Brush-Border Alpha-Actinin?

Comparison of Two Proteins of the Microvillus Core with

Alpha-Actinin by Two-dimensional Peptide Mapping

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ABSTRACT   The bundle of filaments within the intestinal microvillus contains four major polypeptides in addition to actin: calmodulin, a 70-kdalton subunit and two polypeptides with molecular masses similar to that of the Z-line component alpha-actinin (95 and 105 kdaltons). Two-dimensional mapping of tryptic peptides indicates that (a) alpha-actinins from chicken skeletal, cardiac, and smooth muscle are similar but not identical proteins and that skeletal alpha-actinin is more similar to the cardiac subunit than to the alpha-actinin from gizzard; (b) the brush-border 95- and 105-kdalton subunits are closely related to each other, but the smaller subunit is not a proteolytic fragment of the 105-kdalton subunit; and (c) although there is considerable peptide overlap between the brush-border subunits and the three alpha-actinins, the peptide maps of the 95- and 105-kdalton proteins are substantially distinct from the various alpha-actinin maps, suggesting that neither brush-border subunit is a bona fide alpha-actinin. Nevertheless, on the basis of peptide mapping criteria alone, one cannot exclude the possibility that the brush-border subunits are "alpha-actinin-like." However, there is no immunological cross-reactivity between the brush-border subunits and alpha-actinins, using antibodies prepared against gizzard alpha actinin.

Alpha-actinin is a major protein constituent of the Z line in striated muscle (15, 31, 32) and the dense body in smooth muscle (26). Even though it has been shown that alpha-actinin cross-links actin filaments in vitro, the function of this protein in vivo is not understood, because extraction of alpha-actinin from the Z line does not greatly affect the mechanical integrity of this structure (31). A number of years ago, Mooseker and Tilney (20) suggested that an alpha-actinin-like protein is also present in nonmuscle cells and functions as a ligand in actin filament-membrane and/or filament-filament interaction. This speculation was based on morphological and chemical studies of the isolated brush border, a term referring to the apical surface of intestinal epithelial cells. In the microvilli of the brush border, the actin filament core is attached to the plasma membrane at one end, as well as laterally (20, 21). A dense, osmiophilic plaque, morphologically and spatially analogous to the Z line, effects end-on attachment, while numerous periodic cross-filaments with dimensions similar to those of the alpha-actinin molecule (22, 31) connect the filament bundle to the membrane all along its length. Similar cross-filaments attach actin filaments within the bundle to one another (20,

21). That alpha-actinin might be a protein constituent of some or all of these structures in the microvillus was suggested by results of antibody localization studies (27) at the ultrastructural level, and by the fact that a major protein of the isolated, demembranated brush border (which includes proteins of the terminal web) has a molecular weight similar to that of alpha-actinin (17). The original localization studies have never been published *in extenso*. In fact, the only "documentation" of these results that is published (and frequently cited by others) is a line drawing depicting a model, proposed by Mooseker and Tilney (20), for the functional organization of actin and myosin in the brush border (20, 17, 18).

The original antibody localization studies became suspect when no staining of the isolated brush border was observed with the use of a second antiserum that Fujiwara et al. (10) had shown by several criteria to be specific for alpha-actinin. The situation became even more confusing when we discovered that the brush border contains a second, slightly larger protein (105-kdaltons) (19) than the 95-kdalton subunit originally described (17) that is lost from brush-border preparations if proteolysis is not stringently controlled.

In an attempt to unravel this puzzling situation, we have conducted an extensive chemical and immunological comparison of the two brush-border subunits described above (95 and 105 kdalton) and alpha-actinins purified from skeletal, cardiac, and smooth muscle. The technique for comparing these proteins was two-dimensional mapping of tryptic peptides, using methods that permit visualization of essentially all the peptides produced from the digestion of as little as 1 nm of subunit (29). We conclude from our results that the speculations concerning the presence and function of alpha-actinin in the microvilli of the brush border are wrong. While our studies were in progress, several other laboratories presented results that support this conclusion (4–7, 11). The results of these other studies and a comparison with our own results are discussed in detail later. Although the microvilli of the brush border apparently do not contain alpha-actinin involved in the attachment of the core filaments to the plasma membrane, there is immunological evidence indicating that alpha-actinin is present in the terminal web region of the brush border, primarily at the lateral margins of the cell (4, 7, 11). These results were obtained by in situ localization studies on intact cells. Presumably, the alpha-actinin present in the terminal web region of the epithelial cell is lost during brush-border isolation by the procedures employed in this study.

MATERIALS AND METHODS
Brush-Border and Microvillus Isolation

Brush borders were isolated from the small intestines of chicken by the method of Mooseker et al. (19). Microvilli were isolated from purified brush borders by methods modified from procedures of Booth and Kenny (2) and Bretscher and Weber (3), and are described in detail elsewhere (12).

Alpha-Actinin Isolation

Alpha-actinin was partially purified from chicken breast and gizzard by the method of Suzuki et al. (32). A crude fraction of chicken heart alpha-actinin was prepared by the same procedure, except that the chromatographic purification steps were omitted.

Preparative Polyacrylamide Gel Electrophoresis

Final purification of the two brush-border subunits (95 and 105 kdalton) and the three alpha-actinins was achieved by preparative polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) by the method of Stephens (28). All protein samples were amidomethylated before preparative gel electrophoresis by a modification of the method of Craven et al. (8). Proteins were denatured in 5.0 M guanidine HCl and reacted with iodoacetamide rather than iodoacetic acid. A minimum of 2 mg of subunit was isolated for each tryptic digest analyzed.

