heavy chain is readily apparent in lanes *4–10* and detectable but not readily documentable in lane *11*. No detectable myosin heavy chain staining was observed in the BB myosin I lanes (lanes *2* and *3*).

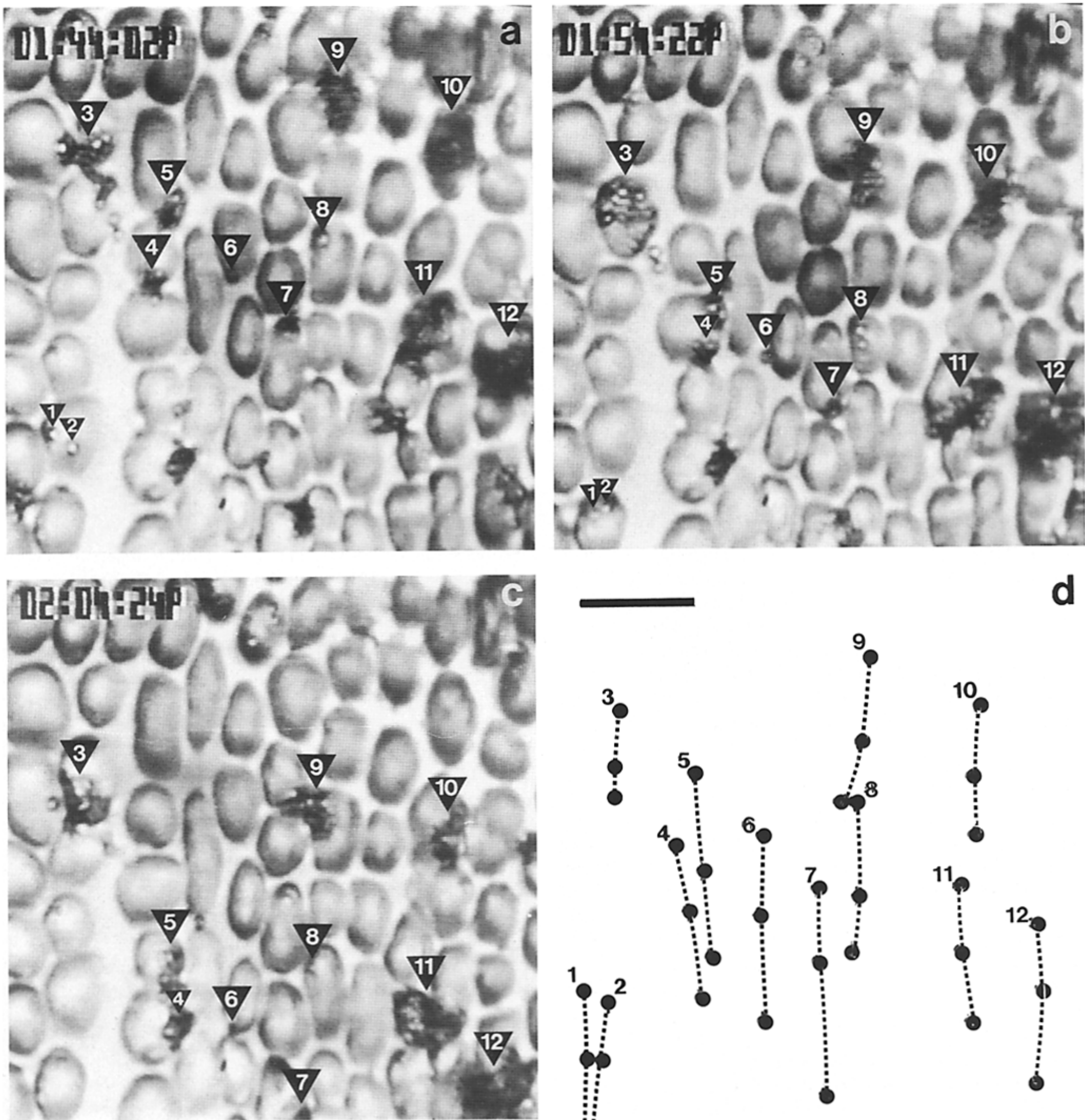


Figure 2. In vitro movement of BB myosin I-coated beads along the actin cables of *Nitella*. Time lapse video frames showing the position of 12 single beads or bead clusters that were moving in a downward, linear fashion along tracks defined by the longitudinal arrays of chloroplasts (*a–c*). The time of day is indicated in the upper left of each micrograph in hours:minutes:seconds. Approximate time between each frame is 10 min. The first time interval (*a*) was taken at ∼15 min after bead injection onto the surface of the dissected *Nitella*. A composite drawing (*d*) depicts the path of motion of the 12 beads or bead cluster indicated in *a–c*. The solid circles indicate the position of a given bead (or the center of a bead cluster) at the three time intervals shown, beginning with the numbered circle. The motion tracks were determined by playback of the video sequence and tracing bead paths on the video monitor. Bar, 10 μm.

important to verify that the preparations of BB myosin I used in the motility assay were free of contamination by conventional BB myosin. Similar studies using skeletal and smooth muscle myosins have determined that a critical concentration of 10–20 μg/ml is required for bead movement in this assay (Sheetz