Zipper Protein, a B-G Protein with the Ability to Regulate Actin/Myosin 1 Interactions in the Intestinal Brush Border*

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We recently identified a 28-kDa protein in the intestinal brush border that resembled tropomyosin in terms of size, homology, and α helical content. This protein contained 27 heptad repeats, nearly all of which began with leucine, leading to its name zipper protein. Subsequent analysis, however, indicated that both a 49-kDa and a 28-kDa immunoreactive protein existed in intestinal brush-border extracts. Using 5'-rapid amplification of cDNA ends analysis, we extended the N-terminal sequence of zipper protein to the apparent translation start site. This additional sequence contained a putative transmembrane domain and two potential tryptic cleavage sites C-terminal to the transmembrane domain which would release a 28-kDa cytoplasmic protein if utilized. The additional sequence was highly homologous to members of the B-G protein family, a family with no known function. Immunoelectron microscopy showed that zipper protein was confined to the membrane of the microvillus where it was in close association with brush-border myosin 1 (BBM1). Recombinant zipper protein (28-kDa cytoplasmic portion) blocked the binding of actin to BBM1 and inhibited actin-stimulated BBM1 ATPase activity. In contrast, zipper protein had no effect on endogenous or K/EDTA-stimulated BBM1 ATPase activity. Furthermore, zipper protein displaced tropomyosin from binding to actin, suggesting that these homologous proteins bind to the same sites on the actin molecule. We conclude that zipper protein is a transmembrane protein of the B-G family localized to the intestinal epithelial cell microvillus. The extended cytoplasmic tail either in the intact molecule or after tryptic cleavage may participate in regulating the binding and, thus, activation of BBM1 by actin in a manner similar to tropomyosin.

The intestinal brush border contains a highly cell-specific form of myosin 1 called brush-border myosin 1 (BBM1)1 (1). BBM1 is part of an ever increasing family of non-muscle myosins (2–6) which are characterized by homology to conventional muscle myosins in terms of their ATP and actin binding domains in the N-terminal head of the molecule, but lack the α helical rod-like tail domain which permits conventional myosins (i.e. myosin 2) to dimerize. Like other myosins, BBM1 has actin-stimulated ATPase activity and, in the presence of ATP and calcium, generates movement along actin filaments (7, 8). BBM1 is capable of binding both to the actin core and to the membrane of the microvillus (7, 9–11). Binding to the membrane alters its activity by as yet uncertain mechanisms (7, 12). Binding to the membrane appears to involve the C-terminal portion of the molecule and requires acidic phospholipids (13). This ability to bind both actin and membranes suggests that BBM1 plays a role in membrane transport (14). Regulation of the binding of BBM1 to the membrane or to actin is not thoroughly understood. Tropomyosin blocks binding of BBM1 to actin (8), but tropomyosin is not found in the microvilli where BBM1 is located (11). We (15) recently described a protein with structural homology to tropomyosin which at the light microscopic level colocalized with BBM1 to the brush border. Because of its extended leucine zipper motif we call this protein zipper protein. Subsequent to the initial report, we have determined that the originally reported structure is the cytoplasmic tail of a larger protein in the B-G protein family, a family of proteins with no known function. The data in this report indicate that zipper protein is a transmembrane protein of the intestinal microvillus whose cytoplasmic tail regulates the binding of BBM1 to actin.