Two-dimensional Tryptic Peptide Mapping

Tryptic digestion was performed at a 100:1 ratio of protein:DCC (diphenylcarbamoyl chloride) -treated trypsin (Calbiochem-Behring Corp., American Hoechst Corp., La Jolla, Calif.). In some of our initial experiments, TPCK (L-[tosylamido 2-phenyl]ethyl chloromethyl ketone) -treated trypsin (Worthington Biochemical Corp., Freehold, N. J.) was used, and in these experiments we experienced some difficulties with incomplete digestion of some of the alpha-actinin samples. The mapping technique of Stephens (29) was used exactly as described. In brief, tryptic digest derived from 1 nm of protein (or, in the case of composite maps, 1 nm of each subunit to be compared) was applied to 20-cm² silica-gel thin-layer chromatography plates (silica gel GHL, Analtech, Inc., Newark, Del.), chromatographed in chloroform-methanol-ammonium hydroxide (2:2:1 vol/vol), and electrophoresed in the second dimension at 1,000 V for 60 min at pH 3.5, using a tank buffer of pyridine–acetic acid water (2:20:978). The resulting peptide maps were visualized by fluorescamine staining (Pierce Chemical Co., Rockford, Ill.), and, for documentation, were photographed under long-wavelength UV illumination. To facilitate map scoring, photographic prints in reverse contrast (dark spots on light background) were prepared by use of internegatives.

Anti-Alpha-Actinin Staining of SDS Gels

SDS gels containing alpha-actinins and brush-border proteins were stained with an antibody for gizzard alpha-actinin by the ^{125}I-labeled protein A procedure of Adair et al. (1). This antibody was prepared by M. E. Porter, K. Fujiwara, and T. D. Pollard and has been characterized in detail elsewhere (10).

Other Methods

Protein concentrations were determined by the method of Lowry et al. (14). Polyacrylamide gel electrophoresis in the presence of SDS was carried out, using the methods of Fairbanks et al. (9), Stephens (28), or Laemmli (13). Light micrographs of purified microvilli were made on Kodak S.O. 115 35-mm film, using a Zeiss Axiomat equipped with differential interference contrast optics.

RESULTS
Proteins of the Brush Border and Isolated Microvillus

The apical, brush-border surface of intestinal epithelial cells consists of two domains—a highly organized contractile apparatus and the plasma membrane to which this apparatus is connected (17, 20, 25, 35). The membrane of the brush border can be removed by detergent treatment with nonionic detergents such as Nonidet P-40 or Triton X-100 (20), and this treatment solubilizes the major proteins of the brush border, most of which are glycoproteins (Fig. 1). The organization of the underlying motile apparatus is not greatly affected by this detergent treatment. The major proteins of this structure include actin (34), myosin (19), tropomyosin (17), a 70-kdalton subunit, several high-molecular-weight subunits, and two subunits in the range of alpha-actinin, at 95 and 105 kdaltons. Preparations of purified microvilli (Fig. 2) are characterized by a simpler spectrum of polypeptides on SDS gels (Fig. 3). All the major glycoproteins of the brush-border membrane are enriched in the microvillus fraction, and are solubilized by detergent treatment. The underlying core consists mainly of actin, the 70-kdalton subunit, a low-molecular-weight protein of ~20 kdaltons, which we have identified as calmodulin (12), and the 95- and 105-kdalton subunits. These results differ substantially from those of Bretscher and Weber (3). Their preparations of microvilli contained only actin and the 95-kdalton protein that they have named villin (5). Results similar to those described here with respect to the composition of the microvillus core have been reported by Matsudaira and Burgess (16).

In our preparations of isolated brush borders and microvilli, the amount of the 95- and 105-kdalton varies from experiment to experiment. However, there is always an increase in both the 95- and 105-kdalton relative to actin in the microvillus as compared with the isolated brush border. The ratio of 95-kdalton:actin increases from 1:10–15 in the intact brush border to 1:7–8 in the isolated microvillus. The ratio of 105-kdalton:actin increases from 1:16–20 to 1:7–10. The ratio of 105-kdalton:95-kdalton in the isolated microvillus is ~1:1–1.5.

Peptide Mapping of the Brush-Border 95- and 105-kdalton Subunits and Alpha-Actinins from Skeletal, Cardiac, and Smooth Muscle

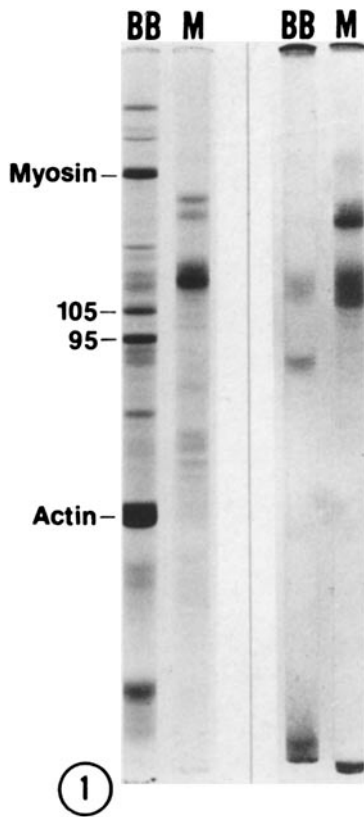Brush-border 95-kdalton, 105-kdalton, and the three alpha-actinins were purified to reasonable homogeneity by prepara-

