Abstract. The actin filament core within each microvillus of the intestinal epithelial cell is attached laterally to the plasma membrane by brush border (BB) myosin I, a protein–calmodulin complex belonging to the myosin I class of actin-based mechanoenzymes. In this report, the binding of BB myosin I to pure phospholipid vesicles was examined and characterized. BB myosin I demonstrated saturable binding to liposomes composed of anionic phospholipids, but did not associate with liposomes composed of only neutral phospholipids. The binding of BB myosin I to phosphatidylserine and phosphatidylglycerol vesicles reached saturation at 4–5 × 10⁻³ nmol protein/nmol phospholipid, while the apparent dissociation constant was determined to be 1–3 × 10⁻⁷ M. Similar to the free protein, membrane-associated BB myosin I bound F-actin in an ATP-sensitive manner and demonstrated actin-activated Mg-ATPase activity. Immunoblot analysis of peptides generated from controlled proteolysis of vesicle-bound BB myosin I provided structural information concerning the site responsible for the membrane interaction. Immunoblot staining with domain-specific mAbs revealed a series of COOH-terminal, liposome-associated peptides that were protected from digestion, suggesting that the membrane-binding domain is within the carboxy-terminal “tail” of the BB myosin I heavy chain.

Two general structural classes of the mechanoenzyme myosin have been identified in eukaryotic cells thus far. Conventional two-headed myosins, or myosins II (based on the terminology suggested by Korn and Hammer, 1988), have been shown to be ubiquitous. The second class, termed myosins I, are single headed and lack the alphahelical tail domain of myosins II. The myosin I class of mechanoenzymes was first characterized in ameboid cells (reviewed in Korn and Hammer, 1988). However, recent studies from several laboratories (Collins and Borysenko, 1984; Conzelman and Mooseker, 1987; Coluccio and Bretschler, 1987; Mooseker and Coleman, 1989; Hoshimaru and Nakanishi, 1987; Garcia et al., 1989; Hoshimaru et al., 1989) have demonstrated the expression of a single-headed, tail-less myosin in the intestinal epithelial cells of the vertebrate intestine. This myosin, previously termed 110K-calmodulin (110K-CM) and now named brush border (BB) myosin I (Mooseker and Coleman, 1989), is a protein complex consisting of a ~110-kD heavy chain and multiple molecules of CM (the calculated M₈ of the heavy chain, based on its deduced sequence, is actually ~119 kD; Hoshimaru and Nakaniski, 1987; Garcia et al., 1989). BB myosin I comprises the lateral bridges that tether the microvillar actin bundle to the plasma membrane (see Coluccio and Bretschler, 1989, for references).

The association of BB myosin I with the microvillar membrane of the BB reflects a general functional property of the myosin I class of actin-based motors. As reviewed by Adams and Pollard (1989b), considerable evidence indicates that the myosins I of both Acanthamoeba and Dictyostelium are, at least in part, membrane associated. Moreover, these myosins may play critical roles in membrane-associated cellular movements such as locomotion and phagocytosis since such movements persist in Dictyostelium amebae that have been molecularly engineered to lack their conventional myosin (Knecht and Loomis, 1987; DeLozanne and Spudich, 1987; Knecht and Loomis, 1988; Wessels et al., 1988). Insight into the possible molecular basis for the association of myosins I with membranes has been recently provided by studies assessing the interaction of Acanthamoeba myosin I with membranes in vitro. Taken together, these studies suggest that myosin I can interact directly with the phospholipid bilayer through interactions with anionic phospholipids. Adams and Pollard (1989a) demonstrated that myosin I binds with high affinity to NaOH-stripped plasma membrane vesicles from Acanthamoeba; similar findings were obtained by Miyata et al. (1989) using KI-stripped membranes. Comparable high affinity binding of Acanthamoeba myosin I was observed using artificial liposomes composed of anionic phospholipids; no binding to neutral phospholipids was observed (Adams and Pollard, 1989a).

In the present study we have assessed whether or not the interaction of myosins I with phospholipids may be a general property of these membrane-associated motors. We demonstrate that purified BB myosin I binds specifically to anionic...
The binding of BB myosin I to phospholipid vesicles via the COOH-terminal domain of its heavy chain, and that the enzymatic and actin-binding properties of vesicle-bound BB myosin I are retained.