EXPERIMENTAL PROCEDURES

Western Analysis—Duodenal mucosa from White leghorn cockerels was extracted with phosphate-buffered saline (PBS) containing 0.1% Triton X-100 and 0.1 mM phenylmethylsulfonyl fluoride. Following homogenization, the extracts were centrifuged at 100,000 × g for 30 min, and the supernatants were collected, flash frozen, and stored at −80 °C until analyzed. 100-μg aliquots of these samples were subjected to sodium lauryl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (12% gels) and blotted onto PVDF membranes using 10 μg CAPS, pH 10.4, and 2% methanol. For selected experiments, non-denaturing gels were employed in which SDS and DTT were not included in the PAGE. The blots were blocked with 10 μg Tris-HCl, pH 8, 150 μg NaCl, 5% non fat dry milk, and 0.05% Tween 20, then incubated overnight with 1/100 × dilution of the following affinity-purified antibodies: 1470 (raised to the epitope KKQYENLASHASE) and 1468 (raised to the epitope RELKRKDAMLGRKD). The first epitope is found near the middle of the cytoplasmic tail (amino acids 351-363 of new sequence, Fig. 1); the second epitope is found near the membrane domain and spans the region containing a potential tryptic cleavage site (amino acids 187–200 of new sequence). A third antibody, 1092, raised against the epitope AKQTEEEYNHAEVE (amino acids 309–321) was used for immunoelectron microscopy and quantitation of zipper protein during the actinBBM1 binding studies. It gave similar results to 1470 on Western analysis. The production of these antibodies is as described previously (15). Detection utilized the ECL kit by Amersham according to the manufacturer’s recommendations. The same blot was used for both antibodies, stripping the blots in between by incubation at 50 °C in 62.5 μg Tris-HCl, pH 6.7, 100 μg β-mercaptoethanol, 2% SDS.

Sequencing—Total RNA was prepared from the duodenal mucosa of...
White leghorn cockerels by the method of Chomczynski and Sacchi (16). 1-µg aliquots were reverse-transcribed and amplified using the 5'-RACE kit from Life Technologies, Inc. The primer used for the reverse transcriptase reaction was TTTTCACTTTTTTCTTCT. The nested primer used for polymerase chain reaction amplification was AGCTCAT-GTACCGGATTGTTGGACTCAGTT which contains a HindIII site for cloning. A second primer used together with the above primer for polymerase chain reaction products and positive clones was TTGGCAGCCTGAATCTAA. The products were subcloned into pGEM4Z (Promega, Madison, WI) using the SmaI and HindIII sites and sequenced using the Sequenase 2 kit (U. S. Biochemical Corp.). All oligonucleotides used for these procedures were prepared by the Biomedical Resource Center, University of California, San Francisco. Sequence analysis and homology searches were performed on-line using selected programs in Intelligentgenes (Mountain View, CA).

Immunoelectron Microscopy—The jejunum from a 6-week-old White leghorn chicken was fixed in 4% paraformaldehyde in 0.05 M Hapes buffer, pH 7.2, containing 3.5% sucrose, or in pyridoxal phosphate-fixed tissues, embedded in Lowicryl HM23 resin, polymerized by UV at -10°C (19, 20). Sections were collected on nickel grids and coated with Formvar and carbon. The sections were first incubated with 50 µg/ml NH4Cl in PBS for 15 min. Non-specific binding was blocked by incubation with 5% newborn calf serum (Life Technologies, Inc.) in 10 mM Tris, pH 7.6, containing 500 mM NaCl (TBS) for 30 min. The grids were then incubated with anti-zipper protein antisera (1092) or with preimmune serum, diluted 1:25 in TBS containing 5% fetal bovine serum for 1 h, followed by washing with 30 min. Binding of the antibody was revealed with goat anti-rabbit IgG, conjugated to 15-nm colloidal gold (Zymed). The grids were washed and stained with 0.3% uranyl acetate in 2% methylcellulose.

BBM1 was detected on pyridoxal phosphate-fixed tissues, embedded into Lowicryl HM23 resin, polymerized by UV at -25°C. The sections were first incubated with 50 µg/ml NH4Cl in PBS for 15 min. Non-specific binding was blocked by incubation with 5% newborn calf serum (Life Technologies, Inc.) in 10 mM Tris, pH 7.6, containing 500 mM NaCl (TBS) for 30 min. The grids were then incubated with anti-zipper protein antisera (1092) or with preimmune serum, diluted 1:25 in TBS containing 5% fetal bovine serum for 1 h, followed by washing for 30 min. Binding of the antibody was revealed with goat anti-rabbit IgG, conjugated to 15-nm colloidal gold (Zymed). The grids were washed and stained with 0.3% uranyl acetate in 2% methylcellulose.

