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Cytovillin and Other Microvillar Proteins of Human Choriocarcinoma Cells

Raimo Pakkanen and Antti Vaheri

Department of Virology, University of Helsinki, 00290 Helsinki, Finland

Microvilli were isolated from cultured human JEG-3 choriocarcinoma cells using a gentle shearing method. The protein components of the isolated microvilli were examined by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting. The major M_r 42,000 and M_r 100,000 polypeptide bands reacted with anti-actin and anti-α-actinin antisera, respectively. Extraction of the isolated JEG-3 microvilli with Triton X-100 left an insoluble cytoskeletal residue containing mainly actin, α-actinin, and polypeptides of M_r 200,000, 55,000 and 35,000. The M_r 35,000 polypeptide remained insoluble only at high concentrations of free Ca^{2+}. Immunoblotting analysis of the JEG-3 microvilli indicated that they were devoid of tropomyosin, although the total JEG-3 protein lysates gave a strong positive reaction with anti-tropomyosin antiserum. The different subcellular localization of cytovillin and tropomyosin was also shown by indirect immunofluorescence microscopy. Cytovillin, an M_r 75,000 microvillus-specific membrane protein of JEG-3 cells, existed in an oligomeric form (dimer or trimer) as shown by gel filtration of Triton X-100 solubilized microvillar proteins and by native polyacrylamide gel electrophoresis of purified cytovillin. Disulfide bridges were not involved in the aggregation, because the mobility of cytovillin was similar under reducing and nonreducing conditions in SDS-PAGE. Cytovillin was shown to be closely related to ezrin, a minor component of chicken intestinal brush border microvilli.

Key words: actin, α-actinin, ezrin, microvillus, tropomyosin

Microvilli and related structures such as blebs and microspikes can be observed on the surface of many cells. On the epithelial cells of intestine and kidney, the microvilli form a special structure called the *brush border,* which increases severalfold the surface area available for absorption of food molecules. The structure of the brush border microvilli has been extensively studied during the past 15 years. The cytoskeletal core of each microvillus is composed of the major structural proteins called *villin* (a 95,000-M_r actin-bundling protein), *fimbrin* (a 68,000-M_r actin cross-linking protein), and a 110 kd protein—*calmodulin complex,* which are bound to actin filaments of the core bundle (for a review, see [1]). Numerous hydrolytic enzymes reside in the microvillar membrane, where they represent a substantial proportion of the total membrane proteins (for a review, see [2]).

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Human placental syncytiotrophoblasts, located at the interface between the maternal circulation and the fetus, are also covered by numerous microvilli. However, these microvilli are more dynamic with a less organized structure than their intestinal counterparts [3]. The placental microvilli contain significant quantities of α-actinin associated with the cytoskeletal core [4], but are apparently devoid of villin and the 110 kd protein [5]. A group of placental microvillar proteins of M_r 72,000, 69,000, 38,000, 36,000, and 32,000 has been reported to be associated at high concentrations of free calcium with the cytoskeleton and the lipids [6].

Microvilli can be generally observed on the surface of cells cultured either in suspension or attached to substratum (for a review, see [7]). However, surprisingly little is known about their structure and biological function(s). The number of microvilli and related microextensions varies during the cell cycle. Microvilli are especially abundant on rounded mitotic cells, but disappear when the cells are spread again [8–11]. Induction of microvilli occurs often after virus infection [12–17], and a microvillous surface is a characteristic of transformed cells [18]. Moreover, microvilli have been observed to mediate cell-cell and cell-substratum contacts [19–21]. Only occasional reports describe proteins, which are specific for microextensions of cultured cells. These proteins include microvillin [22], a chondroitin sulfate proteoglycan [21], fimbrin [23], and ezrin [24]. We have previously characterized an M_r 75,000 protein, cytovillin, of human JEG-3 choriocarcinoma cells [25] and used immunoelectron microscopy to localize it to the cytoplasmic microvillar membrane [26]. Cytovillin is expressed in a number of different cell types in vivo, where it is restricted to microvilli and other cell surface extensions [27]. So far, the only cultured cell line from which the microvilli have been isolated and their protein content analyzed is a MAT-C1 rat mammary adenocarcinoma cell line [28]. We have now purified microvilli from human JEG-3 choriocarcinoma cells and partially characterized their protein composition. The JEG-3 microvilli were found to be closely related but not identical to those of the placenta and the MAT-C1 cells. The most prominent polypeptides of the JEG-3 microvilli were actin, α-actinin, and a M_r 35,000 polypeptide. Cytovillin is found in the microvilli, is observed to form oligomers, but is not associated with the detergent-insoluble cytoskeletal residue of the JEG-3 microvilli. We also show that cytovillin is closely related to ezrin.