et al., 1984; Sellers et al., 1985). Immunoblot analysis of purified BB myosin I preparations using an antibody sufficiently sensitive to detect BB myosin concentrations >∼0.3 μg/ml revealed that the BB myosin I preparations used in this study were free of BB myosin (Fig. 1). Because a fourfold dilution of the BB myosin I preparation assayed here generated movement, the upper limit for contamination by BB myosin was <0.1 μg/ml.

Analysis of Mg-ATPase activity indicated that the BB myo-

sin I preparations used for the present study exhibited similar levels of actin activation to those previously reported (Conzelman and Mooseker, 1987). For example, Mg-ATPase ($-Ca^{2+}$) activities of the motile preparation depicted in Fig. 2 in the absence and presence of actin were 0.014 and 0.094 μmol P_i/mg per min, respectively. The addition of 10 μM Ca^{2+} caused the characteristic (see Conzelman and Mooseker, 1987) actin-independent increase in BB myosin I ATPase (from 0.014 to 0.13 μmol, P_i/mg per min) but twofold activation by actin was still observed (0.13 vs. 0.277 μmol, P_i/mg per min). This level of actin activation in the presence of Ca^{2+} is similar to that observed by Conzelman and Mooseker (1987) when exogenous CM was added to the preparation; thus the purification procedure of Coluccio and Bretscher (1987) probably yields a more active enzyme, possibly because of higher calmodulin content.

BB Myosin I-coated Beads Exhibit Ca^{2+}-dependent Movement on Nitella Actin Cables