BBM1 Purification—BBM1 was prepared using a modification of the method of Svanjung-Collins et al. (21). The duodenal villi of White leghorn cockerels were rinsed with ice-cold saline, then incubated on ice with 10 mM KH2PO4, 150 mM NaCl, pH 7.3, containing 1 mM dithiothreitol (DTT), 0.2 mM 4-[(2-aminophenyl)benzenesulfonyl fluoride (AEBSF), 0.02% NaN3, 5 µg/ml aprotinin, 1 µg/ml leupeptin, 5 µg/ml pepstatin A, and 1 µl diisopropyl fluorophosphate. The tissues were then placed in 76 mM Na2HPO4, 19 mM KH2PO4, 12 mM EDTA, pH 7.3, containing DTT, AEBSF, NaN3 and the above protease inhibitors for 30 min with stirring. The mucosa was scraped off and combined with the eluted cells, washed, and homogenized in 10 mM imidazole chloride, 4 mM EDTA, 1 mM EGTA, pH 7.3, with DTT, AEBSF, NaN3, and the other protease inhibitors. The 800 x g pellet of the homogenate was washed twice in homogenization and twice in wash buffer containing 10 mM imidazole chloride, 75 mM KCl, 5 mM MgCl2, 1 mM EGTA, pH 7.3, with DTT, AEBSF, NaN3 by washing with wash buffer containing 50 mM NaCl, 2% urea, 10% sucrose, and 0.1% wt/vol Triton X-100, then collected the 100,000 x g supernatant. This extract was chromatographed over Sepharose Cl-4B (5 x 90 cm, Pharmacia Biotech Inc.) as described (21), and the BBM1 peak was identified by KEDTA ATPase activity (see below). The second ATPase peak (containing BBM1) was dialyzed into 10 mM imidazole, pH 7.3, 0.1 mM MgCl2, 1 mM EGTA, 50 mM NaCl, 10% sucrose, 0.1 mM AEBSF, loaded unto a CM-Sephadex column (2.5 x 20 cm, Pharmacia), washed with the same buffer but containing 1 mM ATP and 1 mM DTT, then batch-eluted with the same buffer but containing 50 mM NaCl and without ATP and DTT. The BBM1 peak was identified by its ATPase activity. The BBM1 peak was then made up to 10 mM imidazole, pH 7.3, 0.1 mM MgCl2, 1 mM EGTA, 100 mM NaCl, 10% sucrose, 0.1 mM AEBSF, 1 mM DTT, loaded onto a Mono Q column (Pharmacia), and eluted with a linear gradient of NaCl from 100 to 600 mM in the same buffer used for loading. The peak fractions were diluted with 2 volumes of Mono Q loading buffer (without NaCl), loaded onto a Mono S column (Pharmacia), and eluted with the same linear gradient used for Mono Q. The purity of BBM1 in the peak fractions was confirmed by SDS-PAGE. BBM1 is stable for at least 6 weeks if maintained in the elution buffer (containing approximately 400 mM NaCl) at 4°C.

Measurement of Zipper Protein, Actin, and Myosin 1 Levels in Intestinal Preparations—Purified preparations of brush-border membranes were subjected to SDS-PAGE along side purified preparations of BBM1 (prepared as described above), actin (gift from Dr. James Sellers), and zipper protein (prepared as described in Ref. 15) spanning a range (0.15–10 µg, 0.625–10 µg, 0.00625–0.2 µg, respectively) appropriate for the amounts of these proteins expected to be found in the intestinal preparations. The gels were blotted onto PVDF membranes as described above, and BBM1, actin, and zipper protein were detected using the appropriate antibodies and the ECL detection system described above. Using the densitometric quantitation of the purified proteins to prepare standard curves (linear over the ranges employed for all three proteins), the amounts of these proteins in the brush-border membranes were calculated.