MATERIALS AND METHODS

Cells

Human JEG-3 choriocarcinoma cells (ATTC HTB 36; American Type Culture Collection, Rockville, MD) were grown on 64 cm^2 plastic dishes in Eagle's minimal essential medium (MEM) supplemented with 10% (v/v) fetal calf serum (FCS), 100 U/ml penicillin, and 50 μg/ml streptomycin.

Antisera

The preparation and specificity of the rabbit antiserum raised against human cytovillin purified from JEG-3 cells has been described previously [27]. For immunofluorescence microscopy, rabbit antiserum to chicken tropomyosin was kindly provided by Dr. J.J.-C. Lin, University of Pittsburgh, and described elsewhere [29]. In immunoblotting, rabbit antiserum to chicken α-actinin from Bio-Yeda, Rehovot, Israel, was also used. Rabbit antiserum to chicken actin was from Polysciences, Warrington, PA. Rabbit

antiserum to human ezrin was kindly provided by Dr. A. Bretscher, Cornell University, Ithaca, NY.

Immunofluorescence Microscopy

The cell cultures on glass coverslips were fixed with cold methanol (30 min, −20°C). The fixed cells were treated with anti-cytovillin (1:100), anti-human tropomyosin (1:50), or with the control rabbit serum (1:100) diluted in phosphate-buffered saline (10 mM phosphate; 150 mM NaCl, pH 7.4). The cell-bound antibodies were detected with purified sheep IgG raised against rabbit IgG conjugated with fluorescein isothiocyanate (FITC) (Wellcome, Beckenham, UK). The fluorescence was observed with a Leitz Dialux 20 microscope fitted with a ×63 oil immersion objective. Photomicrographs were taken on Agfapan 400 film.

Metabolic Labeling and Immunoprecipitation

Cultures of JEG-3 cells, grown on 64 cm^2 plastic dishes, were first incubated for 1 h in methionine-free MEM containing 0.2% (w/v) bovine serum albumin (BSA). Thereafter, 20 μCi/ml of L-(^{35}S)methionine (1,170 Ci/mmol; Amersham International, England) was applied for 8 h. After the radioactive pulse, the cell cultures were washed twice with phosphate-buffered saline (PBS). The microvilli were isolated as described below, and the labeled proteins were analyzed by SDS-PAGE. The immunoprecipitation was carried out as described previously [27].

Isolation of Microvilli and Microvillar Cytoskeletons

Metabolically labeled JEG-3 cells were washed twice with 20% FCS in phosphate-buffered saline and collected gently with a rubber policeman into a plastic tube. The cells were incubated for 15 min at 37°C in 20% FCS in PBS, and the microvilli were isolated by gentle shearing through a 14-gauge needle followed by differential centrifugation as described previously [30]. Cytoskeletons were prepared from the microvilli by resuspending them in a protein concentration of 1 mg/ml in 10 mM Tris/HCl, pH 7.5; 100 mM KCl; 1 mM dithiothreitol (DTT); 1 mM phenylmethylsulfonyl fluoride (PMSF); 0.2% (w/v) Triton X-100, and either 2.0 mM ethylendiaminetetra-acetic acid (EDTA) or varying concentrations of $CaCl_2$ or $MgCl_2$. After incubation for 20 min at 25°C, insoluble cytoskeletons were collected by centrifugation at 15,000*g* for 15 min at 4°C. The pellets were washed once with appropriate buffers and analyzed by SDS-PAGE under reducing conditions.

SDS-Polyacrylamide Gel Electrophoresis and Immunoblotting

The proteins were separated by SDS-PAGE in 10% (w/v) polyacrylamide gels according to Laemmli [31]. For fluorography, the gels were treated first for 30 min in 10% (v/v) acetic acid and then for 30 min in Amplify® (Amersham). Dried gels were exposed to Kodak X-Omat film. For immunoblotting [32], the proteins were transferred electrophoretically from the gel to 0.45 μm nitrocellulose sheets (BA-85; Schleicher & Schüll, Dassel, FRG). The sheets were treated with anti-cytovillin, 1:2,000; anti-ezrin, 1:1,000; anti-α-actinin, 1:400; anti-tropomyosin, 1:400; anti-actin, 1:400, or normal rabbit serum, diluted 1:1,000 in TEN-Tween (50 mM Tris/HCl, pH 7.0; 5 mM EDTA; 150 mM NaCl; 0.05% (v/v) Tween 20) for 1 h at 37°C. Immunoreactive protein bands

were detected with peroxidase-conjugated anti-rabbit IgG (Dako, Copenhagen, Denmark) diluted 1:200 in TEN-Tween.