FIGURE 1 Proteins of the isolated brush border. SDS-polyacrylamide gels (6.25%) of demembranated brush borders (BB) and the detergent-solubilized brush-border membrane (M). The two gels on the left are stained with Coomassie Blue, and on the right, identical gels stained for carbohydrate with periodic acid–Schiff reagent by the method of Fairbanks et al. (9). In addition to actin and myosin, prominant polypeptides of the demembranated brush border include subunits with molecular masses of 95 and 105 kdaltons, neither of which is PAS positive.
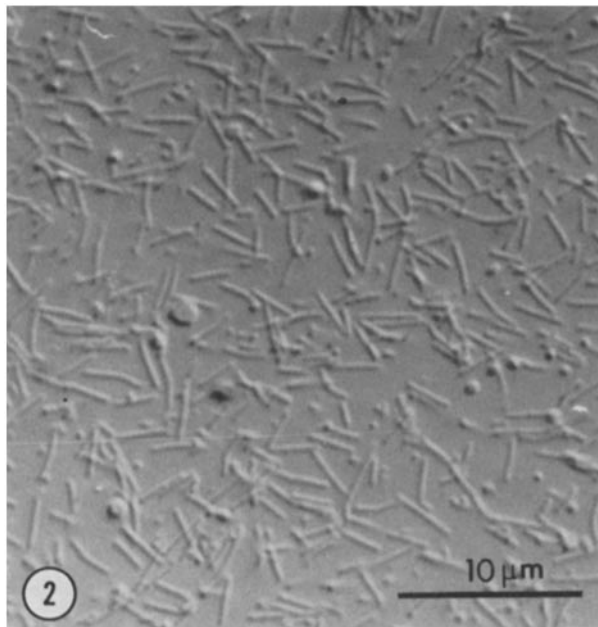

FIGURE 2 Microvilli isolated from purified brush borders. Differential interference light micrograph.
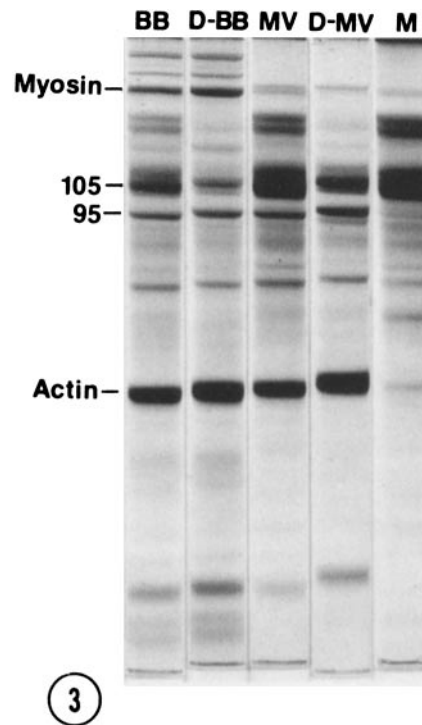
FIGURE 3 Comparison of brush-border and microvillus proteins. SDS gels (6.25%) of membrane intact brush borders (BB) demembranated brush borders (D-BB), intact microvilli (MV), demembranated microvilli (D-MV), and solubilized microvillar membrane (M). Both the 95- and 105-kdalton proteins are major components of the microvillar filament bundle.

tive gel electrophoresis in the presence of SDS (Fig. 4). The electrophoretic mobility of the alpha-actinin subunits relative to the two brush-border proteins depended on the gel system used. On Tris-acetate- (9) and Tris-HCl-buffered gel systems (13), alpha-actinin runs between the 95- and 105-kdalton subunits (Figs. 4a and 8). On Tris-glycine-buffered gels (28) alpha-actinin comigrates with the 105-kdalton subunit (Fig. 4b). All three alpha-actinins co-electrophorese, regardless of the gel system used.

We have obtained reproducible peptide maps of excellent resolution for all the subunits analyzed. The number of major peptide spots resolved ranged from 84 to 101 per subunit. In the case of alpha-actinin, this is in good agreement with the number of peptides expected on the basis of amino acid compositions published for skeletal and cardiac (23, 24, 33) alpha-actinins (~100 lysines and arginines per 100-kdalton subunit). This indicates that the mapping technique used has probably resolved most of the peptides derived from the tryptic digestion of these proteins.

For each pair of protein subunits to be compared, a composite map containing 1 nm of each of the two subunits was prepared. In this study, five proteins have been compared by this technique: skeletal alpha-actinin (S), cardiac alpha-actinin (H), gizzard alpha-actinin (G), brush-border 95-kdalton protein (95), and brush-border 105-kdalton protein (105). Of the ten possible comparisons, seven have been analyzed: S/H, S/G, S/95, S/105, H/95, G/95, and 95/105. Coincident peptide spots were identified on the composite maps by using tracings on transparent acetate sheets. For example, to compare skeletal and cardiac alpha-actinin, a tracing of the skeletal map was used to locate the skeletal peptides on the composite map. A

second tracing of the skeletal alpha-actinin peptide map was then prepared, using the composite map as template. This procedure was repeated for the cardiac subunit, and coincident spots were identified by overlaying tracings of individual maps, because in some instances there was substantial variability in spot migration from plate to plate. The percentage of coincident peptides was calculated for each comparison by dividing the total number of peptides per subunit (Table I) by the number of shared spots on the composite map. These values were calculated for both of the subunits compared, because in some instances one subunit had substantially fewer peptides resolved on the map than the other (e.g., cardiac vs. skeletal alpha-actinin).

