Purification of an 80,000-dalton Protein That is a Component of the Isolated Microvillus Cytoskeleton, and Its Localization in Nonmuscle Cells

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ABSTRACT The microvillus cytoskeleton, isolated from chicken intestinal epithelial cell brush borders, is known to contain five major protein components, the 110,000-dalton polypeptide, villin (95,000 daltons), fimbrin (68,000 daltons), actin (43,000 daltons), and calmodulin (17,000 daltons). In this paper we describe our first step in studying the minor components of the isolated core. We have so far identified and purified an 80,000-dalton polypeptide that was present in the isolated structure in ~0.7% the molar abundance of actin. Antibodies to the 80,000-dalton component did not react with other microvillus core proteins, and, when used in indirect immunofluorescence microscopy, they stained the microvilli of intestinal epithelial cells fixed in situ. The 80,000-dalton component therefore appears to be a newly-identified, authentic component of intestinal microvilli in vivo and of isolated microvillus cores.

Immunological studies demonstrate that the 80,000-dalton component is widely distributed in nonmuscle cells. Indirect immunofluorescence microscopy reveals that it is particularly enriched in surface structures, such as blebs, microvilli, and retraction fibers of cultured cells.

Over the last few years the structure of the microvillus cytoskeleton has received much attention as a model system in which to study the molecular details of a defined microfilament organization. The reason for this interest is that the microvillus is a highly ordered structure that can be isolated ultrastructurally intact in quantities sufficient for biochemical analysis (1, 2). So far, the studies have been encouraging since they have contributed to the current understanding of the biochemical organization of microfilaments in nonmuscle cells (reviewed in 3).

Microvilli isolated from intestinal epithelial cell brush border fragments contain a bundle of actin filaments that attaches to the membrane both at the microvillus tip and laterally through a series of regularly disposed cross-filaments (1, 4). The microvillus cytoskeleton, prepared by Triton X-100 treatment of microvilli, is a compact bundle of actin filaments to which the laterally projecting cross-filaments still adhere (2, 5). The major protein components of this isolated structure are now well documented and consist of polypeptides of molecular weights 110,000 (the 110,000-dalton polypeptide), 95,000 (villin), 68,000 (fimbrin), 43,000 (actin), and 17,000 (calmodulin) (2, 6–8). Except for the 110,000-dalton polypeptide, the purification and detailed characterization of these components have been reported (3, 7, 9–14). These data, together with other studies (6, 15), have led to a model of the microvillus core in which villin and fimbrin are internal F-actin cross-linking proteins and the 110,000-dalton polypeptide and calmodulin make up the laterally projecting cross-filaments. Whether the 110,000-dalton-calmodulin complex attaches directly to F actin and how it interacts with the membrane are not yet clear. Recent experiments, however, indicate that the complex is not an integral membrane protein, as its solubilization from both isolated cores (6, 15, 16) and membrane intact microvilli (15, 16) does not depend on the presence of a detergent.

The minor polypeptide components of the microvillus core have not been described. However, they are potentially interesting, for example, as possible regulators of the structure in vivo and for their probable involvement in the attachment of the microvillus core to the plasma membrane at the microvillus tip. Thus, components that are present on the order of about one copy per actin filament are of potential interest.

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Since the core is ~1 μm long and since there are 14 actin monomers per 38 nm of filament, or ~370 monomers per 1 μm, we are interested in core proteins whose molar abundance relative to actin is in the region of ~0.25%.

In this paper we describe the identification and purification of a minor component of the isolated microvillus core and thereby demonstrate that studying such components is feasible. In addition, we show that an immunologically related protein is widely distributed in nonmuscle cells. A summary of this work has been included in a recent review (16).

MATERIALS AND METHODS

Purification of Brush Borders, Microvilli, and their Cytoskeletons: Brush borders and nuclei from chicken intestinal epithelial cells were prepared as described (13). Pure brush borders were isolated using one (2) or two (17) sucrose gradients. Microvilli were prepared from either the brush border and nuclei mixture, or from highly purified brush borders (2). Cytoskeletons were prepared as described (2).

Purification of Villin, Fimbrin, and the 80,000-dalton Component from Brush Borders: Preparations of brush borders and nuclei, or purified brush borders, in solution I (75 mM KCl, 1 mM EGTA, 0.1 mM $MgCl_2$, 0.25 mM phenylmethylsulfonyl fluoride (PMSF)[1], and 10 mM imidazole, pH 7.3) were made 5 mM in $MgCl_2$ and then 5 mM in $CaCl_2$. After stirring on ice for 15 min, the insoluble material was removed by centrifugations at 20,000 *g* for 15 min and then at 150,000 *g* for 30 min. The extract was made 40% in ammonium sulfate (22.6 g/100 ml extract) and the precipitated material removed by centrifugation and discarded. Additional ammonium sulfate was added to the resulting solution to bring the concentration up to 65% (adding 17.2 g/100 ml of original extract). The material that precipitated was collected by centrifugation and immediately dissolved in a minimal volume of buffer S (0.5 M NaCl, 1 mM $CaCl_2$, 4 mM NaN_3, 0.25 mM PMSF, 0.1 mM DTT (dithiothreitol), and 10 mM imidazole, pH 7.3) and clarified by centrifugation. The preparation contained villin, the 80,000-dalton component, fimbrin, and actin as major components (Fig. 2*a*, lane *3*). The preparation was applied to the first of two columns connected in series, both equilibrated with buffer S. The first was a Sephacryl S300 gel filtration column and the second a Sepharose 4B column to which pancreatic deoxyribonuclease I (DNase I) had been covalently bound (10). After elution through these columns with buffer S, analysis of the resulting fractions revealed that villin and actin had been quantitatively removed from the extract and that fimbrin had been partially separated from the 80,000-dalton component (Fig. 2*a*, lanes 4–19). As described previously, villin and actin bind to DNase I under these conditions, and villin can be recovered from the column by elution with a Ca^{2+}-chelating buffer (10). Fractions enriched in fimbrin or the 80,000-dalton component were pooled separately.