Materials and Methods

Phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylserine (PS), phosphatidylinositol (PI), PI 4,5-bisphosphate (PIP2), α-chymotrypsin, histones, and cytochrome c were purchased from Sigma Chemical Co. (St. Louis, MO). [3H]-labeled triolein (26.8 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Sephacryl S-400, Fast Flow Q-Sepharose, and Fast Flow S-Sepharose were from Pharmacia AB (Uppsala, Sweden). Immobilized soybean trypsin inhibitor was purchased from Pierce Chemical Co. (Rockford, IL). Centriicon-30 microconcentrators were from Amicon Corp. (Lexington, MA). All other reagents were analytical grade and purchased from Sigma Chemical Co.

Purification of Proteins

BBs were isolated from chicken intestines by the method of Mooseker and Howe (1982) with the modifications described by Keller and Mooseker (1982). BB myosin I was purified from ATP extracts of BBs by a modification of the procedure described by Coluccio and Bretscher (1987). Briefly, ATP extracts were chromatographed on a Sephacryl S-400 column and fractions containing BB myosin I were pooled and applied to a column of soybean trypsin inhibitor-agarose to remove residual proteases. The flow-through was loaded onto a Fast Flow Q-Sepharose column from which BB myosin I was eluted with a 0.25–1 M NaCl gradient using the buffer system of Coluccio and Bretscher (1987). BB myosin I-containing fractions were then pooled and chromatographed on a Fast Flow S-Sepharose column; BB myosin I was eluted from this column with a 0.7 M NaCl step gradient and fractions containing BB myosin I were pooled and concentrated to ~1 mg/ml using Centriicon-30 micro-concentrators. The concentrated protein was dialyzed against 10 mM imidazole (pH 7.2), 75 mM KCl, 2 mM MgCl2, 0.1 mM EGTA, 0.2 mM DTT and stored on ice. Buffer solutions used throughout the purification contained aprotinin (0.1 mg/ml) and PMSF (0.2 mM). In addition to these protease inhibitors, the initial homogeneous buffer contained diisopropylfluorophosphate (0.03%). The BB myosin I obtained using this procedure was >95% homogeneous as determined by densiometrical analysis of SDS gels. CM light chain content of the BB myosin I complex was determined as described by Conzelman and Mooseker (1987).

Skeletal muscle actin was isolated from the acetone powder of chicken breast muscle as described by Pardee and Spudich (1982) with further modifications. BB myosin I obtained using this procedure was >95% homogeneous as determined by SDS-PAGE. Immunoblot analysis was performed as described by Shibayama et al. (1987) after transfer of protein bands from gels to Immobilon transfer membranes (Millipore). Immunoreactive bands were visualized by secondary staining with alkaline phosphatase-conjugated anti-mouse IgG (Promega Biotec, Madison, WI), using the methods recommended by the supplier. The binding of BB myosin I to phospholipid vesicles and the release of CM from BB myosin I upon binding of actin or phospholipid vesicles was measured by densiometrical analysis of Coomassie Blue–stained SDS gels.

α-Chymotryptic Digestion of BB Myosin I

Proteolytic digestion of BB myosin I was performed with α-chymotrypsin (6 μg/ml) in buffer A at 4°C for 30 min in the presence or absence of PG vesicles. The reaction was quenched by the addition of both PMSF and benzamidine to 0.5 mM. The mixtures were then centrifuged at 95,000 g for 20 min in an airfuge at 25°C, and the supernatants and pellets were examined by SDS-PAGE. Immunoblots were analyzed as described below using mAbs cross-reactive with either the NH2-terminal domain (mAb CX-1; Carboni et al., 1988) or the COOH-terminal domain (mAb CX-7; Garcia et al., 1989) of the BB myosin I heavy chain. The rationale for assigning the epitope of mAb CX-7 to the COOH-terminus was based on the observation that the antibody reacts with the intact molecule but not the 90-KD chymotryptic fragment described by Coluccio and Bretscher (1988). The 90-KD fragment, like the intact heavy chain (Garcia et al., 1989), has a blocked amino terminus (M. Mooseker, unpublished observation; based on the inability to micro-sequence the fragment).