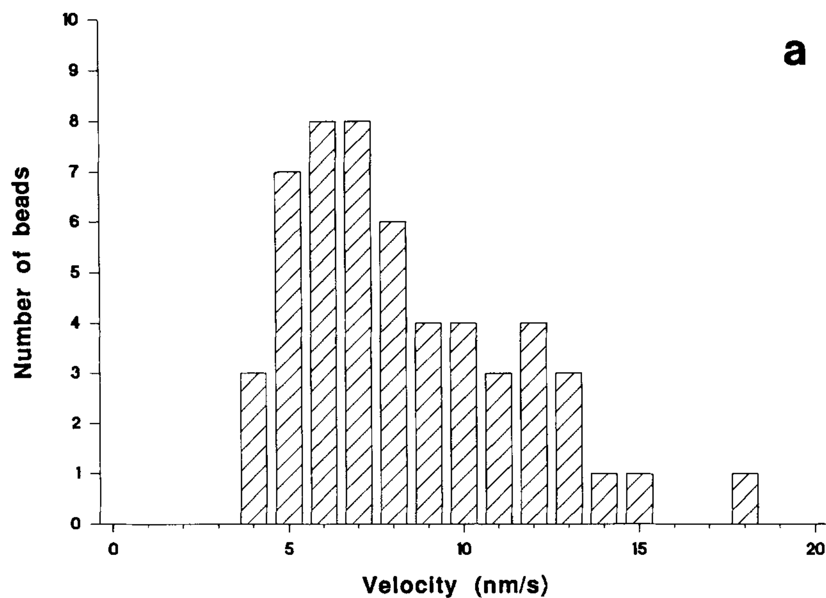
To assess the mechanochemical potential of BB myosin I, the *Nitella* bead movement assay of Sheetz and Spudich (1983; Sheetz et al., 1986) was used. In this assay, internodal cells of *Nitella axillaris* were dissected to expose the uniformly polarized bundles of actin cables that extend the length of this cylindrical cell. Previous studies have shown that latex beads coated with myosin move along these actin cables at rates characteristic of the particular type of myosin used (Sheetz et al., 1984; Warrick and Spudich, 1987). In the motility buffers containing 1 mM EGTA, BB myosin I-coated beads attached to actin cables but no movement was observed (results not shown). Because the Mg-ATPase of BB myosin I is elevated in the presence of Ca^{2+}, we also assayed for motility using a Ca/EGTA buffer that yields maximal Ca^{2+}-dependent enhancement of Mg-ATPase (Conzelman and Mooseker, 1987). In the presence of $\sim$10 μM Ca^{2+}, slow (4–15 nm/s), gliding movements of BB myosin I-coated beads along chloroplast files were observed (Figs. 2 and 3 *a*). Bead movements persisted for $\sim$1 h after injection of the beads onto the *Nitella* cables. At a given time after bead injection, a relatively uniform distribution of rates was observed, although most beads exhibiting movement in a given field of view gradually slowed down. Thus, the fastest and slowest bead movements were observed at the beginning and end of a standard 60-min observation sequence, respectively (Fig. 3 *b*). This deacceleration was not due to a deterioration of the *Nitella* preparation or a significant reduction in ATP concentration because the same preparation would support movements of freshly injected beads (from the same stock preparation of BB myosin I-coated beads) for 5–8 h without significant reduction in initial bead velocities. The BB myosin I-coated beads moved in the same direction as conventional myosin; this was determined by observing the direction of movement of chicken skeletal muscle myosin–coated beads along chloroplast files that had previously supported movement of BB myosin I–coated beads (results not shown).

Since previous studies (Sheetz et al., 1986) have shown that elevated Ca^{2+} has deleterious effects on the *Nitella* preparation, we were concerned that the bead movements might actually be passive movements resulting from breakage and retraction of the actin cables. Several observations ruled out this possibility. First, individual beads or bead clusters moving along the same chloroplast file moved at similar but nonidentical rates. Movement caused by retraction of actin cables should be uniform. Second, all beads in a given field move in the same direction; breakage of fibers should result in bidirectional movement. In fact, rapid, bidirectional movement of beads as a result of cable breakage was occasionally observed. Third, freshly applied beads added to a region where bead movement had ceased consistently moved in the same direction and rates as the previous batch of beads, indicating that the cables were still intact and capable of supporting bead movement.