Although we cannot define unequivocally the properties of a given peptide according to its position on the map, we have, to facilitate the discussion of these results, arbitrarily divided the pH 3.5 peptide map into five regions (Fig. 5). These regions include the extremes of chromatographic and electrophoretic migration as well as clusters or distinct constellations of spots observed for most of the protein subunits mapped. The divisions demonstrated on the peptide map of skeletal alpha-actinin (Fig. 5) could be precisely positioned for the other maps as well by using the composite maps containing skeletal alpha-actinin and each of the other four subunits analyzed. Region 1, at the upper left corner of the map, contains those peptides that have migrated farthest on the vertical axis. These spots represent rather hydrophobic peptides. Region 2 includes those spots just beneath region 1, and is characteristically the most crowded region of the map. As a result, this area is the one of lowest resolution. Nevertheless, discrete spots can be observed in this region, even on the heavily loaded composite maps, if
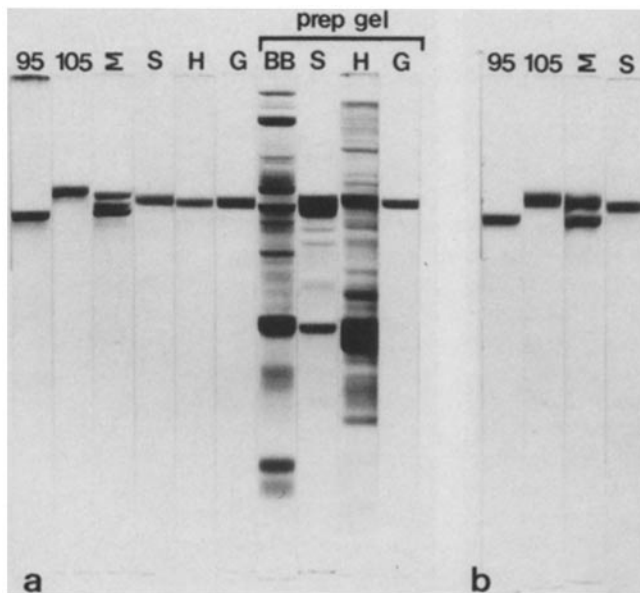

FIGURE 4   Purification of brush-border 95- and 105-kdalton and alpha-actinins. (a) SDS gels of skeletal (S), cardiac (H) and gizzard (G) alpha-actinins purified from crude fractions (prep gel) by preparative SDS gel electrophoresis. On Tris-acetate-buffered gels, alpha-actinin migrates between the 95- and 105-kdalton bands (Σ). (b) On Tris-glycine buffered gels (28), alpha-actinin comigrates with the 105-kdalton subunit (Σ).
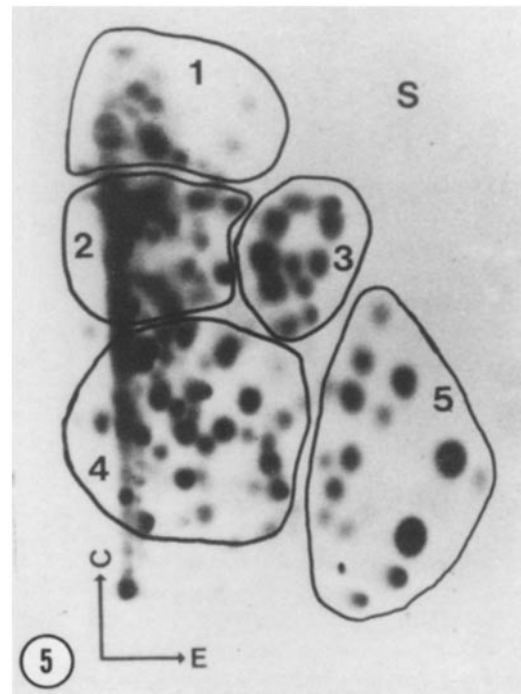

FIGURE 5   Spatial division of the pH 3.5 tryptic peptide map. To facilitate scoring and discussion, each peptide map was arbitrarily divided into five regions, shown here on a map of skeletal (S) alpha-actinin (see text for details). Chromatography (C) was performed in the vertical direction and electrophoresis (E) in the horizontal direction, cathode to the right.

TABLE I

Peptide Spot Coincidence

1 X/Y	2 No. of spots X	3 No. of spots Y	4 No. of spots X + Y	5 No. of shared spots	6 % Coincidence	7 % Coincidence — region 5
S/H	100 (101)	83 (84)	113	70	70/85	65/85
S/G	96 (101)	86 (90)	119	63	65/70	60/70
95/105	83 (90)	76 (85)	102	57	65/70	60/60
95/S	83 (90)	95 (101)	129	49	55/50	50/40
95/H	84 (90)	81 (84)	121	44	50/50	40/40
95/G	87 (90)	86 (90)	127	47	50/50	50/45
105/S	77 (85)	95 (101)	118	53	55/60	45/55

Peptide map comparisons of alpha-actinins from skeletal (S), cardiac (H), and smooth muscle (G), and brush-border 95- and 105-kdalton subunits (95 and 105). For each comparison (X/Y) the following values are listed: Columns 2 and 3: number of spots of proteins X and Y resolved on the composite map, X + Y. The number of spots resolved on the individual map follows in parentheses. Column 4: total number of spots resolved on the composite map. Column 5: number of spots held in common by proteins X and Y. Columns 6 and 7: percentage of coincident peptides (% of Y on X/% of X on Y) calculated with and without the peptides located in region 5 (see text).

one uses a standard light box to transilluminate lightly printed photographs of the maps to be scored. Region *3* consists of a cluster of spots common to all the proteins examined. These peptides have relatively large positive charge at pH 3.5, and moderate hydrophobicity. Region *4* consists of peptides whose summed properties of hydrophobicity, net charge, and size are intermediate. Much of the variability observed among the various subunits examined occurs in this region of the map. Region *5* is the area of greatest peptide spot coincidence for all the comparisons analyzed. This region probably contains small lysyl and arginyl peptides, including free lysine and arginine (29, 30), and consequently, some of the spots observed in this region may be common to most proteins. For example, many of these spots are also seen on maps of alpha- and beta-tubulin (30). We have computed the percentage of coincidences for each comparison, subtracting from the calculation those spots contained in region *5* (Table I).

In scoring the various comparisons, we have identified those spots that are coincident and have calculated the percentage of shared peptide spots. This kind of tabulation does not take into account, however, what is best described as peptide map "morphology." That is, when one looks at two peptide maps, one can immediately assess whether or not the maps are similar or different. This overall impression is the result of visually integrating not only coincidence of spots, but also spot intensity, spot density, and most importantly, the clustering of spots into distinctive groupings or constellations. We have attempted to score the similarities in map morphology by qualitatively assessing, within each of the five regions defined, the degree of similarity in spot grouping, individual spot intensity, and spot density (Table II). In the discussion of results that follows, we use the term "map morphology" to refer to these features.

