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Actin in the Brush-Border of Epithelial Cells of the Chicken Intestine

(electron microscopy/gel electrophoresis/G to F transformation/heavy meromyosin binding)

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ABSTRACT **The major soluble protein of the isolated brush-border of the intestinal epithelium has a molecular weight and net charge indistinguishable from those of skeletal-muscle actin, as determined by polyacrylamide gel electrophoresis. Furthermore, this protein, isolated from acetone powders of the purified brush-border, undergoes a G to F transformation in the presence of Mg^{++}. The filaments have a substructure indistinguishable from muscle actin, as seen by the negative-staining technique, and bind heavy meromyosin with the arrowhead configuration characteristic of actin. The filaments in the microvilli of the intact brush-border also bind heavy meromyosin. Thus, actin seems to be present in intestinal epithelial cells.**

Intracellular filaments, about 5 nm in diameter, are now recognized to be common constituents of eucaryotic cells. Their localization in cell types other than muscle has led to the speculation that they may function in certain types of contractile processes such as cleavage (1–4), neural-tube formation (5), resorption of ascidian tadpole tails (6, 7), certain types of cytoplasmic streaming (8), shortening of secondary mesenchymal processes during gastrulation (9), and pulsations of intestinal epithelial cell microvilli (10). Recent evidence in support of these speculations has come from experiments with cytochalasin B (11, 12). In many, but not all, of the above systems, this compound affects the integrity of the filaments and the contractile function is lost.

5-nm Filaments also occur in primitive cell types, such as the acellular slime mold, *Physarum*, and amoeba. The filaments in both of these organisms have close biochemical homology with skeletal-muscle actin (13–16). Furthermore, the isolated filament protein of both systems has been shown to bind heavy meromyosin (HMM), with the arrowhead configuration characteristic of muscle actin (16–18).

The decoration of filaments by HMM has also been demonstrated in other cell systems which include certain vertebrate cell types that are not muscles. These include chrondrocytes, fibroblasts, the microvilli of intestinal epithelial cells, or kidney tubule cells (19), blood thrombocytes (20), and the cleavage furrow (21). It is important to know if binding of HMM to these nonmuscle filaments truly indicates that the filaments are functionally similar to actin. If so, muscle protein may be ubiquitous to all cells of higher organisms, since these filaments appear not only in dividing cells during cytokinesis, but in the cytoplasm of many nondividing cells.

The brush-border of intestinal epithelial cells is ideal for the study of homology of the 5-nm filaments with skeletal-muscle actin. As is well known, the apical surface of intestinal epithelial cells are characterized by the so-called brush-border, which consists of tightly packed microvilli. Within each microvillus is a core of these 5-nm filaments—in the chicken there are about 20–30 filaments per microvillus. Much is already known about the development of filament organization (22). Purified preparations of the brush-border can be easily obtained in sufficient quantities for biochemical analysis. The filament protein should be a major contributor to the total protein of these preparations.

Abbreviations: HMM, heavy meromyosin; SDS, sodium dodecyl sulfate.

MATERIALS AND METHODS

Preparation of the Brush-Border. Brush-borders from young chickens were isolated by the method of Forstner *et al.* (23). The chickens were starved for 1 day before they were killed. Before and after filtration with glass wool, the pellet was washed several extra times to remove the yellowish unconsolidated layer on top of the pellet. Purity was assessed by light microscopy (phase-contrast and Nomarski interference) and by electron microscopy.

Polyacrylamide Gel Electrophoresis. The molecular weights of proteins from the whole isolated brush-border and from proteins extracted from acetone powders of the brush-border were determined electrophoretically (24, 25) on 5% polyacrylamide gels that contained 0.1% sodium dodecyl sulfate (SDS) at pH 7.1. The purified brush-border, isolated brush-border actin, chick actin prepared from acetone powders (26), and protein standards were heated for 2 min at 100°C in 1.0% SDS–1.0% 2-mercaptoethanol–10 mM phosphate buffer (pH 7.1) before electrophoresis. The gels were calibrated with bovine serum albumin (molecular weight 68,000 and 136,000), catalase (molecular weight 60,000), chick actin (molecular weight 46,000), and pepsin (molecular weight 36,000). Bromphenol blue was used as a tracking dye.