BBM1/Actin Binding—BBM1 prepared as above, rabbit muscle actin (gift from Dr. James Sellers), and recombinant zipper protein prepared as described previously (15) were dialyzed into assay buffer containing 10 mM Tris-HCl, pH 8.0, 1 mM DTT, 100 mM KCl, 1 mM MgCl2. The actin was prepared as a phalloidin:actin mixture at 10:1 molar ratio prior to use in this assay. The assay mixture contained 1 µg of BBM1, 2 µg of actin, 0.2–6 µg of zipper protein, and 0 or 5 mM Tris-HCl. After a 30-min incubation at room temperature, an aliquot (25 µl) was taken for total protein determination; the remaining 25-µl incubates were centrifuged at 200,000 x g for 45 min, and the supernatants (and totals) were spotted onto PVDF membranes using a slot blot apparatus. The pellets were subjected to SDS-PAGE. The BBM1, actin, and zipper protein in the various fractions were quantitated by Western analysis using the appropriate antibodies as described above. Initial experiments demonstrated that the amount of protein used in these experiments fell within the linear range for detection by this method with quantitative recovery of these proteins in the supernatants.

Tropomyosin/Zipper Protein/Actin Binding—Zipper protein and actin were prepared as described above. Chicken gizzard tropomyosin was purchased from Sigma. The binding buffer included 10 mM Tris, pH 8.0, 1 mM DTT, 100 mM KCl, 5 mM MgCl2, and was used to make up the components of the assay. Initial experiments showed essentially complete binding of 0.3 µg of tropomyosin to 1 µg of actin under the conditions of the experiment. 2 µg of the actin-phalloidin complex was incubated for 10 min at 22°C with varying amounts (0–6 µg) of zipper protein in binding buffer prior to the addition of 0.6 µg of tropomyosin for an additional 30 min (total volume 50 µl). 25-µl aliquots were removed to assess total levels of the components, then the mixture was centrifuged at 200,000 x g for 45 min. The pellets and supernatants were treated as for the actin/tropomyosin binding experiments; tropomyosin was detected using 1:100 dilution of anti-tropomyosin raised in rabbits against chicken gizzard tropomyosin (Sigma).

ATPase Activity—The BBM1, actin, and zipper protein were dialyzed into incubation buffer, 10 mM imidazole chloride, pH 7.0, 2 mM MgCl2, 0.1 mM EGTA, 0.4 mM DTT. In initial experiments, activation of BBM1 ATPase activity (1 µg of BBM1) was found to be linear over at least 30 µg of actin. For these experiments, 1 µg of BBM1, 10 µg of actin, and 1–30 µg of zipper protein were combined in 20-µl assay volume. The reaction was initiated with a final concentration of 0.1 µCi of ['gamma-32P]ATP in 1 mM Tris-ATP. The tubes were incubated at 37°C for 60 min, then extracted as described previously (22), and the released ['gamma-32P]P was quantitated by liquid scintillation spectroscopy. KEDTA ATPase activity was measured in similar fashion except that the incubation buffer contained 0.5 mM KCl, 2 mM EDTA, 10 mM imidazole chloride, pH 7.5, and no actin.
RESULTS

Subsequent to our initial report (15), we became aware that our original clones encoding zipper protein may have contained "introns" which interfered with the determination of the beginning of the open reading frame in the 5' end of the sequence, a frequent occurrence in clones of B-G proteins (22, 23). Therefore, we repeated the sequencing of the 5' end of zipper protein, using mRNA from gut mucosa (rather than a cDNA library) as starting material, and 5' RACE technology. Nine clones were fully sequenced in both directions. Although varying in length at the 5' end, all clones encoded the same protein. The consensus sequence when coupled to the previously determined sequence of zipper protein encoded a protein of 49,272 daltons (374 residues) (Fig. 1). This sequence showed a high degree of

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homology with the 5' end of the B-G proteins obtained from an erythroid cell library published by Miller et al. (22) as shown in Fig. 2.