Comparison of Alpha-Actinins to Each Other

Before comparing the brush-border proteins to alpha-actinin by peptide mapping, it was first necessary to establish the degree of variability that one observes among bona fide alpha-actinins with this technique. Peptide maps derived from the three alpha-actinins are very similar but readily discerned from one another (Fig. 6). By way of comparison, there is much more variation observed among these maps than observed for different actins or tubulin subunits (29, 30) with this same mapping technique. The comparison of skeletal and cardiac alpha-actinins indicates a greater similarity between alpha-actinins from striated muscle (70–85% spot coincidence) than

between skeletal and smooth muscle alpha-actinins (60–70% coincidence). One of the main differences observed in the comparison of the maps of skeletal and cardiac muscle alpha-actinin is that the cardiac map has substantially fewer spots (84 vs. 101). Many of the extra spots on the skeletal map are found in regions *1* and *2*. Nevertheless, all of the spots in region *1* and most of those in region *2* on the cardiac map do comigrate with spots on the skeletal map. The difference observed in spot number on these two maps may reflect a real variability in these two alpha-actinins or may be the result of incomplete digestion of the cardiac subunit. We suspect that the latter explanation is correct because several of our initial attempts at mapping the cardiac subunit were thwarted by incomplete tryptic digestion. Of all the peptide map comparisons analyzed, the skeletal and cardiac maps look most similar. This "gestalt" impression is substantiated by the quantitation of peptide coincidence and also by the qualitative assessment of peptide map morphology (Table II). Except for the peptides in region *1*, the similarities in distribution, intensity, and clustering of spots on these two maps is considerably greater than for any of the other comparisons.

The peptide maps of skeletal and smooth muscle alpha-actinins are also quite similar, but less so than observed for the two alpha-actinins from striated muscle. There is a somewhat lower degree of spot overlap (60–70%) that is the result of a more or less uniform reduction of coincidence of spots in regions *2–5*, even though the grouping of spots in these regions is very similar in all three alpha-actinins. The most notable differences between the smooth muscle map and either the skeletal or cardiac maps are in regions *3* and *5*. In region *3*, the smooth muscle map contains fewer spots than the striated alpha-actinin maps (8 vs. 11). Nevertheless, five of the spots on the smooth alpha-actinin map do comigrate with spots on the skeletal map. In region *5*, many of the spots on the smooth muscle map are of lower intensity than those on the skeletal or cardiac map, even though the coincidence of spots in this region is extremely high (Table II). The significance of this difference is questionable, however, because we have observed considerable variability in fluorescamine reactivity from run to run. These two maps were not run at the same time, and were prepared on different batches of chromatography plates. This particular set of peptide maps exemplifies the necessity of using composite maps containing both proteins to be compared. The smooth muscle map looks more unlike the skeletal map than it actually is because of considerable migration differences in both chromatographic and electrophoretic dimensions. Loca-

TABLE II

Comparison of Peptide Map Morphology

X/Y	Region 1 % Coincidence	Morph	Region 2 % Coincidence	Morph	Region 3 % Coincidence	Morph	Region 4 % Coincidence	Morph	Region 5 % Coincidence	Morph
S/H	40/100	−	70/90	+++	80/80	+++	70/80	+++	90/90	+++
S/G	45/45	++	70/70	+++	55/75	++	60/70	++	90/95	++
95/105	55/70	+	60/70	++	90/90	+++	55/50	++	80/80	+++
95/S	65/55	+	60/60	+	50/45	++	40/25	−	65/90	++
95/H	45/80	+	50/65	+	50/45	++	30/20	−	70/100	++
95/G	65/45	+	60/55	+	30/40	+	45/35	−	65/95	++
105/S	65/45	−	60/55	+	70/65	++	50/35	+	75/100	++

Values for percentages of shared peptide spots are given for both subunits (X/Y) compared (% of Y on X/% of X on Y). These values are listed for each of the five regions defined on the pH 3.5 peptide map (see text and Fig. 5). Similarities in spot intensity, density, and spot grouping or peptide map morphology (*Morph*) are ranked qualitatively, using a scale of +++/++/+/− with (+++) indicating a high degree of similarity and (−) to denote dissimilar map morphology.

tion of shared peptide spots, a simple task using the composite map, would be quite difficult if direct comparison of these two maps were required.

Comparison of the Brush-Border 95- and 105-kdalton Subunits

We were surprised to find that the peptide maps of the 95- and 105-kdalton subunits indicate that these two proteins are related (Fig. 7 and Table I). At first glance these two maps look very similar, and as expected, we tabulated a high degree of coincidence of spots (60–70%) as well as a high index of morphological similarity (Table II). The 95-kdalton subunit does not appear to be a proteolytic fragment of the 105-kdalton protein, however. One cannot derive a 95-kdalton map by erasing spots from the 105-kdalton map. Both maps contain unique peptides including spots that are absent from the other

map as well as subtle shifts in spot position within constellations of spots held in common by both proteins. For example, the map of 95-kdalton subunit contains, in region 2, three prominent, vertically oriented spots that are absent from the map of 105-kdalton subunit. An example of shifted spot position is seen in region 4. Both maps contain a closely spaced triplet of spots just to the right of the vertical axis. Each triplet contains one spot that is more intense than the other two, but an examination of the composite map indicates that only two of the three spots are shared by both subunits.