Assay for Enzymatic Activity

The Mg-ATPase activity of BB myosin I bound to PG liposomes and of the free protein was assayed in the presence and absence of 1 mg/ml F-actin by the method of Taussky and Shorr (1953). Assay conditions were 10 mM imidazole (pH 7.5), 4 mM KCl, 5 mM MgCl2, 2 mM ATP, 1 mM EGTA, and 0.4 mM DTT. The final concentrations of BB myosin I and liposomes were 0.1 mg/mI and 1 mM, respectively. In each assay, a correction was made for the hydrolysis of ATP attributable to actin. Under the conditions of the experiment, no ATPase activity was observed in samples that contained liposomes alone.

Other Methods

SDS-PAGE (Laemmli, 1970) was performed using 5-16% linear gradient minigels (Matsudaira and Burgess, 1978). Immunoblot analysis was performed as described by Shibayama et al. (1987) after transfer of protein bands from gels to Immobilon transfer membranes (Millipore Continental Water Systems, Bedford, MA). Immunoreactive bands were visualized by secondary staining with alkaline phosphatase–conjugated anti-mouse IgG (Promega Biotec, Madison, WI), using the methods recommended by the supplier. The binding of BB myosin I to phospholipid vesicles and the release of CM from BB myosin I upon binding of actin or phospholipid vesicles was measured by densiometrical analysis of Coomassie Blue–stained SDS gels.

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**Results**

**BB Myosin I Associates with Liposomes Containing Anionic Phospholipids**

The binding of BB myosin I to phospholipids was assessed by cosedimentation analysis. BB myosin I bound pure phospholipid vesicles containing the anionic phospholipids PG and PS, but it showed no apparent interaction with vesicles containing only the neutral phospholipid PC. When binding to mixed liposomes containing PC plus either PE, PI, or PIP2 was examined, similar results were obtained, in that binding was observed only with vesicles containing the anionic phospholipids PI or PIP2 (Fig. 1). The binding of BB myosin I to PS or PG liposomes was not affected by ATP at concentrations of 2 mM, but it could be reversed by high concentrations of salt. The addition of 1 M NaCl to solutions of PG and BB myosin I caused the release of ~90% of the membrane-bound BB myosin I (Fig. 2).

To determine the apparent affinity of the interaction of BB myosin I with phospholipid vesicles, binding was measured by densitometric analysis of SDS gels and analyzed by the method of Scatchard (1949). Binding of PS vesicles was saturable, with a binding capacity of 5.5 pmol protein/nmol phospholipid and an apparent dissociation constant (Kd) of 300 nM (Fig. 3). Taking into account the standard error of the slope in Fig. 3, a Kd range of 260-390 nM was obtained. Similar binding characteristics were observed for the interaction of BB myosin I with PG vesicles, where values of 4.1 pmol protein/nmol phospholipid and 100 nM (range of 80-130 nM) were calculated for the maximum binding capacity and the Kd, respectively (data not shown). Although a binding capacity of 4-5 pmol/nmol phospholipid is within the range reported for other protein/liposome interactions (Fukushima et al., 1981; Lau et al., 1983; Kaiser and Kezdy, 1984; Cohen et al., 1988; Benfenati et al., 1989), this value is likely to be an underestimate; the preparation of liposomes employed for these studies most probably yields a mixture of large unilamellar and multilamellar vesicles (Szoka, 1980) so that the phospholipid available for binding is less than the calculated total phospholipid concentration.

Given the basic nature of BB myosin I heavy chain (pl ~10), it was of interest to compare its interaction with anionic phospholipid vesicles to that of other basic proteins. Under the conditions used for measuring the binding of BB myosin I, no measurable binding of cytochrome c to PG vesicles was observed (data not shown), a finding consistent with a previous report (Mimms et al., 1981). We did observe

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**Figure 1.** Cosedimentation analysis of BB myosin I and phospholipid vesicles. BB myosin I (0.2 mg/ml) was added to either buffer A alone (MI) or to buffer A containing 1 mM of the phospholipids indicated (PC, PG, PS, PE, PI, PIP2). After centrifugation, pellet (P) and supernatant (S) fractions were analyzed by SDS-PAGE. Positions for BB myosin I heavy chain (MI; 110 kD) and CM (17 kD) are indicated. BB myosin I cosediments only with liposomes that contain anionic phospholipids.