Fimbrin fractions were dialyzed into 50 mM NaCl, 0.1 mM DTT, and 10 mM imidazole, pH 6.7 and applied to a 5 ml Whatman DE52 ion exchange column (Whatman Chemical Separation, Inc., Clifton, NJ) equilibrated with this buffer. The column was then eluted with a linear gradient of 50–250 mM NaCl in 10 mM imidazole, pH 6.7. This yielded homogeneous fimbrin (not shown, but see 3, 13).

Fractions containing the 80,000-dalton component were dialyzed into 20 mM NaCl, 0.1 mM DTT, and 10 mM imidazole, pH 6.7 and then applied to a 5-ml Whatman DE52 ion exchange column (Whatman Chemical Separation, Inc.) equilibrated with this buffer. Elution was performed using a linear gradient of 20–220 mM NaCl in 0.1 mM DTT, and 10 mM imidazole, pH 6.7 (Fig. 2*b*). Fractions rich in the 80,000-dalton component, eluting at about the middle of the gradient, were pooled and diluted twofold with cold water. This material was applied directly to a 5-ml hydroxylapatite column equilibrated with 50 mM KH_2PO_4, and 0.1 mM DTT adjusted to pH 7.0 with KOH. The column was developed with a gradient of 50–600 mM potassium phosphate, pH 7.0. Homogeneous 80,000-daltons eluted at about two-thirds of the way through the gradient (Fig. 2*c*).

Immunological Techniques: Antibodies to the 80,000-dalton component were elicited in rabbits. Prior to immunization the purified 80,000-dalton protein was subjected to preparative SDS PAGE, the band excised, and the polypeptide electrophoretically eluted. About 200 μg of protein was injected subcutaneously with 1 ml of Freund's complete adjuvant. On days 21 and 42 the animals were immunized with the same amount of protein but in Freund's incomplete adjuvant. On day 49 a small amount of blood was taken and the serum tested for the presence of antibodies to the 80,000-dalton component. Over the course of these experiments, three animals were immunized with the 80,000-dalton protein and in all cases, antibodies to this component could be detected after 49 d. All results presented in this paper were obtained with antibodies from blood taken 51 d after the original immunization.

Antibodies to be used in immunofluorescence microscopy, and in some of the immune blotting experiments, were purified by affinity column purification on the purified 80,000-dalton component. To prepare the affinity column, the isolated 80,000-dalton component was subjected to preparative SDS PAGE, the band excised, and the protein recovered by electrophoretic elution. The protein was dialyzed extensively against 0.1% SDS and the solution lyophilized. The resulting powder was extracted with −20° acetone to remove excess SDS and remaining Tris and glycine. After recovering the insoluble material by centrifugation and allowing the pellet to air dry, the material was dissolved in 0.1% SDS in borate buffer (0.1 M H_3BO_3, 0.025 M $Na_2B_4O_7$, and 0.075 M NaCl, pH 8.4). To ensure that no glycine or Tris remained with the protein, it was gel filtered on a Sephadex G50 column in this buffer. Fractions containing the 80,000-dalton component (~0.5 μg) were then mixed with 1.5 ml of CNBr-activated Sepharose and gently shaken for 4 h at room temperature. The resin was then put in a column and washed extensively with borate buffer to remove excess SDS. The resin was treated as described in the manufacturer's instructions (Pharmacia Fine Chemicals Div., Pharmacia, Inc., Uppsala, Sweden). Using this method we have routinely obtained essentially quantitative coupling of proteins prepared by SDS PAGE to activated Sepharose. Affinity-purified antibodies to the 80,000-dalton component were prepared from serum using this affinity matrix as described (5).

Immune blotting analysis was performed essentially as described (20). Immunofluorescence microscopy on dissociated intestinal epithelial cells after in situ fixation (19) and on cultured cells was done as described (8). Antibodies to the 80,000-dalton component were used at 32 μg/ml for the indirect immunofluorescence studies.

Other Procedures: Protein concentration was determined according to the method of Bradford (18) using ovalbumin as standard. Methods for the determination of the sedimentation coefficient and Stokes radius by gel filtration have been described (10).

RESULTS

The Isolated Microvillus Core Contains Minor Polypeptide Components

The polypeptide composition of brush borders, brush border cytoskeletons, microvilli, and microvillus cytoskeletons (cores) is shown in Fig. 1. As is evident from this figure, and has been reported previously (1, 2, 8), the components that make up the cytoskeletons are a clear subset of the total proteins of the membrane-intact structures. The components of the brush border cytoskeleton (lane *c*) can be divided into those also present in isolated cores (lane *e*), and those not present in cores that must therefore be located in the terminal web (2). It is now well established that myosin (with heavy chains at 200,000 daltons) (2, 19, 21) and some higher molecular weight polypeptides, designated TW 260/240 and shown to be related to erythrocyte spectrin (22, 23), are major components of the terminal web.

The major components of the isolated microvillus core (Fig. 1, lane *e*) are the 110,000-dalton polypeptide, villin (95,000 daltons), fimbrin (68,000 daltons), actin (43,000 daltons), and calmodulin (17,000 daltons) as has been reported by several laboratories (2, 6–8). Careful inspection of the polypeptide composition of isolated cores reveals the presence of some minor species. Among the most prominent of these are polypeptides with apparent molecular weights of ~200,000 (that are distinct from the heavy chains of myosin, see Fig. 1) and 80,000. Our initial studies on the minor polypeptides of the core have been directed at investigating the 80,000-dalton component.