Native Polyacrylamide Gel Electrophoresis and Immunoblotting

Cytovillin was purified as described previously [25] and made up to a protein concentration of 1 mg/ml in 20 mM Tris/HCl; 150 mM NaCl, pH 7.4. Native polyacrylamide gel electrophoresis was performed by using a PhastSystem apparatus (Pharmacia, Uppsala, Sweden) and 10–15% polyacrylamide gels in 0.112 M acetate, 0.112 M Tris/HCl, pH 6.4 according to the manufacturer's instructions. The proteins were visualized by using automated silver staining [33] of the gel in the PhastSystem development unit. Immunoblotting of cytovillin from the native polyacrylamide gels was carried out as described previously [17].

Gel Filtration

Isolated microvilli were lysed in 20 mM Tris/HCl, pH 7.5; 100 mM KCl; 2 mM $MgCl_2$; 1 mM $CaCl_2$; 0.5 mM DTT; 1 mM PMSF containing 0.2% (w/v) Triton X-100 for 20 min at 25°C. Insoluble material was separated by centrifugation at 15,000*g* for 15 min at 4°C. Gel filtration of the supernatant was carried out by using an UltroPac TSK-G 3000 SW gel-filtration column (7.5 × 600 mm) (LKB, Bromma, Sweden) and h.p.l.c. (Varian 5000 liquid chromatograph). The running buffer was the same as the lysis buffer, and the flow rate was 0.7 ml/min. Fractions of 0.7 ml were collected and analyzed by immunoblotting with anti-cytovillin antiserum.

Electron Microscopy

Aliquots of the isolated microvilli were fixed in 3.0% (v/v) glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 30 min. A drop of the suspension was placed onto a carbon/Formvar electron microscopy grid and stained with 0.25% (w/v) potassium phosphotungstate, pH 7.0. The samples were examined in a Jeol JEM 100 CX electron microscope at 60 kV at the Department of Electron Microscopy, University of Helsinki.

RESULTS

Purification of Microvilli

Electron microscopy showed that gentle shearing of the JEG-3 cells followed by differential centrifugation allowed the separation of the microvilli from the cells (Fig. 1). The microvillus-specific membrane protein, cytovillin, was also found to be enriched in the isolated microvillus preparations (Fig. 2). By contrast, these preparations were devoid of tropomyosin, which gave a strong positive reaction in immunoblotting of the total JEG-3 protein lysates (see below). The results indicated that the microvillus preparations were not significantly contaminated by cellular debris.

Identification of Microvillar Proteins

The protein components of the isolated JEG-3 microvilli were examined by SDS-PAGE of metabolically radiolabeled proteins and immunoblotting. The most prominent polypeptide band had a relative molecular weight of 42,000 (Fig. 2, track 2). In immunoblotting this polypeptide was recognized with anti-actin antiserum, as expected (Fig. 2, track 3). A second major component, an M_r 100,000 polypeptide, gave a positive reaction with anti-α-actinin antiserum (Fig. 2, track 4). These two proteins are

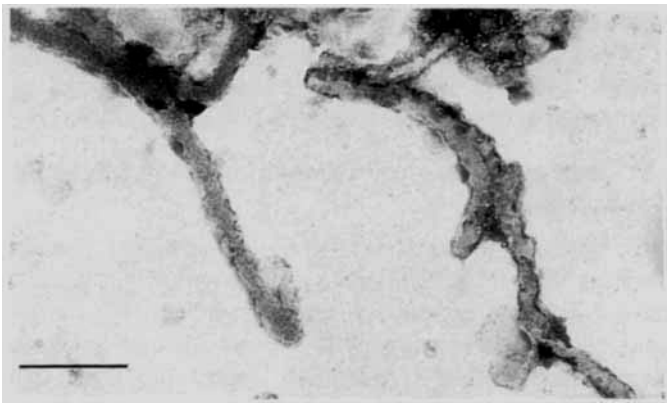

Fig. 1. Electron microscopy. Isolated JEG-3 microvilli were fixed in 3.0% (v/v) glutaraldehyde and visualized for electron microscopy by negative staining. Bar = 500 nm.