For the determination of net protein charge, we electrophoresed brush-border samples and skeletal-muscle actin in 5% gels that contained 8 M urea, in a running buffer of 5 mM Tris–glycine at pH 7.5 (27). Actin and brush-border samples were reduced and acetylated (28) before electrophoresis. Gels were run at 100 V for 1 hr before protein samples were layered on them.

HMM Binding to the Isolated Brush-Border. HMM binding to the filaments in the microvilli of isolated brush-border was determined by the method of Ishikawa (19). The isolated brush-border was treated with glycerin for 3–4 hr, and incubated with HMM for 4–6 hr, then fixed, dehydrated, and

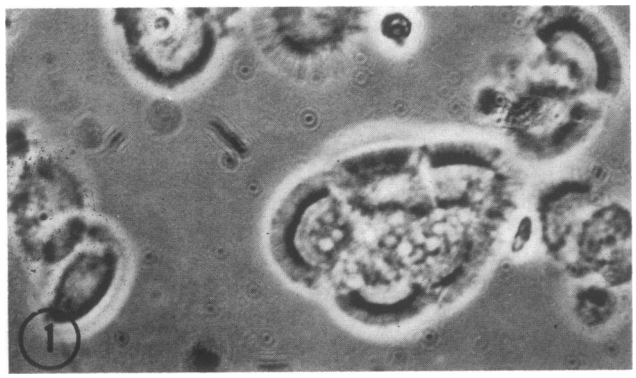

FIG. 1. Light micrograph of the isolated brush-border. Phase-contrast microscopy. ×925.

embedded. HMM, prepared from chicken-breast-muscle myosin, was kindly supplied by Dr. Hal Ishikawa.

Isolation of Brush-Border Actin. Acetone powders were prepared from the purified brush-borders of 10–12 chickens. Fresh cold acetone was added until most of the lipid was dissolved from the preparations. The yield from 10 chickens was about 1 g of dried powder. The powder was then extracted with a total of 10–12 ml of cold CO_2-free water per gram of powder and filtered through a Buchner funnel (26). (The powder was extracted twice to increase the protein yield.) The filtrate was centrifuged at 80,000 × *g*. The G-actin in the supernatant was then polymerized by the addition of 0.15 M KCl–0.01 M $MgCl_2$–0.01 M Tris·HCl buffer (pH 6.5) (16). The F-actin was concentrated by centrifugation at 80,000 × *g*. A glassy pellet was seen in the bottom of the centrifuge tube.

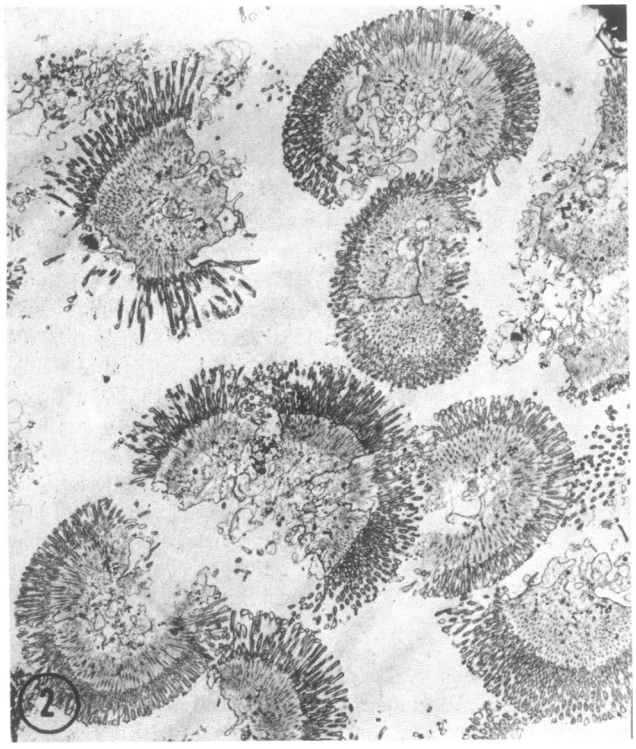

FIG. 2. Low-magnification electron micrograph of the isolated brush-border. ×4,000.