The 80,000-dalton component appears to be an authentic constituent of isolated cores as we have observed it in all our

[1] *Abbreviations used in this paper*: DDT, dithiothreitol; DNase I, deoxyribonuclease I; PMSF, phenylmethylsulfonyl fluoride.

preparations, and it co-purifies with the structures as they are put through various steps of purification. For example, in experiments where microvilli were prepared from either a crude preparation of brush borders and nuclei, as described in our original procedure (2), or from highly purified brush borders in a modified procedure (17), the ratio of the 80,000-dalton component to known core proteins remained constant, despite the higher purity of the later preparations.

The abundance in the core of the 80,000-dalton component, estimated by scanning Coomassie Blue-stained gels, was about one-tenth that of the major actin-associated proteins. Thus, the molar abundance of the 110,000-dalton polypeptide, villin, and fimbrin, and the 80,000-dalton component, estimated from six different preparations, was found to be 8, 13, 6.5, and 0.7%, respectively, relative to actin. These values for the major components are in reasonable agreement with published data (6) and put the abundance of the 80,000-dalton component in the region of interest (see introduction).

Purification of the 80,000-dalton Component

We have recently described (3, 13) a method for the purification of villin and fimbrin from brush borders in which fractions enriched in the 80,000-dalton component are normally discarded. We have therefore adapted the method to allow the purification of all three proteins from the same preparation. The purity of the resulting isolated proteins using this method is shown in Fig. 1, lanes *f–h*. The polypeptide composition of the steps relevant to the purification of the 80,000-dalton component is shown in Fig. 2. Since details of the procedure are given in Materials and Methods, only an outline will be presented here.

The 80,000-dalton component and many other cytoskeletal proteins were solubilized from isolated brush borders by treatment with millimolar Ca^{2+} (Fig. 2*a*, lane *1*). The extract was clarified and subjected to ammonium sulfate fractionation to give a preparation enriched in villin, fimbrin, the 80,000-dalton component, and actin (lane *3*). The preparation, after being dissolved in a Ca^{2+}-containing buffer, was subjected to gel filtration on a Sephacryl S300 column followed immediately by passage through a Sepharose 4B column to which pancreatic DNnase I was covalently bound. The S300 column separated some proteolytic activity from the cytoskeletal components (3) as well as partially separating fimbrin from the 80,000-dalton component. The material eluting from the Sepharose-DNase I column was free of actin and villin as they bind quantitatively to DNase I under these conditions (3, 10, 13). Fractions eluting from the two columns were therefore enriched in either the 80,000-dalton component or fimbrin (Fig. 2*a*, lanes *5–19*). The DNase I column was washed and homogeneous villin recovered by elution with a Ca^{2+}-chelating buffer (lane *20*; [10]). Fractions containing the 80,000-dalton component or fimbrin were pooled separately and, after appropriate dialysis, subjected to anion exchange chromatography. This yielded a homogeneous preparation of fimbrin (Fig. 1, lane *h*; [3, 13]). Anion exchange chromatography for the 80,000-dalton component did not yield a pure protein (Fig. 2*b*), so the peak fractions were pooled, dialyzed, and subjected to chromatography on hydroxylapatite. This removed the remaining contaminants to give an apparently homogeneous preparation (Fig. 2*c*). The yield of the 80,000-dalton component was somewhat variable. Between 0.1 and

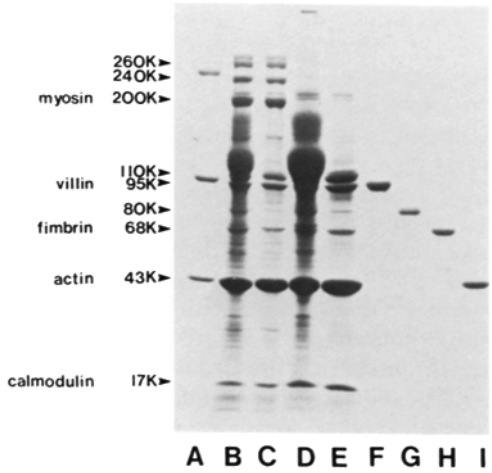

FIGURE 1 5–20% SDS PAGE of the polypeptide components of brush borders and microvilli. (*A*) Molecular weight markers, filamin (250,000 mol wt), α-actinin (100,000 mol wt), and actin (43,000 mol wt); (*B*) purified brush borders; (*C*) brush border cytoskeletons; (*D*) purified microvilli; (*E*) microvillus cytoskeletons (cores); (*F*) purified villin; (*G*) purified 80,000-mol-wt component; (*H*) purified fimbrin; and (*I*) purified actin. *K*, kilodalton.