Comparisons between the Brush-Border 95- and 105-kdalton Subunits and Alpha-Actinin

We have compared the 95-kdalton subunit to skeletal, cardiac, and smooth muscle alpha-actinins, and the 105-kdalton subunit to skeletal alpha-actinin (Fig. 8). The results of these
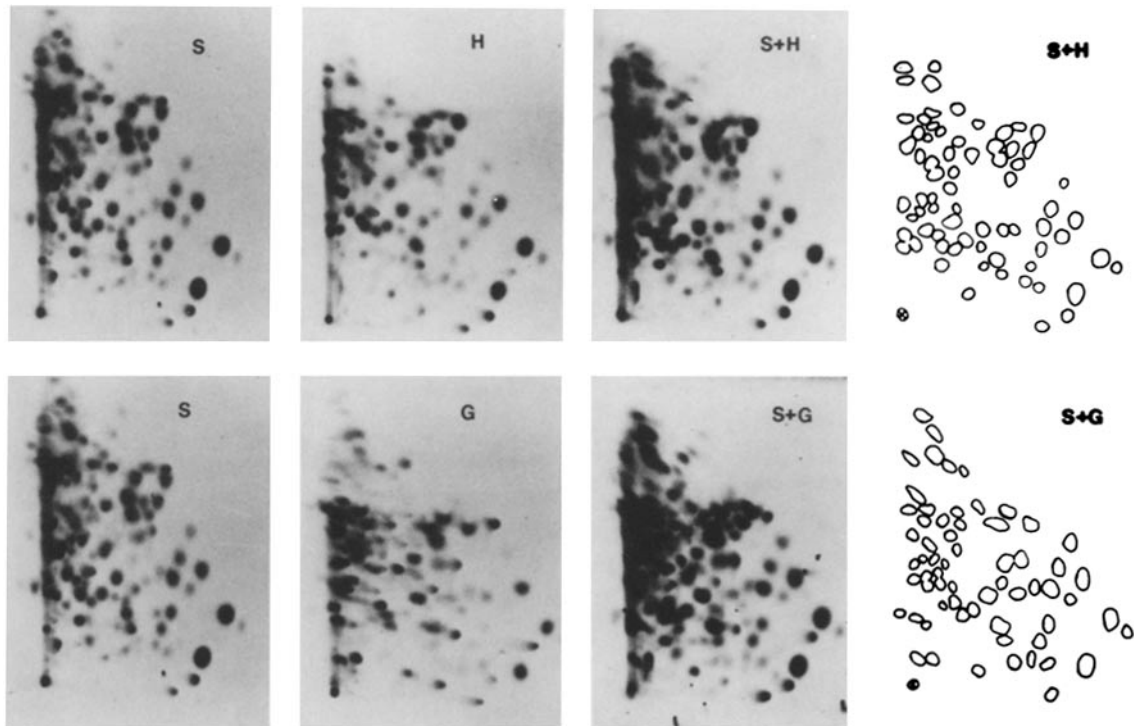

FIGURE 6   Peptide map comparisons of skeletal (S), cardiac (H), and smooth (G) alpha-actinins. Coincident spots were identified using composite maps (S + H, S + G) and are traced in the drawings on the right. The three alpha-actinins have very similar but distinct peptide maps. Skeletal alpha-actinin is more similar to cardiac alpha-actinin than to the subunit isolated from smooth muscle.
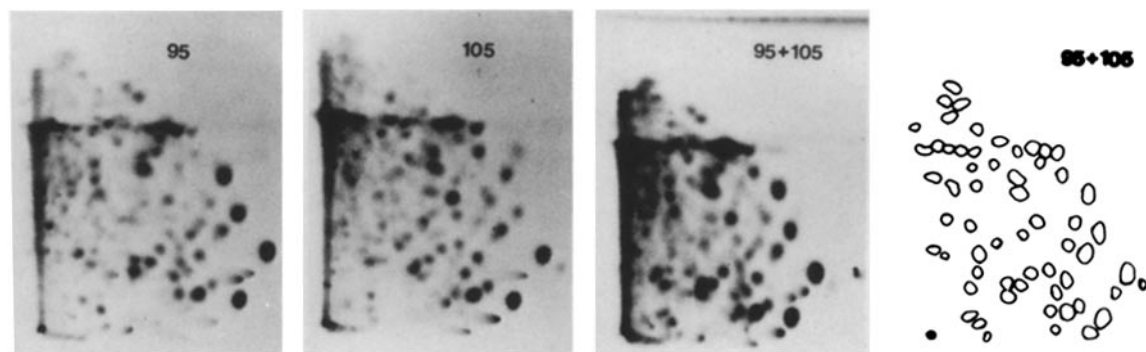