also the major microvillar proteins of human placenta [4] and of MAT-C1 mammary adenocarcinoma cells [28]. A third major band had an M_r value of 35,000. Additionally, approximately 10 minor bands could be seen (Fig. 2, track 2). One of these bands was detected by anti-cytovillin antiserum in immunoblotting (Fig. 2, track 5). As mentioned above, tropomyosin was not observed in the JEG-3 microvilli (Fig. 2, track 6), although it

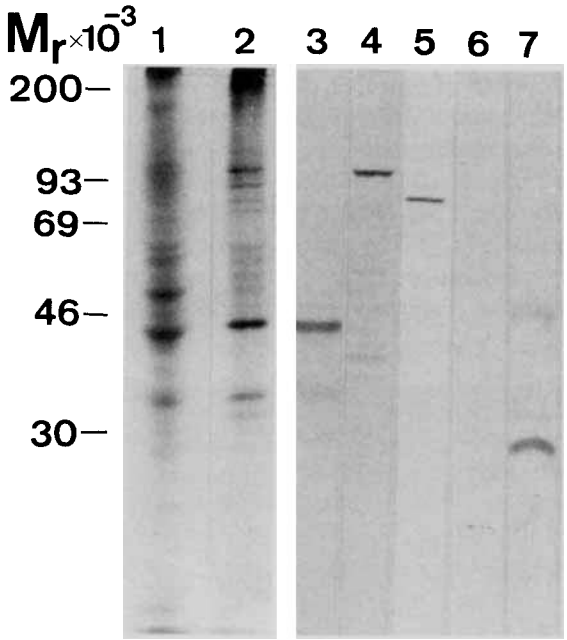

Fig. 2. Polypeptide components of isolated microvilli. L-(^{35}S)methionine-labeled JEG-3 microvilli were subjected to SDS-PAGE followed by fluorography. **Track 1:** total JEG-3 proteins; **track 2:** isolated microvilli. **Tracks 3–6** and **track 7** represent immunoblotting of the purified microvilli and total JEG-3 proteins, respectively. The nitrocellulose sheets were stained with anti-actin, 1:400 (**track 3**); anti-α-actinin, 1:400 (**track 4**); anti-cytovillin, 1:2,000 (**track 5**); anti-tropomyosin, 1:400 (**tracks 6, 7**). The M_r values of the marker proteins are indicated on the left.

was present in the total JEG-3 protein lysates (Fig. 2, track 7). No distinct band was seen at the position of the 110 kd protein. In immunofluorescence microscopy, cytovillin-containing microvilli could be seen as dots or short spikes on cell surface, whereas tropomyosin was located in filamentous structures outside of the microvilli (Fig. 3).

Effects of Ca^{2+} and Mg^{2+} on the Interaction of the Microvillar Proteins with the Cytoskeleton

To study the effect of Ca^{2+} and Mg^{2+} on the interaction of the microvillar proteins with the cytoskeleton the L-(^{35}S)methionine-labeled microvillus preparations were exposed to Triton X-100 in the presence of varying concentrations of Ca^{2+}, Mg^{2+}, and EDTA. The insoluble material, containing the microvillar cytoskeletons, was separated by centrifugation and analyzed by SDS-PAGE. The results showed that the cytoskeletons contained mainly actin, α-actinin, M_r 200,000, M_r 55,000, and M_r 35,000 polypeptides (Fig. 4). The M_r 35,000 polypeptide bound to cytoskeletons in a Ca^{++}-dependent manner. The association of the M_r 35,000 polypeptide with the cytoskeletons was half maximal at a calcium concentration of about 0.05–0.25 mM. In the presence of 1.0 mM Mg^{2+} the protein was almost completely soluble (Fig. 4). Overexposure of the fluorograph in Figure 4 showed the absence of the 35 kd band also in the EDTA extract (data not shown). Thus, the M_r 35,000 polypeptide closely resembles the calcium-sensitive microfilament-binding microvillus protein, AMV-p35, of MAT-C1 microvilli [34].

The microvillus polypeptides of M_r 55,000 and 200,000 were insoluble both in the absence or presence of divalent cations (Fig. 4), suggesting a tight association of these polypeptides with the cytoskeletal core of the JEG-3 microvillus.