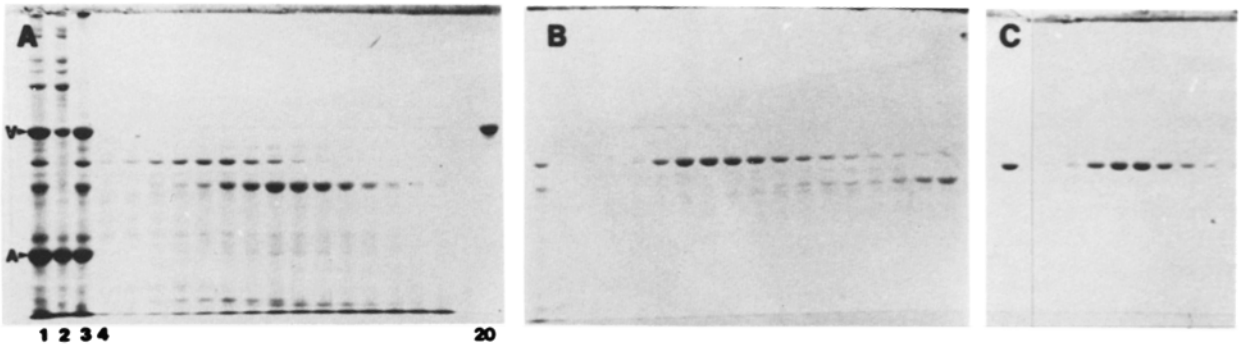

FIGURE 2 7.5% SDS PAGE of the fractionation steps in the purification of the 80,000-dalton component. (*A*, lane *1*) Material extracted from brush borders by treatment with calcium; (lane *2*) material precipitated from the extract by 40% ammonium sulfate; (lane *3*) additional material precipitated by increasing ammonium sulfate to 65% that was then redissolved and applied to the Sephacryl S300 and Sepharose 4B-DNase I columns in series; (lanes *4–19*) fractions eluting from the double column; (lane *20*) villin eluted from DNase I column by calcium-chelating buffer. (*B*) Material applied (*left* lane) and fractions eluting from the DEAE ion exchange column. (C) Material applied (*left* lane) and fractions eluting from the hydroxylapatite column. *V*, villin; *A*, actin.

1.0 mg was obtained from preparations involving the small intestines from 30 chickens.

Biochemical Properties of the Isolated 80,000-dalton Component

The isolated protein has a polypeptide molecular weight of 80,000 as estimated by SDS PAGE (Fig. 1, lane *g*). Two-dimensional gel electrophoresis of either the preparation applied to the gel filtration column or of the purified protein shows that it has an isoelectric point slightly more basic than villin and exists as a major and a minor isoelectric species (Fig. 3). The Stokes radius of the purified protein was estimated by gel filtration on a Sephacryl S300 gel filtration column, calibrated with known standards, and found to be ~43 Å (Fig. 4). This value is sufficiently different from that of fimbrin (Stokes radius 38 Å, [13]) to allow their partial separation on the Sephacryl S300 column during the purifiation of these proteins (see above). The sedimentation coefficient of the 80,000-dalton component, estimated on sucrose gradients to which known proteins were also added, was found to be 3.9 ± 0.15 S in the presence or absence of millimolar calcium. This value, which is intermediate between that of ovalbumin (M_r 43,000 and 3.6S) and that of BSA (M_r 68,000 and 4.3S), taken together with the Stokes radius of 43 Å, sugests that the isolated protein exists as a monomeric species in solution that is probably somewhat elongated. Chemical cross-linking experiments with dimethylsuberimidate also suggest that the isolated protein is monomeric, as no higher molecular weight species could be obtained (data not shown).

We have studied the 80,000-dalton component in vitro in an effort to elucidate its role in the microvillus core. The isolated protein was sedimented in sucrose gradients with villin and/or fimbrin in the presence and absence of calcium. In all cases the proteins sedimented independently, suggesting that the 80,000-dalton component did not form a tight association with either of these components. Sedimentation assays of the 80,000-dalton component with F actin, or with F actin-villin bundles, or with F actin-fimbrin bundles, or with F actin-villin-fimbrin bundles did not reveal any association with these structures. However, such experiments would probably not identify a weak or substoichiometric association of the 80,000-dalton component with sedimented components.

Antibodies to the 80,000-dalton Polypeptide

The material isolated by the scheme described above appears homogeneous as determined by SDS PAGE (Fig. 2*c*). However, to ensure purity of the protein used for immunization, we subjected the preparation to preparative SDS PAGE, excised the 80,000-dalton band, and eluted the protein. Over the period of these studies, three rabbits were immunized with the 80,000-dalton component and antibodies were elicited in all the animals. Double-diffusion analysis with purified 80,000-dalton component gave a single precipitin line (Fig. 5), and no reaction was seen with the preimmune sera (not shown) or between the 80,000-dalton component and villin antibody or fimbrin antibody (Fig. 5). These data suggest that the 80,000-dalton component is not related to fimbrin and is not a proteolytic degradation product of villin. This conclusion is confirmed by the finding that antibodies to the 80,000-dalton component recognize only this component when used in immune blotting procedures among the total proteins of microvillus cores or brush border cytoskeletons (Fig. 6). A minor polypeptide at ~60,000 daltons is sometimes recognized by this antibody. This is probably a degradation product of the 80,000-dalton component because it has not been detected in fresh preparations but appeared in samples that had been stored for several days. Immune blotting procedures also revealed that the 80,000-dalton component is present in other cells of chicken origin. The component is readily detected among the total proteins of cultured chicken embryo fibroblasts and total proteins of brain tissue

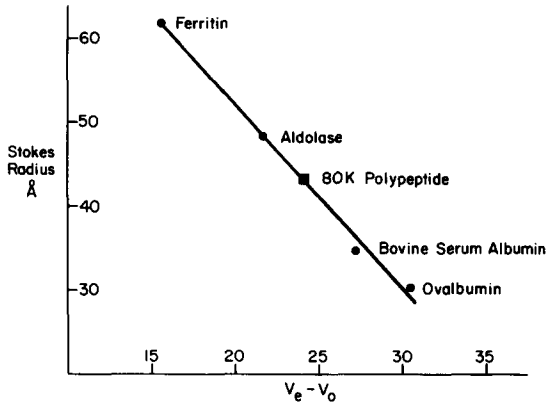

FIGURE 4 Determination of the Stokes radius of the 80,000-dalton component by gel filtration. Marker proteins (from Pharmacia Fine Chemicals, Uppsala, Sweden) were mixed with the purified 80,000-dalton component in 0.2 M KCl, 1 mM $CaCl_2$, 0.1 mM DTT, and 10 mM imidazole-HCl (pH 7.3) and applied to a 200-ml Sephacryl S300 column equilibrated with this buffer. The fraction number after the void volume (V_e-V_o) is plotted against the published Stokes radii for the marker proteins.