FIGURE 7   Comparison of brush-border 95- and 105-kdalton proteins. These two brush-border proteins have similar peptide maps, exhibiting a high degree of spot coincidence. The 95-kdalton protein does not appear to be a proteolytic fragment of the larger subunit.
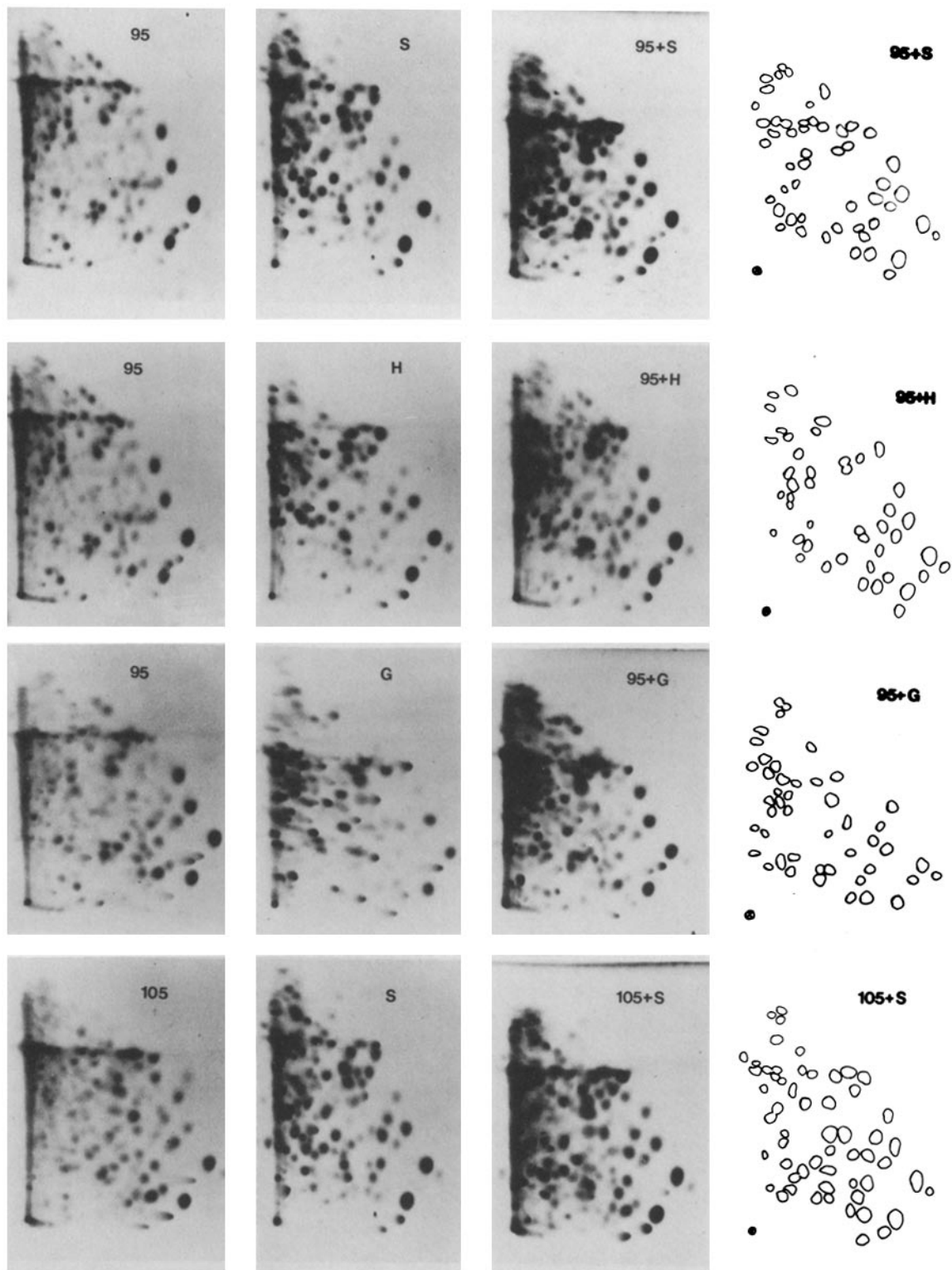
FIGURE 8   Comparison of brush-border 95- and 105-kdalton proteins with alpha actinin. Although both the 95- and 105-kdalton subunits have substantial numbers of peptides that coincide with spots on the three alpha-actinin maps, the overall distribution, intensity, and clustering of spots on the two brush-border maps is substantially different from that observed on the maps of skeletal, cardiac and smooth muscle alpha-actinin, indicating that neither the 95- nor the 105-kdalton subunit is an alpha-actinin. Skeletal (S), cardiac (H), and smooth muscle (G) fractions.

comparisons indicate that the three alpha-actinins are more similar to each other than to either of the brush-border subunits (Tables I and II), arguing that neither protein is a bona fide alpha-actinin. Nevertheless, our results leave open the possibility that these two proteins contain regions of primary struc-

ture similar to those of alpha-actinin. Even though the peptide maps of the 95- and 105-kdalton proteins look substantially different from the alpha actinin maps (Table II), there is still substantial spot coincidence in all the comparisons analyzed: 95/S: 50–55%, 95/H: 50%, 95/G: 50%, 105/S: 55–60% (Table

I). This raises the important question of how much overlap one might expect on peptide maps derived from functionally unrelated proteins of the same molecular weight. It seems reasonable to expect that two polypeptides of ~100 kdaltons containing similar amounts of lysine and arginine might contain identical peptides, particularly those consisting of only a few amino acids, such as those spots found in region 5. It is also possible that two substantially different peptides could migrate together on the map because three variables, peptide size, charge, and relative hydrophobicity, affect migration in only two dimensions. In view of these considerations, the subjective indexing of spot intensity and spot density and the comparison of spot clustering become an important element in assessing the similarities and differences between the brush-border proteins and alpha-actinin. Such an analysis (Table II) indicates that the two brush-border maps may be considerably less similar to alpha-actinin than one would estimate on the basis of spot coincidence alone. The most striking differences in map morphology are observed in regions 2 and 4. The 95- and 105-kdalton subunits have fewer peptides in these regions (95: 48 spots, 105: 44 spots) than the alpha-actinins (S: 60 spots, H: 51 spots, G: 53 spots), but a more significant contrast is seen in the comparison of spot intensities, and, in particular, in the groupings of spots in these two regions. All three alpha-actinin maps contain several clusters of intensely staining spots directly on and just to the right of the vertical axis of the map. Although both brush-border subunits contain substantial numbers of spots that coincide with peptides within these clusters (Fig. 8 and Table II), most of these spots are of lower intensity, and the distinctive arrangements of spots are absent. This suggests that the alpha-actinins contain greater numbers of relatively acidic peptides than either the 95- or 105-kdalton subunits. Conversely, the two brush-border proteins have more spots in region 5, the area of the map containing highly basic peptides (22 vs. 15–16). These results indicate that the brush-border proteins are both quite distinct from alpha-actinin but, on the basis of mapping data alone, we cannot exclude the possibility that the two brush-border subunits are "alpha-actinin-like."

Immunological Cross-Reactivity of Alpha-Actinins and Brush-Border 95- and 105-kdalton Subunits

We have stained SDS gels containing brush-border proteins and alpha-actinins from skeletal, cardiac, and smooth muscle with antibodies prepared against chicken gizzard alpha-actinin according to the technique of Adair et al. (1). Results indicate that there is substantial cross-reactivity among the three alpha-actinins, but there is no staining of the 95-kdalton, 105-kdalton, or any other protein of the isolated brush border (Fig. 9).