Characterization of Cytovillin

It has been previously shown that cytovillin could be almost completely released from JEG-3 cells with Triton X-100 [26], and that the solubilized protein migrated as a single broad peak in preparative gel filtration of Triton X-100 lysates of JEG-3 cells [25]. However, when the Triton X-100 extracts of the JEG-3 microvilli were subjected to an analytical gel filtration on a TSK-G 3000 SW column, cytovillin was found to be eluted out as two distinct peaks (Fig. 5). The major fraction corresponded to a molecular weight of 75,000 and represents most probably the monomeric form of the molecule. To study whether the minor fraction, corresponding to a molecular weight of 200,000, contained a

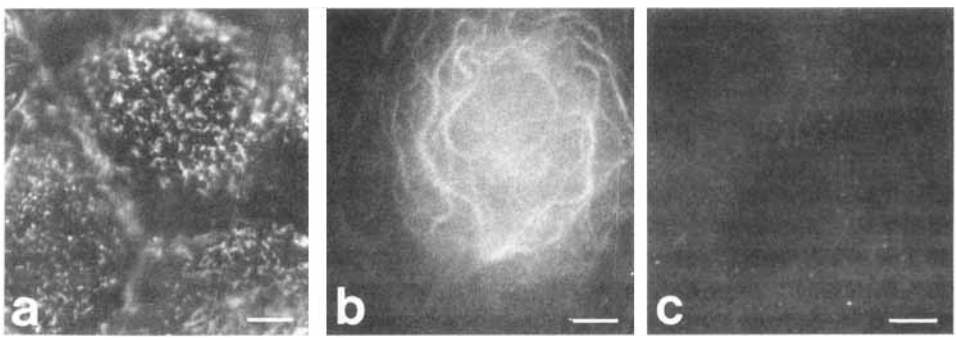

Fig. 3. Immunofluorescence microscopy. The cells were labeled with anti-cytovillin (1:100) (**a**), anti-tropomyosin (1:50) (**b**), or normal rabbit serum (1:100) (**c**). Bars = 15 μm.