Discussion

The key conclusion from the results presented here is that BB myosin I, shown to be free of contamination by conventional BB myosin, is a mechanoenzyme. The observed rates of bead movement are considerably slower than that recorded for both conventional myosins and myosin Is examined thus far using the *Nitella* bead movement assay. Velocities range from 6 μm/s for skeletal muscle myosin to $\sim$0.4 μm/s for smooth muscle myosin (Sheetz et al., 1984; Sellers et al., 1985) and 20-80 nm/s for purified *Acanthamoeba* myosin Ib (Albanesi et al., 1985). The slow velocity observed may be characteristic of BB myosin I or additional factors may regulate the activity of this motor. For example, previous studies have shown that the isolated BB contains kinase activity that phosphorylates the 110-kD subunit (Keller and Mooseker, 1982); it will be important to establish what if any role this kinase plays in regulating BB myosin I. Another factor that may affect the velocity of BB myosin I, is its association state with the microvillar membrane. Studies by Adams and Pollard (1986) have demonstrated that vesicles containing *Acanthamoeba* myosin I move along *Nitella* actin cables at rates that are considerably faster (0.24 μm/s) than that observed for beads coated with purified myosin Ib. Similarly, we have observed that beads coated with detergent-resistant microvillar membrane fragments enriched in bound BB myosin I move at velocities which on average are four times faster (33 nm/s) than purified BB myosin I (Mooseker, M., K. Conzelman, T. Coleman, J. Heuser, and M. Sheetz, manuscript submitted for publication). Assuming that the observed movements are mediated by the membrane bound BB myosin I in these preparations, then like *Acanthamoeba* myosin I, the interaction of BB myosin I with membranes may play a critical role in regulating its mechanochemical activity.

The observed Ca^{2+} dependence of BB myosin I motility in vitro suggests that this mechanoenzyme is regulated by Ca^{2+}. Future experiments will hopefully help determine if such regulation is mediated by direct Ca^{2+} binding to the 110-kD subunit, or the calmodulin light chains. Two additional sets of as yet unpublished observations have clouded the significance of the observed Ca^{2+}-dependence of bead movement by BB myosin I, however. First, the movement of beads coated with the BB myosin I–enriched membrane fraction described above is Ca^{2+}-independent, and, in fact, is observed most reproducibly in buffers free of Ca^{2+}. Second, coupling of BB myosin I to beads via an mAb directed to the COOH-terminal domain of the 110-kD subunit results in beads that move both in the presence and absence of Ca^{2+}, at rates identical to those described here for movement of BB myosin I-coated beads (M. Mooseker, T. Coleman, and J. Carboni, unpublished observations). Future studies, focus-



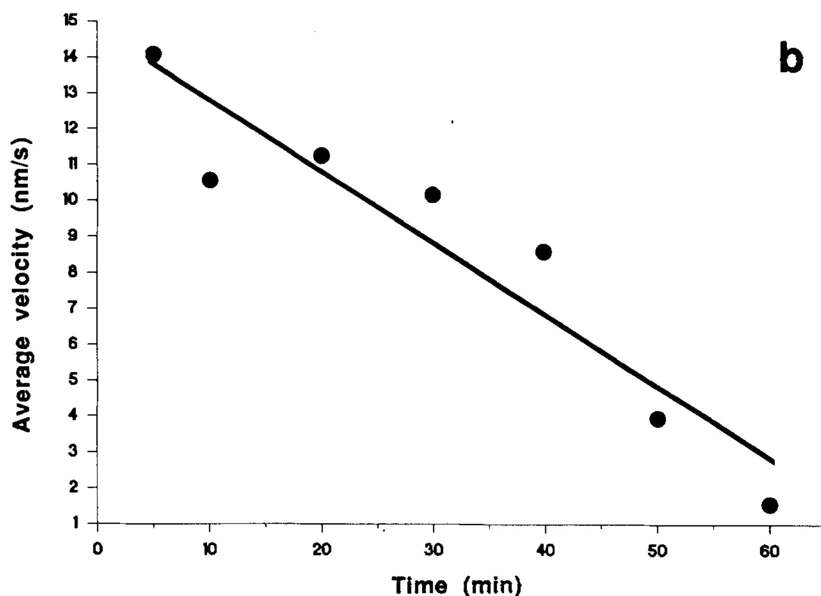


Figure 3. (*a*) Average velocities of movement of BB myosin I-coated beads along *Nitella* actin cables. These values represent measurements of five different video fields each tracking the movement of beads over a 30–60-min time period. The fastest rates were observed at early time points (immediately after beads settled). (*b*) Average velocity of motile beads (average velocity of ~30 separate bead or bead clusters is plotted) from a single video field (same as in Fig. 2) as a function of time after onset of motility. Movement of BB myosin I-coated beads gradually slows and generally stops within 60–70 min.