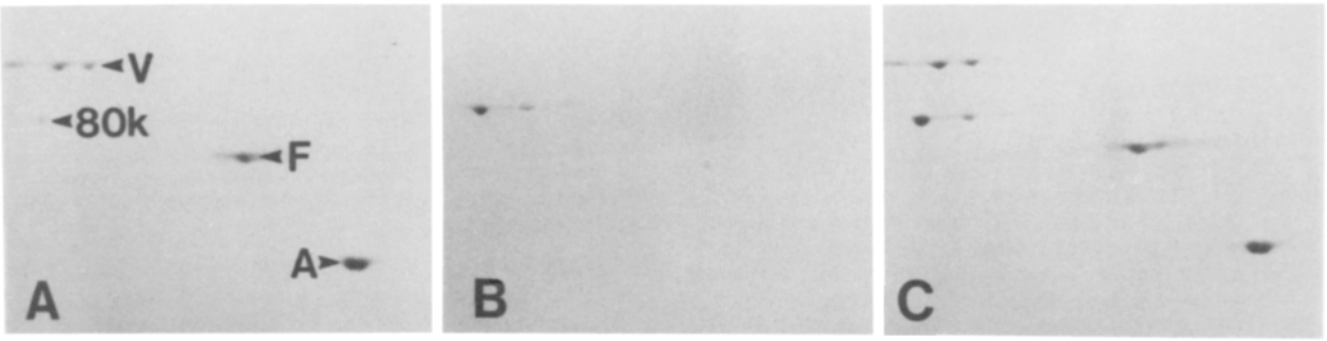

FIGURE 3 Two-dimensional gels (isoelectric focusing horizontally, 8% SDS PAGE vertically) of (*A*) material applied to the Sephacryl column during the purification of the 80,000-dalton component, (*B*) pure 80,000-dalton component, and (*C*) a mixture of the material applied to the gels in *A* and *B*. The basic ends of the gels are at the left. *V*, villin; *80k*, 80,000-dalton component; *F*, fimbrin; *A*, actin.

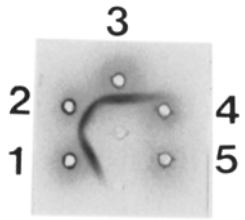

FIGURE 5 Double diffusion analysis of the antisera to the 80,000-dalton component. Purified 80,000-dalton component was placed in the center well and three different anti-80,000-dalton sera in wells marked *1–3*. A positive reaction is seen for all three sera. Fimbrin antiserum (well *4*) and villin antiserum (well *5*) showed no reaction.

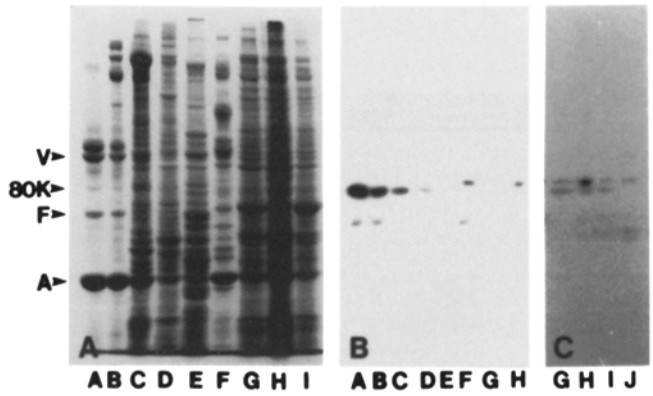

FIGURE 6 Immunological reactivity of the 80,000-dalton antisera to various protein mixtures as revealed by immune blotting. Duplicate samples of each protein mixture were separated by 7.5% SDS PAGE and one sample of each stained with Coomassie Blue (*A*). The other sample was electrophoretically transferred to a nitrocellulose filter, incubated sequentially with anti-80,000-dalton serum, washed, treated with ^{125}I-labeled protein A, washed, and subjected to autoradiography. *B* shows a short exposure of part of the filter and *C* shows a longer exposure of the same filter. The samples applied were (*A*) microvillus cores; (*B*) brush border cytoskeletons, (*C*) total proteins from chicken embryo fibroblasts, (*D*) total proteins from chicken brain, (*E*) total proteins from chicken liver, (*F*) mouse brush border, (*G*) total proteins of mouse NIH 3T3 cells, (*H*) total proteins of mouse Swiss 3T3 cells, (*I*) total proteins of cultured rat mammary cells, (*J*) total proteins of rat kangaroo PtK_2 cells. *V*, villin; *F*, fimbrin; *A*, actin; and *80K*, 80,000-dalton component.

(Fig. 6, lanes *d* and *e*). A very faint response was seen when total proteins from chicken liver were probed, suggesting that this tissue contains little 80,000-dalton component.

The immune blotting experiments also showed that components in cells from other species were recognized by the antibodies to 80,000-daltons. All the mammalian cell lines tested (Fig. 6, lanes *f–j*) gave a positive response to a protein of apparently slightly higher molecular weight and, in some cases, to one of slightly lower molecular weight than 80,000-daltons. These data imply that the protein in mammalian tissues related to the avian 80,000-dalton component has a slightly higher apparent molecular weight, and that a second species, or a degradation product of the first species, exists in some cells. It seems unlikely that these results came from nonspecific interactions as (*a*) we have never observed a labeled band at these molecular weights with antibodies to other microvillus core components, (*b*) a strong positive response occurred when brush borders from mice were probed (Fig. 6, lane *f*), (*c*) the same result was obtained with all three sera to the 80,000-dalton component, and (*d*) the same results were obtained when antigen affinity-purified antibodies to the 80,000-dalton component were used in place of the crude sera (not shown). We therefore conclude that a protein related to the microvillus core 80,000-dalton polypeptide is present in a wide variety of nonmuscle cells, whether they are of avian or mammalian origin.