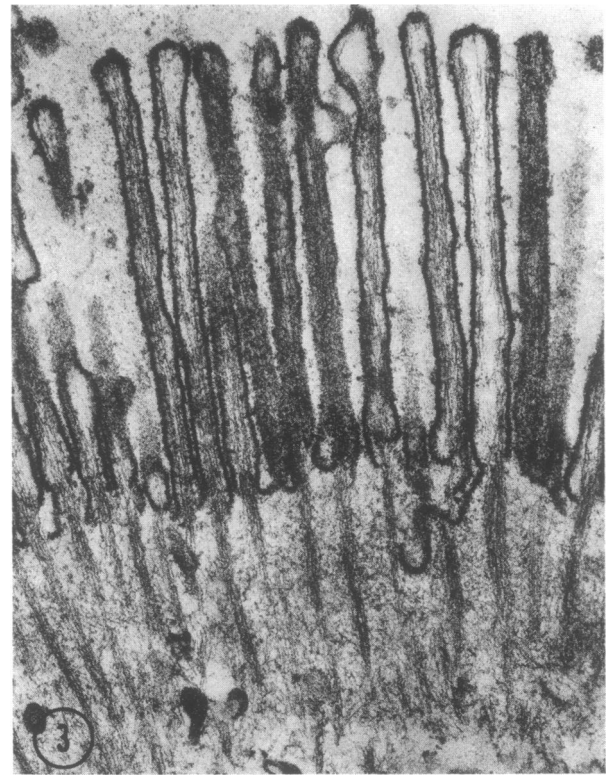

FIG. 3. Higher magnification of an isolated brush-border. The microvilli are prominent. Each has a central core of filaments that extends into the apical part of the cell. ×15,000.

Electron Microscopy. The purified brush-borders or brush-borders treated with glycerin were fixed for 1 hr in 2% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.4). The pellet was washed in buffer and post-fixed in 1% OsO_4 in 0.1 M cacodylate buffer for 45 min, dehydrated in acetone, and embedded in araldite. Fixation was at 0°C. Thin sections were cut with a diamond knife on a Servall Porter–Blum ultramicrotome and stained with uranyl acetate and lead citrate. The F-actin isolated from acetone powders of the brush-border and F-actin decorated with chick HMM were negatively stained (29, 16).

RESULTS

Purity of the brush-border preparations

We have been unable to find any contamination from the lamina propria, such as erythrocytes, collagen, or smooth

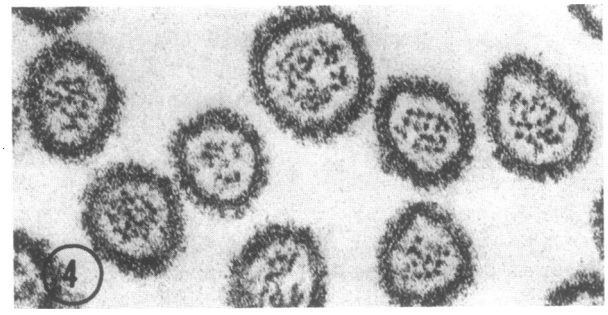

FIG. 4. Transverse section through several microvilli from an isolated brush-border preparation. The central core of 20–30 filaments within each microvillus is clearly visible. ×140,000.