ing on the COOH-terminal, calmodulin binding domain (and possibly membrane binding domain) of the 110-kD subunit will be required to decipher the molecular basis for these conflicting observations on the Ca^{2+} requirement for motility.

An unusual characteristic of the motility of BB myosin I-coated beads, compared to that of other myosins, is the gradual reduction in bead velocity with time. Myosin-coated beads generally move at a constant rate and then stop abruptly (Sheetz et al., 1984; Sheetz, M., and M. Mooseker, unpublished observations). Because previous studies have shown a partial dissociation of calmodulin from BB myosin I in the presence of actin and Ca^{2+} (Coluccio and Bretscher, 1987) perhaps the observed deacceleration is due to gradual loss of calmodulin. Unfortunately, biochemical analysis of beads after incubation on the *Nitella* substrate to assess for such loss of calmodulin is not technically feasible.

The demonstration that the 110K-CM complex (BB myosin I) is an active mechanoenzyme provides firm evidence for the expression of myosins of the myosin I class in vertebrate species. Although the function for BB myosin I is unknown, several aspects of its structural organization within the brush border may be of general import in considering the function of this class of myosins. BB myosin I is associated with the plasma membrane and membrane-associated actin filaments in a region of the cell that is devoid of conventional myosin. This mode of actin filament organization is characteristic of the organization of membrane-associated actin filaments in many cells, particularly within protrusions of the cell surface such as lamellapodia, filopodia, and nerve growth cones. In general, such protrusions are structurally analogous to the microvillus in that they contain uniformly polarized arrays of actin filaments (barbed ends at the membrane) and lack

conventional myosin (for review and references see Schliwa, 1986). The results presented here provide support for the involvement of membrane-associated myosin Is in the motility of such cellular protrusions. The validity of this idea has been greatly strengthened by the recent studies on *Dictyostelium* that have shown that cells lacking conventional myosin, although unable to divide, can still locomote and respond to chemotactic stimuli (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987; Wessels et al., 1988; Peters et al., 1988; Knecht and Loomis, 1988). These studies indicate that either myosins are not involved in such movements, or that the myosin I(s) present in these cells is responsible.

We would like to acknowledge the important contribution of Dr. Mike Sheetz, Washington University School of Medicine (St. Louis, MO), who was instrumental in teaching us the *Nitella* bead assay; he was also an active participant in our initial, unsuccessful attempts to assess the motility of BB myosin I, which led to the examination of the motility of membrane fractions enriched in associated BB myosin I to be described elsewhere (Mooseker, M., K. Conzelman, T. Coleman, J. Heuser, and M. Sheetz, manuscript submitted for publication). We thank Dr. Haig Keshishian for both the use of his video equipment and help with the photography of video images. We thank all the members of the Mooseker lab group for their help and input; this includes the superb technical assistance of Deborah Sliker and Deborah Braun.

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References

Adams, R. A., and T. D. Pollard. 1986. Propulsion of organelles isolated from *Acanthamoeba* along actin filaments by myosin I. *Nature (Lond.).* 322: 754-756.

Albanesi, J. P., H. Fujisaki, J. A. Hammer, E. D. Korn, R. Jones, and M. P. Sheetz. 1985. Monomeric *Acanthamoeba* myosin Is support movement in vitro. *J. Biol. Chem.* 260:8649-8652.