Localization of the 80,000-dalton Component in Nonmuscle Cells

Indirect immunofluorescence microscopy was used to localize the 80,000-dalton component in a variety of nonmuscle cells. In all cases, only antigen affinity-purified antibodies were used, and controls in which nonspecific rabbit IgG replaced the 80,000-dalton specific antibodies resulted in too low a response to record photographically.

Immunofluoresence microscopy on fixed intestinal epithelial cells of either chicken or mouse origin (Fig. 7) resulted in a strong fluorescence from the microvilli of the brush border. Careful examination of such images gave the impression that the fluorescence came from all along the microvilli and not, for example, from the tip alone. Staining of a group of intestinal epithelial cells (Fig. 8) gave a picture very reminiscent of images obtained with antibodies to actin (20), villin, or fimbrin (not shown), and clearly very distinct from images obtained for terminal web components, such as myosin, α-actinin, tropomyosin, filamin (20), or TW 260/240 (23). These data imply that the 80,000-dalton component is indeed a constituent of the microvilli of intestinal epithelial cells fixed immediately after removing the intestine from a freshly sacrificed animal.

Immunofluorescence microscopy using 80,000-dalton antibody on populations of cultured cells generally gave a rather weak uniform stain that was not possible to document photographically. However, among these were many cells having intensely staining surface structures (Figs. 9–11). Studies with various cell lines showed staining in a number of different cell surface structures, many of which were most evident on cells

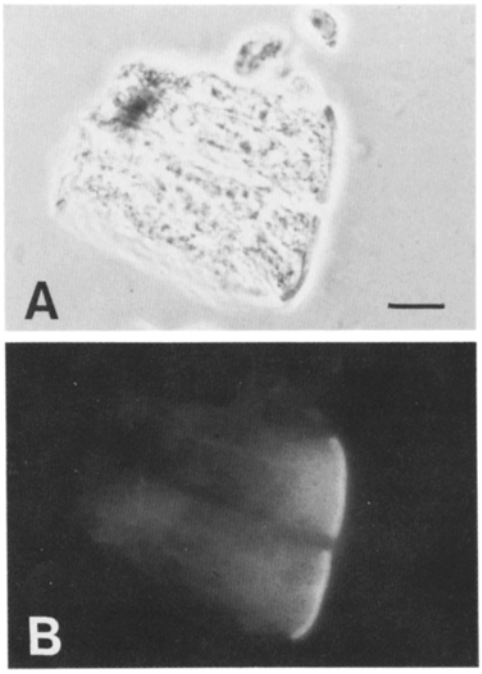

FIGURE 7 Phase (*A*) and fluorescence (*B*) micrographs of the same mouse intestinal epithelial cells stained with antibody to the 80,000-dalton component. Note that the microvilli of the brush border are heavily stained. Bar, 10 μm. × 770.

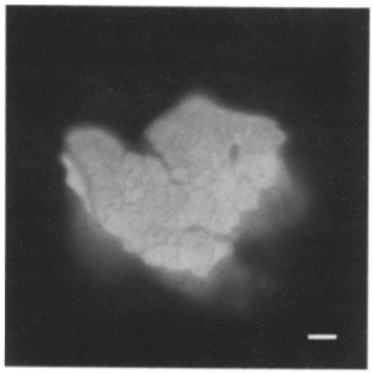

FIGURE 8 Fluorescence micrograph of a small sheet of mouse intestinal epithelial cells stained with 80,000-dalton antibody and viewed from the apical end. Intense fluorescence is seen coming from the brush borders of the cells. Bar, 10 μm. × 440.

apparently in various stages of mitosis. Fig. 9 shows several views of the same chicken embryo fibroblast stained with 80,000-dalton antibody and photographed with the plane of focus at different depths in the cell. Surface structures such as blebs, microspikes, and microvilli were stained. Fig. 10 shows two views of a cultured mouse 3T3 cell with numerous surface microvilli that are clearly enriched in the 80,000-dalton component. Similarly, retraction fibers and surface microvilli of a cell probably rounding up during mitosis can be seen to be enriched in the 80,000-dalton component (Fig. 11).

DISCUSSION

In this paper we described a newly identified component of the microvillus cytoskeleton. The component was present in the isolated structure in ~0.7% the molar abundance of monomeric actin, which corresponds on average to ~2–3 copies per filament. This demonstrates the feasibility of isolating and characterizing minor core components and so adds credibility to the idea that one can reasonably expect to eventually understand the entire structure in molecular detail.

Although the 80,000-dalton component is clearly a minor constituent of the isolated microvillus core, it may be a more major constituent of the microvillus in vivo. This seems likely, as we have previously found that its association with the core in vitro is easily disrupted by a variety of rather gentle extraction treatments (16).

Since the stoichiometry of the 80,000-dalton component with the other known components of the core is low, and since the yield of purified protein was somewhat variable, we were initially concerned that it may represent a contaminant that adhered to the structure during its isolation. Two observations make this possibility unlikely: (*a*) In any given experiment we have found that the molar ratio of the 80,000-dalton component to other known components of the core, such as actin, villin, and fimbrin, is constant during the final stages of the purification of microvillus cores and is not changed by the purity of the preparation, and (*b*) three different preparations of antibodies specific for the 80,000-dalton component reveal it to be present in microvilli on intestinal epithelial cells that were fixed in situ immediately after removing the small intestine from the animal.