DISCUSSION

We have listed below three statements that summarize our current understanding of the localization and function of alpha-actinin in the intestinal epithelial cell. This summary is based on the results presented here and those of Bretscher and Weber (4, 5), Craig and co-workers (6, 7), Geiger et al. (11), and Matsudaira and Burgess (16).

(a) The epithelial cells of the small intestine may contain a bona fide alpha-actinin localized in the terminal web region of the brush border, predominantly at the junctional margins of the cell. This is based on the localization studies of Bretscher
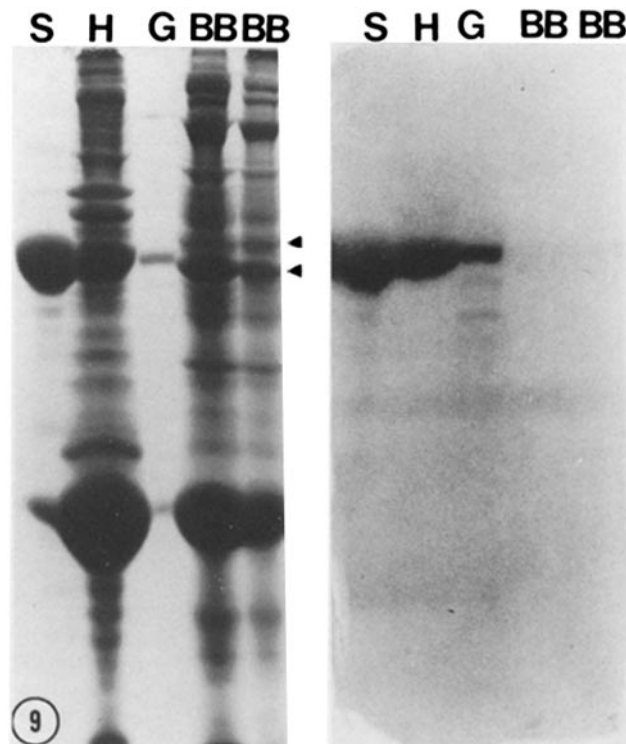

FIGURE 9   Anti-alpha-actinin staining of SDS gels, using ^{125}I-labeled protein A. Gel on the left contains crude fractions of alpha-actinin from skeletal (S), cardiac (H), and smooth muscle (G), and proteins of the demembranated brush border (BB). On the right, an autoradiograph of an identical gel incubated with anti-gizzard alpha-actinin, followed by incubation in iodinated protein A, using the technique of Adair et al. (1). There is an intense staining of the three alpha-actinins but neither the 95-kdalton, 105-kdalton (arrows for each), nor any other brush-border protein cross-reacts with this antibody.

and Weber (4), Craig and Pardo (7), and Geiger et al. (11). Although no chemical characterization of this "terminal web" alpha-actinin has been reported, Craig and Pardo (7) have immunoprecipitated a 100-kdalton polypeptide from homogenates of intestinal epithelial cells using anti-gizzard alpha-actinin. This alpha-actinin is probably lost from the brush border during isolation, as we have not been able to detect alpha-actinin either chemically or immunologically in our brush-border preparations. Similar findings have been reported by Craig and Lancashire (6).

(b) Although the isolated microvillus core contains two major polypeptides with molecular weights similar to but distinct from that of alpha-actinin, neither the 95- or 105-kdalton microvillar subunit is a bona fide alpha-actinin. Our results and those from several other laboratories (5, 6, 11) indicate a lack of immunological cross-reactivity between the 95-kdalton (villin) and alpha-actinin. We have demonstrated a similar lack of cross-reactivity for the 105-kdalton subunit. In addition to the peptide mapping studies reported here, two other groups have compared the 95-kdalton subunit with gizzard alpha-actinin by one- and two-dimensional mapping techniques. Both Geiger et al. (11) and Craig and Lancashire (6) conclude from their mapping studies that these two proteins are not related. Using techniques that resolve total tryptic peptides as opposed to the 10–30% resolution obtained in the studies mentioned above, we have observed a degree of peptide coincidence (a maximum of 55%) much greater than that observed

by either Craig and Lancashire (6) or Geiger et al. (11). Nevertheless, by establishing the degree of similarity among alpha-actinins isolated from three different muscle types, we have been able to demonstrate that neither the 95- nor the 105-kdalton subunit is an alpha-actinin. The functional significance of the considerable peptide overlap we have observed for comparisons of both 95- and 105-kdalton subunits with alpha-actinins has not been determined. Perhaps certain portions of these two proteins have alpha-actinin-like sequences, but the bulk of the evidence from immunological studies and, in particular, studies on the native properties of these proteins strongly suggest that neither the 95- nor the 105-kdalton subunit is related to alpha-actinin. For example, the 95-kdalton is a basic protein; alpha-actinin is acidic (M. S. Mooseker, unpublished observations).

(c) Although the 95- and 105-kdalton subunits may have two separate structural functions in the microvillus core, these two polypeptides may be chemically similar to each other. Using selective extraction procedures, Matsudaira and Burgess (16) have obtained indirect evidence that suggests that the 105-kdalton subunit (110 kdaltons on their gel system) is the cross-filament protein linking the microvillar actin bundle laterally to the plasma membrane. Conversely, they have also presented evidence suggesting that the 95-kdalton and/or the 70-kdalton subunits are proteins cross-linking the actin filaments to each other. Despite these different functions, the analysis of peptide maps of the 95- and 105-kdalton subunits indicate that these two proteins may be closely related. We are presently attempting to isolate and characterize the 95- and 105-kdalton subunits. Hopefully, these studies will enable us to determine whether any functional similarities exist between these two proteins.

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