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."
contains 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, 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 Nittla 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 Ca²⁺ on the Mg-ATPase of BB myosin I in the presence and absence of actin was assayed by inclusion of 5 mM EGTA/CaCl₂ buffer (1:1 molar ratio to achieve a final free ion concentration of ~10 μM free Ca²⁺; Portzehl et al. 1964) in the standard assay (Conzelman and Mooseker, 1987). The free Ca²⁺ 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 electrophoresed to nitrocellulose (Towbin et al., 1979). Immunosigning was performed as described in Shibayama et al. (1987) using a commercially available polyclonal antiserum raised against brain 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 Nittla 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-CI, pH 7.2, 2 mM MgCl₂, 75 mM KCl, 10 mM EGTA, 0.5 mM EDTA) with 1 vol of Covasphere bead (1 μm diameter) 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 Nittla dissection buffer to stabilize the actin cables. The dissection/motility buffer contained 2 mM ATP and either 1 mM EGTA or 1 mM CaCl₂ buffer (1 mM CaCl₂, 1 mM EGTA, ~5–10 μM free Ca²⁺).

Our preparations of dissected Nittla generally sustained movement of BB myosin I or skeletal muscle myosin-coated beads for up to 12 h. Motilities of BB myosin I-coated beads along Nittla 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 copies 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 Ca²⁺-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
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-
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 (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 0.1-10 \(\mu\)M Ca\(^{2+}\), slow (4-15 nm/s), gliding movements of BB myosin I-coated beads along chloroplast files were observed (Figs. 2 and 3a). 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. 3b). This deceleration 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 \(\mu\)m/s for skeletal muscle myosin to \(\sim\)0.4 \(\mu\)m/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 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 \(\mu\)m/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 \(\mu\)m/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 secreted by Acanthamoeba (Mooseker, M., T. Coleman, and J. Carboni, unpublished observations). Future studies, focus-
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 lamellipodia, 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 I 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.

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