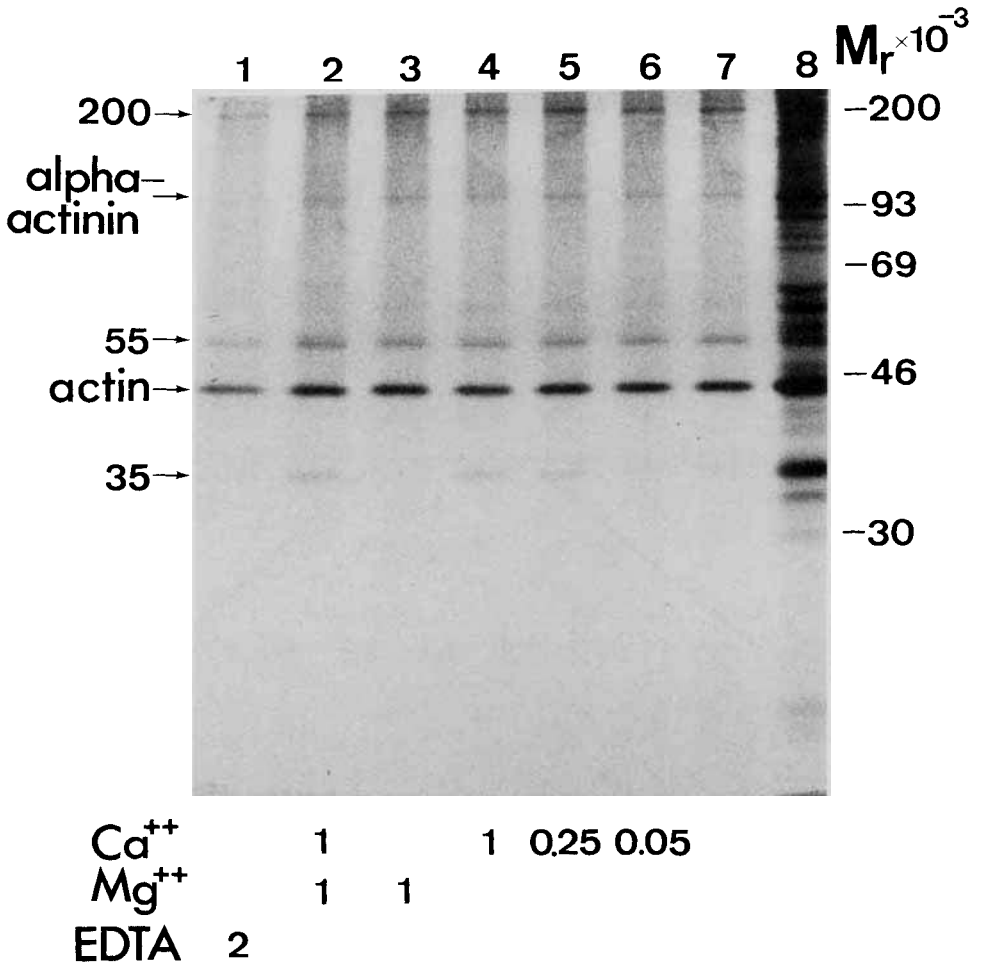

Fig. 4. Effect of divalent cations on the interaction of microvillar proteins with the cytoskeletons. Samples of L-(^{35}S)methionine-labeled microvillar preparations were resuspensed in buffers containing the indicated concentrations of Ca^{2+}, Mg^{2+}, and EDTA. The insoluble cytoskeletal materials were separated by centrifugation at 15,000*g* for 15 min. After washing with appropriate buffers, the pellets were examined by SDS-PAGE followed by fluorography. The concentrations of Ca^{2+}, Mg^{2+}, and EDTA used are indicated in mM (**tracks 1–7**). **Track 8:** total microvillar proteins. The positions of actin, α-actinin, the M_r 200,000, M_r 55,000, and M_r 35,000 polypeptides are indicated on the left (arrows) and the M_r values of the marker proteins on the right.

complex of cytovillin with other component(s) or an oligomeric form of the protein, we analyzed purified cytovillin on a native polyacrylamide gel under non-denaturating conditions. After silver staining of the gel, two bands could be seen (Fig. 6a, track 1). The relative molecular weights of the bands were 80,000 and 190,000. Both bands also gave a positive reaction with anti-cytovillin serum in immunoblotting of the gel (Fig. 6a, track 2). The similar mobility of cytovillin under reducing and nonreducing conditions in SDS-PAGE of JEG-3 lysates (Fig. 6b) indicated that disulfide bonds were not involved in the aggregation. When cytovillin was immunoprecipitated from the L-(^{35}S)methionine-labeled JEG-3 cells, using the same buffer conditions as in gel filtration and analyzed by