Carboni, J. M., K. A. Conzelman, R. A. Adams, D. A. Kaiser, T. D. Pollard, and M. S. Mooseker. 1989. Structural and immunological characterization of the myosin-like 110-kD subunit of the intestinal microvillar 110K-calmodulin complex: evidence for discrete myosin head and calmodulin binding domains. *J. Cell Biol.* 107:1749-1757.

Collins, J. H., and C. W. Borysenko. 1984. The 110,000-dalton actin- and calmodulin-binding protein from intestinal brush border is a myosin-like ATPase. *J. Biol. Chem.* 259:14128-14135.

Coluccio, L. M., and A. Bretscher. 1987. Calcium-regulated cooperative binding of the microvillar 110K-calmodulin complex to F-actin: Formation of decorated filaments. *J. Cell Biol.* 105:325-333.

Coluccio, L. M., and A. Bretscher. 1988. Mapping of the microvillar 110K-calmodulin complex: Calmodulin-associated or free fragments of the 110kD polypeptide bind F-actin and retain ATPase activity. *J. Cell Biol.* 106: 367-373.

Conzelman, K. A., and M. S. Mooseker. 1987. The 110-kD protein-calmodulin complex of the intestinal microvillus is an actin-activated Mg^{2+}-ATPase. *J. Cell Biol.* 105:313-324.

Cote, G. P., J. P. Albanesi, T. Ueno, J. A. Hammer, and E. D. Korn. 1985. Purification from *Dictyostelium discoideum* of a low-molecular weight Imyosin that resembles myosin I from *Acanthamoeba castellanii. J. Biol. Chem.* 260:4543-4546.

De Lozzane, A., and J. A. Spudich. 1987. Disruption of the *Dictyostelium* myosin heavy chain gene by homologous recombination. *Science (Wash. DC).* 236:1086-1091.

Hoshimaru, M., and S. Nakanishi. 1987. Identification of a new type of mammalian myosin heavy chain by molecular cloning: Overlap of its mRNA with preprotachykinin B mRNA. *J. Biol. Chem.* 262:14625-14632.

Howe, C. L., and M. S. Mooseker. 1983. Characterization of the 110-kdalton actin-calmodulin-, and membrane-binding protein from microvilli of intestinal epithelial cells. *J. Cell Biol.* 97:974-985.

Jung, G., E. D. Korn, and J. A. Hammer III. 1987. The heavy chain of *Acanthamoeba* myosin IB is a fusion of myosin-like and nonmyosin-like sequences. *Proc. Natl. Acad. Sci. USA.* 84:6720-6725.

Keller, T. C. S., K. A. Conzelman, R. Chasan, and M. S. Mooseker. 1985. Role of myosin in terminal web contraction in isolated intestinal epithelial brush borders. *J. Cell Biol.* 100:1647-1655.

Keller, T. C. S., and M. S. Mooseker. 1982. Ca^{++}calmodulin-dependent phosphorylation of myosin and its role in brush border contraction in vitro. *J. Cell Biol.* 95:943-959.

Knecht, D. A., and W. F. Loomis. 1987. Antisense RNA inactivation of myosin heavy chain gene expression in *Dictyostelium Discoideum. Science (Wash. DC).* 236:1081-1085.

Knecht, D. A., and W. F. Loomis. 1988. Developmental consequences of the lack of myhosin heavy chain in *Dictyostelium Discoidium. Dev. Biol.* 128:178-198.

Krizek, J., L. M. Coluccio, and A. Bretscher. 1987. ATPase activity of the microvillar 110 kDa polypeptide-calmodulin complex is activated in Mg^{2+} and inhibited in K^{+}-EDTA by F-actin. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 225:269-272.

Korn, E. D., M. A. L. Atkinson, H. Brzeska, J. A. Hammer, G. Jung, and T. L. Lynch. 1988. Structure-function studies on *Acanthamoeba* myosins 1A, 1B, and II. *J. Cell. Biochem.* 36:37-50.

Korn, E. D., and J. A. Hammer III. 1988. Myosins of nonmuscle cells. *Annu. Rev. Biophys. Chem.* 17:23-45.