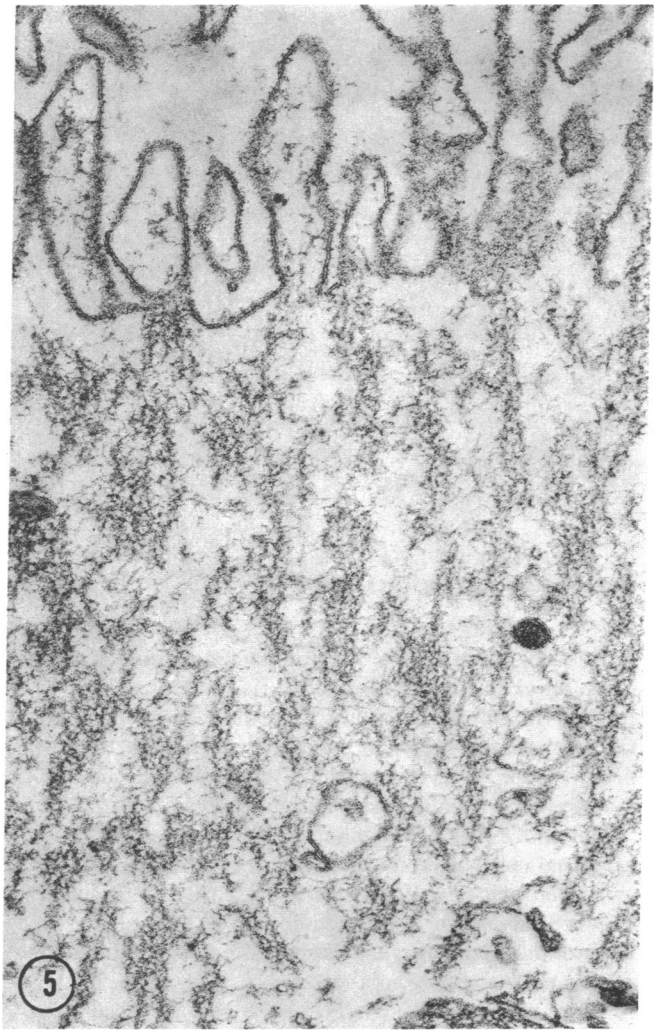

FIG. 5. Electron micrograph of a portion of a brush-border to which HMM has been added. Note the arrowhead decoration of the filaments by the HMM. The binding is more visible where the bundles of filaments are somewhat separated. This is due to the superposition of material in a thin section. ×75,500.

muscle, at any stage in the preparation, as assessed by light microscopy (Fig. 1) or electron microscopy (Fig. 2). We occasionally found a few nuclei from the epithelial cells, but the preparation seemed virtually free of other organelles.

Structures of the isolated brush-border

As shown by many others (see ref. 22), the brush-border is composed of microvilli. Within each microvillus is a full complement of 5-nm filaments (Figs. 3 and 4). These extend from the tips of the microvilli into the terminal web region.

HMM binding to the isolated brush-border

Our observations on the isolated brush-border confirmed those of Ishikawa *et al.* (19) from whole segments of the intestine. The 5-nm filaments present within the microvilli are decorated with HMM in the arrowhead pattern characteristic of decorated actin filaments (Fig. 5).

Polyacrylamide gel electrophoresis

The rate of migration of proteins in SDS–acrylamide gels is linearly proportional to the log of the molecular weight of the

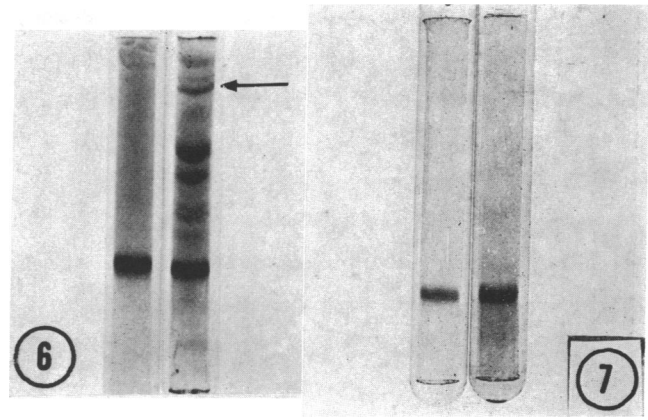

FIG. 6 (*left*). SDS–polyacrylamide gels stained with commassie blue. The purified brush-border fraction was run on the *right* gel, actin isolated from skeletal muscle was electrophoresed on the *left* gel. The *arrow* points to a band with the same molecular weight as myosin.