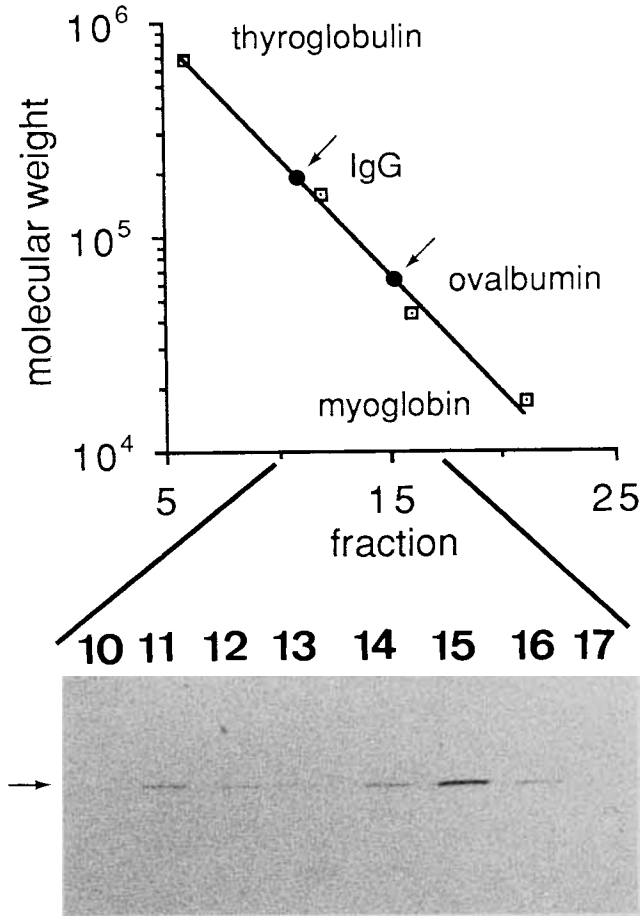

Fig. 5. Gel filtration of Triton X-100-soluble microvillar proteins. Marker proteins (thyroglobulin, M_r 670,000; IgG, M_r 158,000; ovalbumin, M_r 44,000; myoglobin, M_r 17,000) were mixed with the soluble microvillar proteins in 20 mM Tris/HCl, pH 7.5; 100 mM KCl; 1 mM $MgCl_2$; 1 mM $CaCl_2$; 0.5 mM DTT; 1 mM PMSF containing 0.2% (w/v) Triton X-100 and applied on an UltroPac TSK-G 3000 SW column equilibrated with this buffer. The flow rate was 0.7 ml/min, and 0.7 ml fractions were collected. The fractions were analyzed by immunoblotting conditions with anti-cytovillin antiserum. Cytovillin was eluted as two major peaks (fractions 11 and 15, arrows) corresponding to molecular weights of 75,000 and 200,000, respectively.

SDS-PAGE, no other polypeptide co-precipitated (Fig. 6c). Together with the previous finding that purified cytovillin migrated as a single band at pI 6.1 in isoelectric focusing [27], the results suggest that a portion of cytovillin exists as an oligomer.

Ezrin (p80) is a microvillar protein, which was originally purified from the chicken intestinal brush border [24]. Later it was also reported to be detected in human placental microvilli [6]. Because of the similar characteristics of cytovillin and ezrin, we tested whether they were related proteins. In immunoprecipitation of radiolabeled JEG-3 proteins both anti-ezrin and anti-cytovillin antisera precipitated an M_r 75,000 polypeptide, which migrated identically in SDS-PAGE (Fig. 7a). In addition, antiserum to

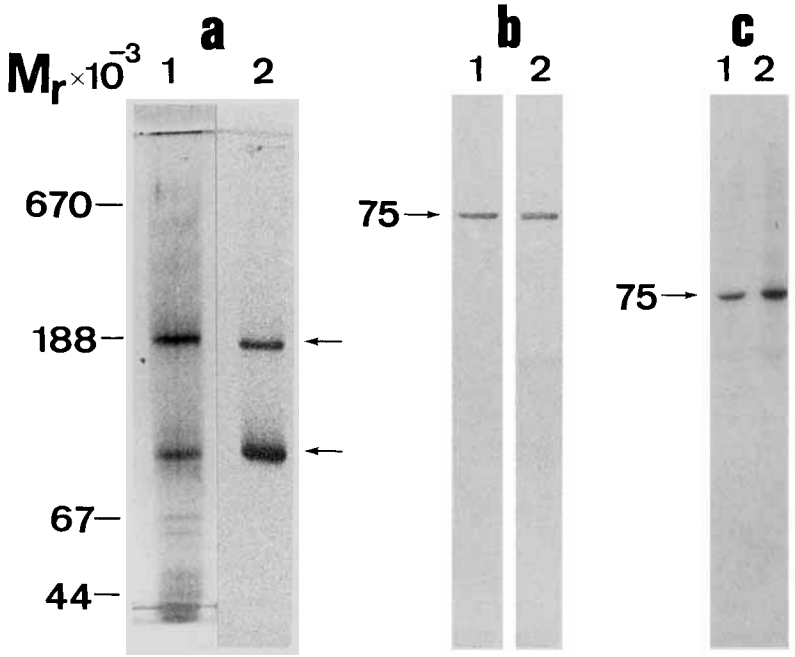

Fig. 6. Characterization of cytovillin. Purified cytovillin was subjected to native polyacrylamide gel electrophoresis without SDS and β-mercaptoethanol. The protein bands (arrows) were visualized by silver staining of the gel (**track a1**) and by immunoblotting with anti-cytovillin serum (diluted at 1:2000 in TEN-Tween buffer) (**track a2**). The positions of the marker proteins thyroglobulin (M_r 670,000), phosphorylase A (M_r 188,000), BSA (M_r 67,000), and ovalbumin (M_r 44,000) are indicated on the left. The JEG-3 proteins were separated by SDS-PAGE under reducing (**track b1**) and nonreducing (**track b2**) conditions followed by immunoblotting with anti-cytovillin antiserum. Cytovillin migrated identically in the two gels as shown by the arrow. Cytovillin was immunoprecipitated in the presence of 0.1% (w/v) SDS and 0.5% (v/v) Triton X-114 in 50 mM Tris/HCl, pH 8.0, 150 mM NaCl, and 1 mM PMSF (**track c1**) and by using the same buffer as in gel-filtration of Triton X-100 solubilized microvillar proteins (**track c2**) followed by separation in SDS-PAGE under reducing conditions. Only cytovillin bands (arrow) can be seen. The gel was run over to detect the possible oligomers.

human ezrin reacted with the purified cytovillin in immunoblotting (Fig. 7b). Thus, the results suggested that ezrin and cytovillin are closely related if not identical proteins.