The isolated 80,000-dalton component appears to exist in solution as a monomeric protein. In vitro reconstitution experiments with actin, villin, and fimbrin have so far failed to demonstrate a specific binding to any of these components. Other experiments have not revealed any obvious association of the 80,000-dalton component with known core proteins when isolated cores are subjected to differential solubilization

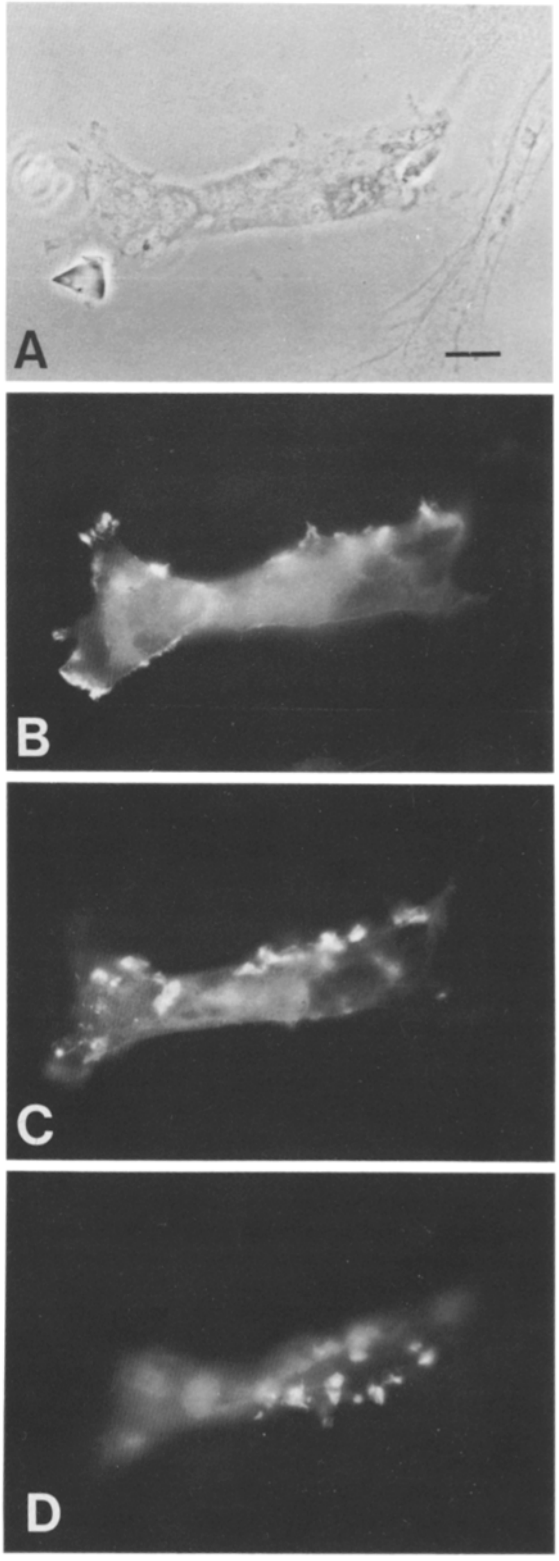

FIGURE 9 Phase (*A*) and fluorescence (*B–D*) micrographs of the same cultured chicken embryo fibroblast stained with antibodies to the 80,000-dalton component. In *B–D* the plane of focus was positioned at different levels in the cell to demonstrate the various surface structures that are stained. Bar, 10 μm. × 720.

studies (16). Other approaches are now being taken to try and identify the role of the 80,000 dalton component in the microvillus core.

Immunological experiments show that antigens related to

the microvillus core 80,000-dalton component are present in a wide variety of nonmuscle cells. The distribution of this new protein is completely different from that of the currently known microfilament-associated proteins. During interphase in cultured cells, the 80,000-dalton component does not generally appear associated with identifiable structures, at least at the light microscope level. However, in cells with surface blebs, with microvilli, or with retraction fibers, which are commonly associated with cells in the mitotic stage of the cell cycle, the 80,000-dalton component appears to be preferentially enriched in these surface structures. As it is not yet known whether the 80,000-dalton component is present in all these types of cellular protrusions, it is too early to evaluate whether 80,000-dalton specific antibodies will provide a general marker for such surface structures. However, it is interesting that all structures containing the 80,000-dalton component are small surface protrusions that have F actin as an internal skeleton. It will therefore be of considerable interest to elucidate the function of this protein and to determine