**Figure 2.** Effect of NaCl on the binding of BB myosin I to phosphatidylglycerol vesicles. Increasing amounts of NaCl were added to PG vesicles (1 mM) that had been preincubated with BB myosin I (1.1 μM) for 10 min at 4°C in buffer A. Binding measured in the absence of NaCl (1.1 pmol BB myosin I/nmol phospholipid) was adjusted to 100%, and other values were normalized accordingly.
Figure 3. Binding of BB myosin I to phosphatidylinerine vesicles. (a) BB myosin I was incubated with 10 nmol PS vesicles and binding was measured as described in Materials and Methods. (b) Binding data plotted according to Scatchard where protein bound is expressed as pmol BB myosin I heavy chain bound/nmol phospholipid. A value of 300 nM was measured for the apparent dissociation constant (Kd) of the BB myosin I-phospholipid interaction, with a maximum binding capacity of 5.5 pmol protein/nmol PS.

Figure 4. Binding of proteolytic fragments of BB myosin I to PG vesicles. BB myosin I (0.1 mg/ml) was digested with α-chymotrypsin for 30 min in the absence (−PG) or presence (+PG) of PG vesicles. (a) SDS-PAGE analysis of pellet (p) and supernatant (s) fractions obtained from cosedimentation with liposomes. (b) Immunoblot analysis of a gel identical to that shown in a treated with a mouse mAb specific for the NH2-terminal region of the BB myosin I heavy chain. (c) Immunoblot analysis of a gel identical to that shown in a treated with a mouse mAb specific for the COOH-terminal region of the BB myosin I heavy chain. Carboxy-terminal fragments of 30-45 kD are protected from digestion when BB myosin I is bound to liposomes. All of these fragments cosediment with phospholipid vesicles.

Figure 5. MgATPase activity of BB myosin I bound to phosphatidylglycerol vesicles. BB myosin I (0.1 mg/ml), in buffer A containing 4 mM KCl, 2 mM ATP, 5 mM MgCl2, and 1 mM EGTA, was incubated with F-actin (1 mg/ml) or an equivalent volume of buffer in the absence (−PG) or presence (+PG) of PG vesicles. ATPase activity was measured after 20 min at 35°C. Standard deviations are indicated by bars (n = 3).

Carboxy-terminal Proteolytic Fragments of the BB Myosin I Heavy Chain Bind Phospholipid Vesicles

To determine the domain of BB myosin I that is involved in membrane binding, peptides generated by controlled α-chymotryptic proteolysis were analyzed for membrane binding and identified as either amino- or carboxy-terminal fragments. Immunoblot analysis, using an mAb specific for the amino terminal domain of the BB myosin I heavy chain (mAb CX-1), showed that the NH2-terminal digestion pattern was similar for both the free and membrane-bound protein (Fig. 4 b). All of the NH2-terminal peptides identified separated into the supernatant fraction following sedimentation of liposomes. Analysis with the COOH-terminal specific antibody, mAb CX-7, showed that the COOH-terminal digestion pattern for the free protein differed significantly from that for membrane-bound BB myosin I. In the absence of vesicles, digestion of peptides containing the carboxy-terminal mAb CX-7 epitope was nearly complete. In the presence of PG liposomes, membrane binding afforded partial protection to a series of COOH-terminal peptides of 30-45 kD, all of which remained associated with membrane vesicles following sedimentation (Fig. 4 c). In agreement with these findings, the NH2-terminal 90-kD chymotryptic fragment (Coluccio and Bretscher, 1988), which retains bound CM but lacks the mAb CX-7 epitope, did not bind liposomes (data not shown). These results suggest that the carboxy-terminal domain of the BB myosin I heavy chain contains the site responsible for membrane binding.
Membrane-bound BB Myosin I Demonstrates Actin-activated MgATPase Activity

The finding of a specific interaction between BB myosin I and phospholipid vesicles prompted a study of the effect of membrane binding on the functional activity of the protein. As a first step, we measured the MgATPase activity of free and membrane-bound BB myosin I. Cosedimentation studies showed that neither ATP nor F-actin affected the binding of BB myosin I to phospholipid vesicles (data not shown). Under low ionic strength conditions (4 mM KCl, 5 mM MgCl₂) the MgATPase activity of free BB myosin I was found to be activated 13-fold by F-actin (Fig. 5). Actin activation of membrane-bound BB myosin I was also observed but was dependent on the order of addition of BB myosin I, F-actin, and vesicles. When PG vesicles were incubated with BB myosin I before the addition of F-actin, an activation of sevenfold occurred. Levels of actin activation that were similar to those of the free protein (10-fold) were recorded when BB myosin I was first preincubated with F-actin before the addition of PG vesicles (Fig. 5). The reasons for this discrepancy are not clear.