The hydropathy plot of the N-terminal region of zipper protein (Fig. 3) indicates a 20-amino acid stretch of hydrophobic amino acids (amino acids 159–178) which is highly conserved among the B-G proteins; as seen in Fig. 2, conservative amino acid substitutions are the only differences in the 7 positions not completely homologous among these proteins in this region with the exception of a proline in D39371 instead of an alanine in the other proteins. This likely represents a transmembrane domain. Immediately C-terminal to this putative transmem-

Fig. 3. Hydropathy plot of zipper protein. Hydrophobicity is indicated by positive values. Charged residues are indicated by a + or − as appropriate. The boxed region includes the hydrophobic region from amino acids 159–178, the likely transmembrane domain, and a hydrophilic region from amino acids 179–194 which contains a number of basic residues in doublets or triplets providing a likely site for proteolytic cleavage.
brane domain is a highly hydrophilic domain (amino acids 179–194) which contains 7 basic residues in pairs or triplets. Proteolytic cleavage in this basic region would release a 28-kDa protein. This region also is highly homologous with the B-G proteins. The homology among the B-G proteins in the remainder of the structure is less well preserved, although all proteins demonstrate the extended leucine zipper motif. The leucine zipper motif suggests that these proteins exist as coiled coils in their C-terminal cytoplasmic domains. Comparing the migration of the C-terminal portion of zipper protein on denaturing versus non-denaturing gels, we found the expected size (28 kDa) on denaturing gels but an apparently much larger protein (approximately 120 kDa) on non-denaturing gels suggesting multimer formation and/or aberrant migration of an asymmetric molecule (data not shown).

To determine whether zipper protein existed as the full-length or cleaved product in the intestinal epithelium, we examined extracts of intestinal mucosa by Western analysis using antibodies raised to different regions of the deduced sequence. The results are shown in Fig. 4 in which the same blot is probed with both antibodies. The antibody (1470) raised to a region near the tail of the sequence (amino acids 351–363) detected two proteins, one with the expected size for the cytoplasmic domain of zipper protein (28 kDa) and one substantially larger (49–50 kDa). A second antibody (1468) raised to the basic region adjacent to the putative transmembrane domain (see below) (amino acids 187–200) recognized only the 49-kDa band. Both antibodies recognized only the 49-kDa species in purified brush-border membranes (data not shown). The structure for zipper protein shown in Fig. 1 has a calculated molecular mass of 49.3 kDa, suggesting that it is the complete sequence. The smaller band (28 kDa) detected by antibody 1470 is consistent with cleavage in the polybasic region of zipper protein C-terminal of the transmembrane domain and in a region that would disrupt the epitope recognized by antibody 1468.

To determine more precisely the location of zipper protein within the brush border, we performed immunogold localization at the electron microscopic level and compared the results to the immunolocalization of BBM1. The results are shown in Fig. 5. The left panel shows the immunolocalization of zipper protein; the right panel shows the immunolocalization of BBM1. Although BBM1 could be detected in embedded tissue, zipper protein localization at the ultrastructural level required cryosections. The zipper protein was found exclusively in the microvilli of the intestinal epithelial cells where it appeared closely associated with the membrane. No labeling of the basolateral membrane or cytoplasmic organelles was seen. Goblet cells and cells in the submucosa did not express detectable levels of zipper protein. BBM1 was also found exclusively in the microvilli. However, BBM1 had both a membrane-associated and intravesicular localization. As for zipper protein, BBM1 was not found in the intermicrovillar domain of the apical plasma membrane or invaginations of the apical plasma membrane, basolateral membrane, or other subcellular organelles. When the anti-zipper protein or anti-BBM1 antiserum was replaced with preimmune serum, no gold particles were seen over the brush border.

We then assessed the amounts of zipper protein in purified brush-border membranes in comparison with actin and myosin 1. In purified brush-border membranes, zipper protein existed only in the 49-kDa form at a concentration of 0.4 ng/µg of protein, whereas in intestinal extracts its concentration was considerably higher (2.5 ng/µg of protein) and both 49-kDa and 28-kDa forms were present. These data suggest that the cleaved product of zipper protein is lost during purification of the membranes. Actin and myosin were found in purified membranes at a concentration of 47 and 56 ng/µg of protein, respectively.