FIG. 7 (*right*). SDS–polyacrylamide gels. An acetone powder of the purified brush-border was extracted and the protein was polymerized with Mg^{++}. The pellet (*right* gel) was treated with SDS. Skeletal-muscle actin was run on the *left* gel.

proteins (25). Purified brush-borders were heated in the presence of SDS and mercaptoethanol and electrophoresed on 5% SDS gels. The protein separated into about 10 bands. The most prominent band migrated at a rate indistinguishable from that of chick-muscle actin (Fig. 6). Also present was a band at a position that corresponded to the molecular weight of myosin (Fig. 6, *arrow*). This band was much less intense than the actin band, however. We could not find a band that corresponded to the molecular weight of tropomyosin (35,000).

When acetone powders of purified brush-borders are extracted with CO_2-free water and the extract is run on SDS gels, one major band and a number of weaker bands are present. When this extract is polymerized with magnesium and KCl and the pellet is electrophoresed, there is only a single band, which has a molecular weight indistinguishable from that of muscle actin (Fig. 7). A faint dimer band (92,000) can sometimes be seen.

The purified brush-border was reduced and acetylated and electrophoresed on 5% gels that contained 8 M urea. A single band, indistinguishable in its migration rate from muscle actin, was seen (Fig. 8). Some background staining was pres-

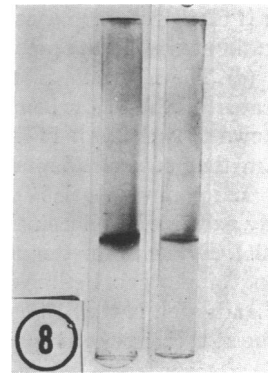

FIG. 8. Polyacrylamide gels containing 8 M urea at pH 7.1. The brush-border fraction was run on the *left* gel, skeletal-muscle actin on the *right* gel.

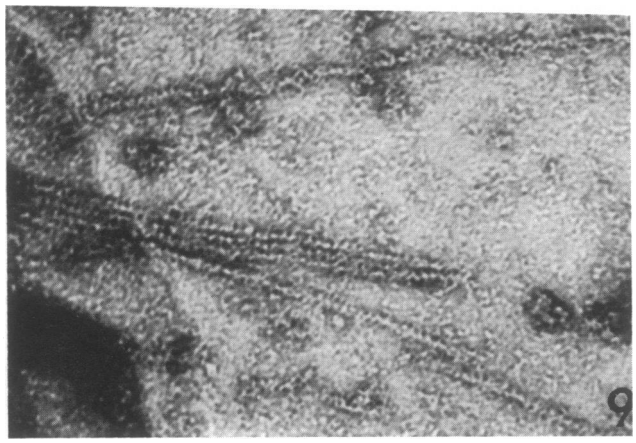

FIG. 9. High-magnification electron micrograph of a negatively stained preparation of the magnesium-polymerized pellet mentioned in Fig. 7. ×310,000.

ent in the brush-border gel. When actin and brush-border samples were added to the same gel, they migrated as a single band. Thus, the major band has a net charge indistinguishable from that of actin.

Examination of F-actin extracted from the acetone powder of isolated brush-borders

The pellet, when examined by negative staining, contained filaments about 5-nm in diameter. These filaments showed a beaded substructure indistinguishable from the substructure of actin isolated from skeletal muscle (Fig. 9). Furthermore, the filaments from the pellet bind HMM with the arrowhead configuration characteristic of skeletal-muscle actin (Fig. 10).