Membrane-associated BB Myosin I Binds and Cross-links F-actin in an ATP-sensitive Manner

In addition to its enzymatic properties, BB myosin I also retained its ability to interact with F-actin in an ATP-dependent fashion. Cosedimentation analysis of mixtures of BB myosin I and PG vesicles plus F-actin revealed that the vesicles pelleted with F-actin in the absence, but not the presence, of ATP. Vesicle-bound BB myosin I caused the cross-linking of actin filaments into aggregates detectable by either low-speed sedimentation (Fig. 6) or dark field light microscopy (Fig. 7). The latter technique also verified that the BB myosin I most likely remains associated with the vesicles in the presence of F-actin since all the liposomes present in these mixtures were incorporated and uniformly distributed within the actin aggregates even after repeated pipetting to free nonspecifically trapped vesicles. Similarly, in cosedimentation studies of mixtures of BB myosin I and PG vesicles labeled with [³H]triolein, >99% of the total radioactivity was recovered in pellets of cross-linked actin aggregates after low-speed centrifugation (data not shown).

It is evident from Fig. 6 that actin binding by the free protein in the absence of ATP resulted in an apparent partial release of CM light chains. Apparent CM dissociation was also a consequence of vesicle association (Fig. 1). The two effects did not appear to be additive, however, because the extent of CM release was identical in mixtures of BB myosin I plus either actin or phospholipid vesicles or both (Fig. 6; compare lanes 6 and 10). In all cases, CM dissociation amounted to ~50% as determined by densitometric analysis of gels similar to those in Figs. 1 and 6. In a subsequent study, it was found that exogenous CM added in excess had no effect on liposome binding by BB myosin I (data not shown). It should be noted that the observed dissociation of CM from the BB myosin I complex in the presence of actin or vesicles could also represent the presence of free CM in the BB myosin I preparation before the addition of actin or vesicles.

Discussion

The results presented here demonstrate a saturable binding of BB myosin I to anionic phospholipid vesicles. These findings are consistent with the recent studies of Adams and Pollard (1989a), who found that Acanthamoeba myosin I associated specifically with liposomes composed of the anionic...
Figure 7. ATP-dependent cross-linking of actin filaments by membrane-bound BB myosin I. (a) Dark field microscopy of PG vesicles (1 mM) alone. (b) PG vesicles plus 0.2 mg/ml BB myosin I. (c) PG vesicles plus both BB myosin I and 0.3 mg/ml F-actin in the presence of 2 mM ATP. (e) PG vesicle plus both BB myosin I and F-actin in the absence of ATP. (d) BB myosin I plus F-actin in the absence of ATP. Addition of free or vesicle-bound BB myosin I to solutions of F-actin in the absence of ATP induces the formation of cross-linked actin aggregates (d and e). Bar, 10 μm.

Phospholipids PS or PIP₂. Moreover, the apparent Kₐ of 100–300 nM that we measured for binding is comparable to a Kₐ value of 140 nM determined by Adams and Pollard (1989a) for the binding of Acanthamoeba myosin I to NaOH-extracted Acanthamoeba organelles, and to a Kₐ of 30–50 nM measured by Miyata et al. (1989) for the binding of Acanthamoeba myosins I to KI-stripped plasma membranes.

As observed for the free protein, membrane-associated BB myosin I cross-linked actin filaments in the absence of ATP. It is important to note, however, that the presumed mechanism of filament cross-linking by vesicle-bound BB myosin I is probably distinct from that for cross-linking by the free protein (Fig. 7; see also Conzelman and Mooseker, 1987). The BB myosin I vesicles are multivalent complexes which could mediate filament cross-linking by the actin site on the myosin heads. The mechanism of filament cross-linking by free BB myosin I has not yet been determined, but recent studies in our laboratory suggest that the most likely explanation is an actin-induced tail to tail association of the molecule (J. Wolenski, S. Hayden, and M. Mooseker; unpublished observations), rather than the presence of a second actin binding site on BB myosin I.