The structure of the presumed cytoplasmic portion of zipper protein resembles tropomyosin, raising the possibility that it functions like tropomyosin in regulating myosin/actin interactions. To test this possibility, we evaluated first the ability of zipper protein to block the binding of actin to BBM1, a property previously found for tropomyosin (8). The results are shown in Fig. 6. The labeling of this figure reflects the fact that one-half of the original incubate was used for the binding analysis (i.e. 0.5 µg of BBM1, 1 µg of actin, and 0.1 to 3 µg of zipper protein). The first three panels (A, B, and C) depict the same blot of supernatant samples probed for BBM1, actin, and zipper protein, respectively. The final three panels (D, E, and F) depict the same blot of pellet samples probed for BBM1, actin, and zipper protein, respectively. The graphs indicate the percent of the total BBM1 and actin in the supernatants and pellets,
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Fig. 6.
respectively. In the absence of zipper protein and ATP, all BBM1 was precipitated with actin (A and D). As zipper protein was increased from 0.1 to 3 μg, an increasing amount of BBM1 (up to 30%) was displaced from the actin precipitate. Maximal inhibition occurred at 1 μg of zipper protein or approximately a 2:1 molar ratio with actin. ATP alone released 70% of the BBM1 from the actin pellet, and zipper protein potentiated this effect of ATP (100% release at 3 μg). Actin (B and E) remained in the precipitate in the absence of ATP despite increasing concentrations of zipper protein. In the presence of ATP, 18% of the actin was found in the supernatant; zipper protein from 0.1 to 0.3 μg increased actin solubilization to 40%, but at higher concentrations inhibited this solubilization such that, at 3 μg of zipper protein, all actin was found in the precipitate regardless of ATP concentration. Zipper protein (C and F) remained primarily in the supernatant where it showed the expected increase with increasing addition of zipper protein.

We repeated this approach to determine whether zipper protein and tropomyosin share the same binding site on actin. The results are shown in Fig. 7. In panel A of this figure, increasing amounts of zipper protein (from 0 to 3 μg) were added to 1 μg of actin prior to incubation with 0.3 μg of tropomyosin. In the absence of zipper protein, essentially all the tropomyosin was pelleted with the actin. In the presence of increasing amounts of zipper protein, less tropomyosin was pelleted with actin, such that displacement was nearly complete at the highest concentration of zipper protein (3 μg). In panel B, the experiment shown tested the ability of tropomyosin to displace zipper protein in its binding to actin. In the absence of tropomyosin, 64% of the zipper protein (0.3 μg) was removed from the supernatant by 1 μg of actin. Coincubation with 0.3 μg of tropomyosin released all the actin-bound zipper protein into the supernatant. In a separate experiment, we found that maximum binding of zipper protein to 1 μg of actin occurred at 1–3 μg of zipper protein indicating saturation of the actin at a molar ratio of approximately 2:1 zipper protein:actin (data not shown).

The ability of zipper protein to inhibit actin-stimulated (A) but not K/EDTA-stimulated (B) BBM1 ATPase is shown in Fig. 8. In this experiment, 10 μg of actin was used to stimulate the ATPase activity of 1 μg of BBM1. This concentration of actin stimulated endogenous BBM1 ATPase activity by 2.17-fold. Increasing the zipper protein from 1 to 10 μg eliminated completely the actin stimulation with no further inhibition at 30 μg. Zipper protein had no effect on BBM1 ATPase activity in the absence of actin. As for actin binding (Fig. 6), zipper protein had its maximal effect at a 2:1 molar ratio with actin. In contrast to its inhibition of actin-stimulated BBM1 ATPase, zipper protein had no effect on K/EDTA-stimulated ATPase (B).

**DISCUSSION**

In our original report of zipper protein (15), we identified 3 clones which differed primarily at their 5' ends but contained an essentially identical open reading frame encoding a 28-kDa protein which we called zipper protein. Although the nucleotide sequences were homologous to the family of proteins called B-G proteins, proteins originally purified from chicken erythrocytes but found also in a variety of immune tissues (24), the lack of hybridization of the zipper protein cDNAs to marrow and...
spleen cells indicated to us that we were dealing with a different protein (15). Subsequent to our original report, we became aware of the potential inclusion of introns into the cDNAs from which zipper protein was cloned (22, 23) which could account for the variation in the 5' end and mislead us in terms of identifying the full open reading frame. Therefore, we repeated the sequencing of zipper protein using mRNA directly from the chick intestinal mucosa. Nine clones were sequenced and all encoded the same protein. The additional N-terminal sequence had a much higher homology with the equivalent region of the B-G proteins sequenced by Miller et al. (22) which were cloned from a library prepared from erythroid cells of a 14-day-old chick embryo. Furthermore, Western analysis of intestinal mucosal extracts demonstrated the existence of a 49-kDa protein indicating that the predicted B-G-like protein was actually produced. Thus zipper protein is clearly a member of the B-G family.