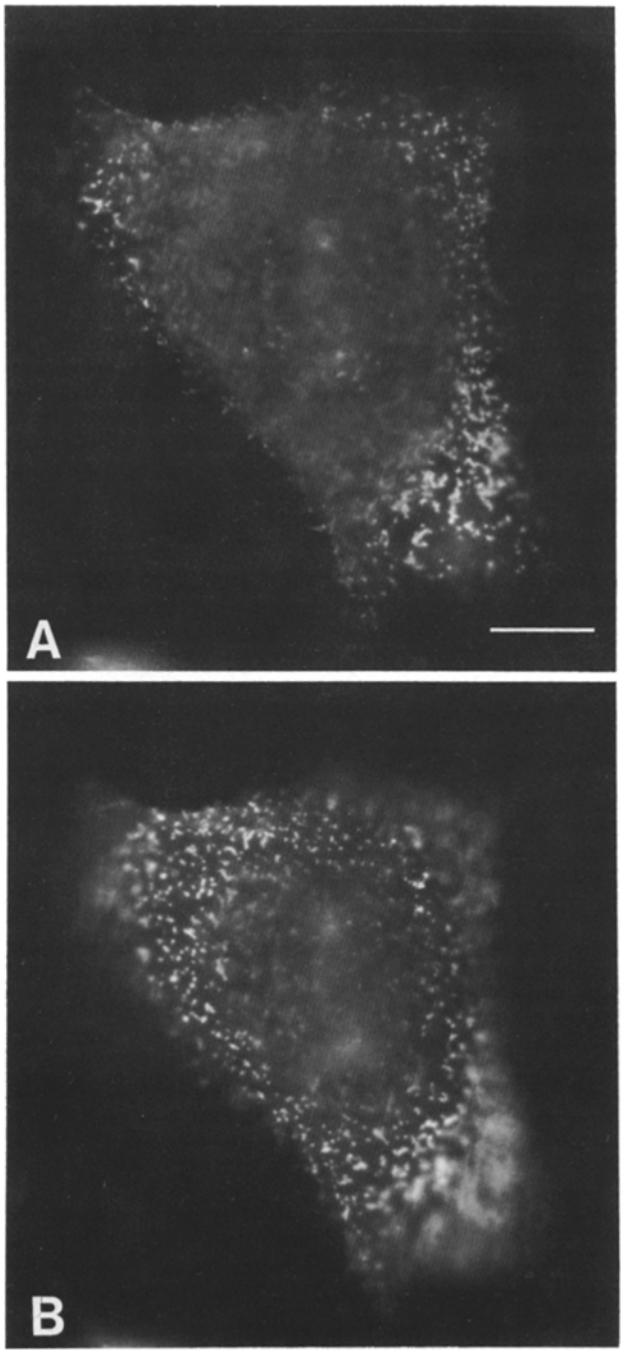

FIGURE 10 Fluorescence micrographs of the same mouse 3T3 cell stained with antibody to the 80,000-dalton component. Note the numerous surface microvilli that are labeled. Bar, 10 μm. × 1,300.

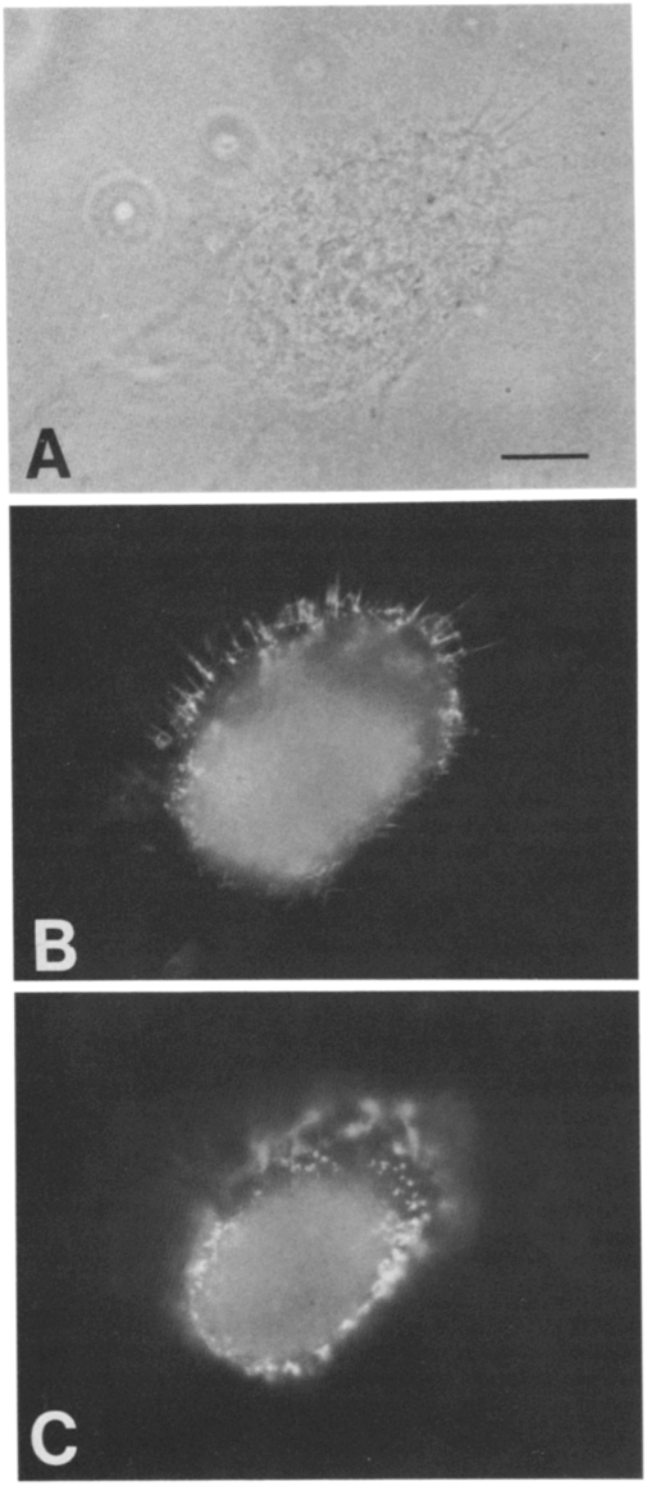

FIGURE 11 Phase (*A*) and fluorescence (*B* and *C*) views of the same mouse 3T3 cell stained with 80,000-dalton antibody. This cell had rounded up before fixation and shows the presence of the 80,000-dalton component in retractions fibers (*B*) and surface microvilli (*C*). Bar, 10 μm. × 1,150.

whether it plays a role in membrane attachment of this class of microfilaments in nonmuscle cells.

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