Several lines of evidence indicate that the interaction of BB myosin I with vesicles is mediated by site(s) distal to the CM binding domains located in the COOH-terminal “tail” of the heavy chain (Fig. 8). The positioning of CM binding sites within a 10–15 kD domain adjacent to the head–tail junction was first proposed based on proteolytic domain mapping of chymotryptic and tryptic fragments (Coluccio and Bretscher, 1988; Carboni et al., 1988). We show here that peptides
Figure 8. Domain structure of chicken BB myosin I. (a) Model of the proteolytic domain structure of BB myosin I (see text for references and discussion). The subfragment-1 (S1) domain comprises the "head," while the "tail" domain contains subdomains for CM and phospholipid binding. As noted, different values (three to four) for the number of associated CM light chains have been reported (Coluccio and Bretscher, 1988; Konzelman and Mooseker, 1987); denisitometric analysis of SDS gels of the preparations used in this study indicated ~4 mol CM/mol heavy chain. (b) Primary structural domains of BB myosin I heavy chain. The solid region represents the S1 domain. The cross-hatched areas indicate the two putative CM-binding domains first reported for the bovine protein (Hoshimaru et al., 1989). The bar below indicates the position of a putative CM-binding sequence based on comparison with the CM-binding domain of the erythroid Ca++ ATPase (residues 1,100-1,121, Vorherr et al., 1990; see Fig. 8 c). The domain exhibits ~60% homology (30-43% identity) with the erythrocyte protein and is positioned NH2-terminal to, and partially overlaps, one of the potential domains noted by Hoshimaru et al. (1989; see Fig. 8, b and c). Positioning of the phospholipid binding domain distal to the CM binding region is supported by sequence comparisons of bovine BB myosin I with Acanthamoeba myosins IB and IC (Jung et al., 1989 and Hoshimaru et al., 1989). These workers have noted that bovine BB myosin I residues 850-1,031 shares limited sequence homology with domains present in the COOH-terminal "tails" of Acanthamoeba myosins IB and IC. In the ameboid myosins I, this region may contain the site for the binding of phospholipids (see Jung et al., 1989; Hoshimaru et al., 1989 for discussion). In chicken BB myosin I, the region of shared homology is positioned at residues 808-989 and exhibits sequence similarity comparable to that determined for the bovine protein (~50% homology, ~20% identity). These structural comparisons are consistent with the positioning of the phospholipid binding site near the COOH-terminus of BB myosin I (in contrast to its localiza-
tion near the head–tail junction in the ameboid proteins; Fig. 8 d) and suggest a conserved basis for the interaction of myosins I with phospholipids. However, one of our colleagues (S. Doberstein, Department of Cell Biology and Anatomy, Johns Hopkins University School of Medicine; personal communication) has convinced us that the basis for the observed sequence homologies is in large part due to the high percentage of basic residues in these domains (14% for chicken and bovine BB myosin I; 19 and 20% for Acanthamoeba IB and IC, respectively). Four consecutive randomizations of this domain sequence in the chicken protein produced nearly identical levels (~20%) of sequence identity when compared with the ameboid proteins (S. Doberstein, personal communication). Clearly, it will be important to examine (e.g., through peptide inhibition studies) this domain and compare the relative contributions of primary structure versus overall composition to the interaction of BB myosin I with phospholipids.

In a final point concerning the structural basis for the BB myosin I–phospholipid interaction, it should be noted that the sequence described above does not contain a 50 aa sequence, called the SH3 domain, identified in a diverse population of membrane-associated cytoplasmic proteins and in the COOH-terminal domain of Acanthamoeba IC (see Rodaway et al., 1989 for discussion). Jung et al. (1989) identified a 53 aa sequence, analogous to SH3, within the COOH-terminal domains of both Acanthamoeba myosins IB and IC but found no indication of a similar sequence in bovine BB myosin I. This domain is also absent in the avian BB myosin I heavy chain.

Although the results described here demonstrate a specific association of BB myosin I with phospholipid vesicles, it should be noted that they do not necessarily preclude an interaction of BB myosin I with other microvillar membrane-associated proteins. Coudrier et al. (1983) reported evidence for a proteolytic fragment (GP-140) of a 200-kD porcine gycroprotein from BB membrane that binds BB myosin I transferred to nitrocellulose. Interestingly, a preparation of detergent-resistant membrane sheets enriched in BB myosin I contains a glycoprotein(s) of ~140 kD (Mooskeeper et al., 1989) which may represent the avian analog of GP-140. It is left for additional studies to determine whether the membrane association of BB myosin I is facilitated by other peripheral or integral membrane proteins and whether or not other mechanisms are involved (e.g., fatty acylation).

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