B-G proteins are characterized by a highly conserved N-terminal domain with a variable C-terminal domain (22, 23, 25). Zipper protein fits this description in that it is highly homologous to the N-terminal portion of the erythroid B-G proteins but not to the C-terminal region. B-G proteins appear to be integral membrane proteins oriented with the N-terminal region extracellular and with a single transmembrane domain. The putative transmembrane domain of zipper protein is highly homologous to that in the erythroid B-G proteins (Fig. 2). The extracellular domain of B-G proteins is not N-glycosylated and shows homology with members of the immunoglobulin superfamily (22). B-G proteins are encoded by a polygenic region of the chick major histocompatibility locus (26) and were originally thought to be limited in distribution to erythroid tissues. With their demonstration in thrombocytes and lymphoid tissue (24), a potential role for B-G proteins in immunologic function has been proposed. However, B-G proteins have also been described in the intestine (27), and an understanding of their role remains elusive.

We (15) were impressed with the high degree of homology between the cytoplasmic portion of zipper protein and tropomyosin and proposed that zipper protein might function as the tropomyosin of the microvillus in terms of regulating BBM1/actin interactions (8, 15). Tropomyosin, although present in the intestinal epithelial cell, is not present in the microvillus (28). Zipper protein, on the other hand, is found exclusively in the microvillus. Our immunolocalization studies indicate that zipper protein is inserted into the microvillus membrane, consistent with its possession of a transmembrane domain. However, Western analysis indicates that the cytoplasmic portion can be cleaved from its anchor in the membrane. During preparation of purified brush-border membranes, all of the cleaved product is lost, such that the measured levels of zipper protein in the purified brush-border membrane preparation are considerably below those found in crude intestinal extracts. The highly basic region adjacent to the transmembrane domain is a likely target for proteolytic cleavage and, if utilized, would release the 28-kDa protein found on Western analysis. The antibody that spans the putative cleavage site (1468) did not detect the 28-kDa band, only the intact 49-kDa protein, providing additional evidence that this region is the site of cleavage. Although protease inhibitors were employed during the extraction of the intestinal mucosa for Western analysis, we cannot be certain that the proteolysis occurred in vivo. In either case, the cytoplasmic portion of zipper protein does have tropomyosin-like properties in that it blocks the binding of actin to BBM1 and tropomyosin and inhibits actin-stimulated BBM1 ATPase activity. A 2:1 molar ratio of zipper protein to actin appears to be maximally effective and is sufficient to saturate binding of zipper protein to actin. This ratio is greater than that seen for tropomyosin which saturates actin binding at a molar ratio of approximately 1:3.5 tropomyosin:actin (29). This would imply that zipper protein has a lower affinity for actin than does tropomyosin or binds to a different site on the actin molecule. The latter possibility seems unlikely in view of the ability of zipper protein to displace tropomyosin from actin and vice versa. At this point, it is not clear whether zipper protein can perform these tropomyosin-like functions while anchored to the membrane or whether the cytoplasmic portion needs to be released from its membrane anchor to be effective. Our measurements of zipper protein levels in purified brush-border membranes relative to actin and myosin indicate that the concentration of intact zipper protein is far below that required to saturate actin suggesting that its regulatory role may be limited. Conceivably, the intact molecule acts as a receptor for some as yet undefined signal leading to release of the intracellular domain which acts locally to regulate actin/BBM1 interactions. Whether other B-G proteins will have similar functions remains for future investigation. But the concept that B-G proteins can control membrane movement by regulating the activity of the submembrane cytoskeleton is intriguing.

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