DISCUSSION

We have demonstrated by SDS–gel electrophoresis that the most prominent protein component of the isolated brush-border preparations or from acetone powders of the brush-border has a molecular weight indistinguishable from that of skeletal muscle actin. We believe that this protein corresponds to the 5-nm filaments because: (*a*) the purity of the sample eliminates the possibility of muscle contamination. Furthermore, the band is too prominent to be a contaminant, even if we were mistaken about its purity. (*b*) The filament core occupies a large portion of the internal microvillus volume, and thus one would expect the filament protein to be a major contributor to the total protein content of the brush-border samples. (*c*) If there were muscle contamination, there should be tropomyosin present in the preparations; we found no evidence of it. (*d*) The filaments in the microvilli bind HMM, which indicates at least a close homology to actin.

We have also shown that the protein has a net charge indistinguishable from that of skeletal-muscle actin. The absence of multiple banding in urea–gels is somewhat puzzling, but can be partially explained by incomplete disaggregation in the 8 M urea (25). Evidence for this may be seen in the background staining of the gel. Perhaps some of the high molecular weight bands seen in the SDS gels are lipids. Some proteins may also be at their isoelectric point and, thus, may not migrate.

Finally, by procedures analogous to those for the isolation of skeletal-muscle actin, we have succeeded in isolating a protein from the brush-border with a molecular weight indis-

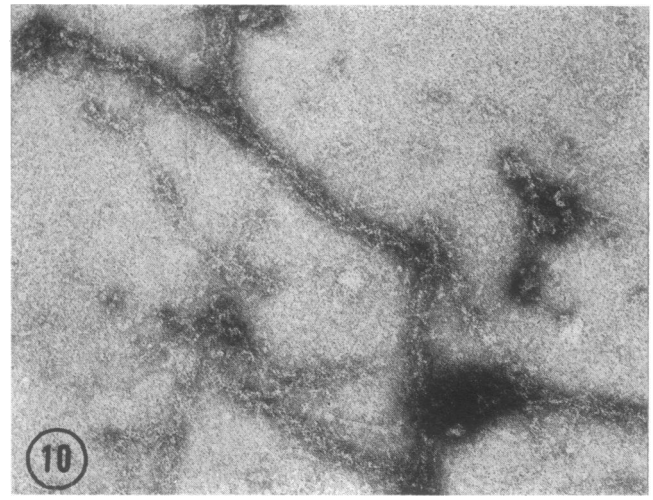

FIG. 10. Brush-border actin extracted from acetone powder and polymerized with Mg^{++} was decorated with HMM. Notice the arrowhead pattern. ×147,000.

tinguishable from that of skeletal-muscle actin. This protein polymerizes under the same conditions necessary for G- to F-actin transformation. Direct electron microscopic observations of isolated brush-border F-actin and HMM-decorated brush-border actin show that this protein is closely homologous structurally to skeletal-muscle actin.

Ishikawa's (19) HMM-binding studies on 5-nm filaments in intestinal epithelia and other cell types that are not muscles give good evidence that there is at least a close homology between these 5-nm filaments and muscle actin. Our findings confirm this homology, and support the contention that these filaments are composed of the muscle protein, actin.

There may be considerable significance attached to these findings. This is the first instance of the isolation and characterization of an "actin-like" protein from a vertebrate tissue cell that is not a muscle. If HMM-binding is indeed indicative of the presence of actin, then actin might well be ubiquitous in cell types that have contractile or motile processes. This does not necessarily mean that there is a ubiquitous mechanism for contraction of these filaments, however. On SDS–gels there is a protein with the same molecular weight as myosin, but it appears to be far less concentrated than myosin from smooth muscle run at the same time on adjacent gels. In addition, there does not appear to be tropomyosin present. Thus, the mechanism for control of contraction and relaxation of the microvillus has to be different from that for skeletal muscle.

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