Matsudaira, P., and D. Burgess. 1978. SDS microslab linear gradient polyacrylamide gel electrophoresis. *Anal. Biochem.* 87:386-396.

Mooseker, M. S. 1985. Organization, chemistry and assembly of the cytoskeletal apparatus of the intestinal brush border. *Annu. Rev. Cell Biol.* 1:209-241.

Mooseker, M. S., and C. L. Howe. 1982. The brush border of intestinal epithelium: a model system for analysis of cell-surface architecture and motility. *Methods Cell Biol.* 25:144-175.

Peters, D., D. A. Knecht, W. F. Loomis, A. De Lozanne, J. A. Spudich, and P. J. M. Haastert. 1988. Signal transduction, chemotaxis, and cell aggregation in *Dictyostelium Discoideum* cells without myosin heavy chain. *Dev. Biol.* 128:158-163.

Pollard, T. D., and E. D. Korn. 1973. *Acanthamoeba* myosin I: Isolation from *Acanthamoeba castellanii* of an enzyme similar to muscle myosin. *J. Biol. Chem.* 248:4682-4690.

Portzehl, H., P. C. Caldwell, and J. C. Ruegg. 1964. The dependence of contraction and relaxation of muscle fibres from the crab *Mara squinado. Biochim. Biophys. Acta.* 79:581-591.

Sellers, J. R., J. A. Spudich, and M. P. Sheetz. 1985. Light chain phosphorylation regulates the movement of smooth muscle myosin on actin filaments. *J. Cell Biol.* 101:1897-1902.

Schliwa, M. 1986. The cytoskeleton. Cell Biology Monographs. Vol. 13. Springer-Verlag, Vienna, New York. 323.

Sheetz, M. P., and J. A. Spudich. 1983. Movement of myosin-coated fluorescent beads on actin cables *in vitro. Nature (Lond.).* 303:31-35.

Sheetz, M. P., S. M. Block, and J. A. Spudich. 1986. Myosin movement *in vitro*: quantitative assay using oriented actin cables for *Nitella. Methods Enzymol.* 134:531-543.

Sheetz, M. P., M. Chasan, and J. A. Spudich. 1984. ATP-dependent movement of myosin in vitro: characterization of a quantitative assay. *J. Cell Biol.* 99:1867-1871.

Shibayama, T., J. M. Carboni, and M. S. Mooseker. 1987. Assembly of the intestinal brush border: Appearance and redistribution of microvillar core proteins in developing chick enterocytes. *J. Cell Biol.* 105:335-344.

Spudich, J. A., and S. Watt. 1971. The regulation of skeletal muscle contraction. I. Biochemical studies of interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. *J. Biol. Chem.* 246:4866-4871.

Swanljung-Collins, H., J. Montibeller, and J. H. Collins. 1987. Purification and characterization of the 110-kDa actin- and calmodulin-binding protein from intestinal brush border: a myosin-like ATPase. *Methods Enzymol.* 139:137-148.

Taussky, H. H., and E. Shorr. 1953. A microcolorimetric method for the determination of inorganic phosphorous. *J. Biol. Chem.* 202:675-686.

Towbin, H. T., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350-4354.

Verner, K., and A. Bretscher. 1985. Microvillus 110K-calmodulin: Effects of nucleotides on isolated cytoskeletons and the interaction of the purified complex with F-actin. *J. Cell Biol.* 100:1455-1465.

Warrick, H. M., and J. A. Spudich. 1987. Myosin structure and function in cell motility. *Annu. Rev. Cell Biol.* 3:379-421.

Wessels, D., D. R. Soll, D. Knecht, W. F. Loomis, A. De Lozanne, and J. Spudich. 1988. Cell motility and chemotaxis in *Dictyostelium* amebae lacking myosin heavy chain. *Dev. Biol.* 128:164-177.