DISCUSSION

This work was carried out in order to obtain more information about the poorly characterized microvillar proteins of cultured cells. We applied the gentle shearing method [30] to isolate microvilli from cultured human JEG-3 choriocarcinoma cells and analyzed their protein composition. As expected, the major structural protein of the JEG-3 microvilli was found to be actin. Actin filaments form the microvillar core of the intestinal brush border (for a review, see [1]) and the placental microvilli [3]. Moreover, actin was the most prominent protein of the isolated MAT-C1 microvilli [28]. Besides actin, α-actinin was found to be one of the major components of the JEG-3 microvilli. Moreover, α-actinin has been found in placental [4] and in MAT-C1 microvilli [28], but in the intestinal brush border α-actinin is located in the terminal web region beneath the

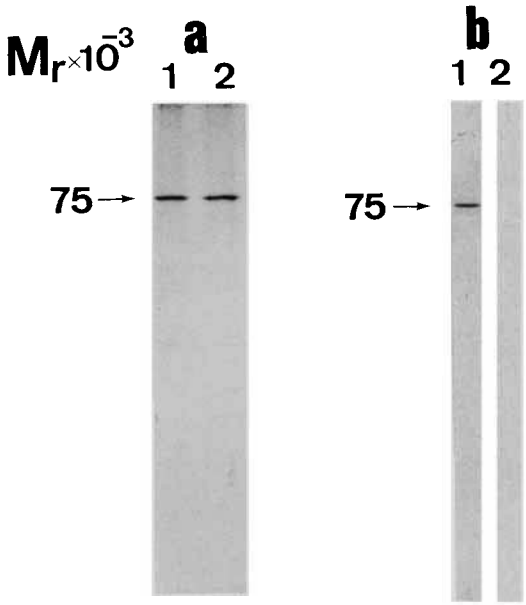

Fig. 7. Comparison of cytovillin to ezrin. JEG-3 cells were labeled metabolically with L-(^{35}S)methionine. Immunoprecipitations were carried out by using anti-cytovillin (**track a1**) and anti-ezrin (**track a2**) antisera. Immunoblotting of the purified cytovillin with anti-ezrin antiserum (1:1,000) and normal rabbit serum (1:1,000) are shown in **track b1** and **track b2,** respectively. The M_r 75,000 bands are indicated by the arrow.

microvillar array [35]. The major apparent difference between the JEG-3 and the MAT-C1 microvilli is the absence of tropomyosin in the JEG-3 microvilli, although the protein was present in the rest of the JEG-3 cell. Three major isoforms of tropomyosin (31K-a, 31K-b, and 29K) have been purified from MAT-C1 microvilli, where their amount was estimated to be sufficient to saturate about half of the microvillar F-actin [36]. In addition, no diffuse M_r 75,000–80,000 band corresponding to a prominent cell surface glycoprotein (CAG) of the MAT-C1 microvilli [37] was observed in the JEG-3 microvilli.

The presence of the M_r 35,000 polypeptide in the Triton X-100 lysates of the JEG-3 microvilli is of interest. In its relative molecular weight and calcium-dependent association with the cytoskeleton, it resembles AMV-p35, a calcium-sensitive membrane-binding protein of the MAT-C1 microvilli. AMV-p35 is closely related to calpactin I [34], which belongs to a large family of calcium-sensitive membrane-binding proteins [38]. Calpactin I is a peripheral membrane protein, which is expressed in high concentrations in the mammalian intestine and is localized primarily in the cytoskeletal region of the brush border beneath the microvillar array [39]. Moreover, it is the major tyrosine kinase substrate of $pp60^{v\text{-}src}$ in Rous sarcoma virus-infected cells [40]. Similar M_r 32,000–38,000 proteins have also been detected in human placental microvilli [6].

The minor JEG-3 microvillar polypeptides of M_r 55,000 and 200,000 remained insoluble during Triton X-100 extractions in the presence or absence of divalent cations, indicating a tight interaction of the proteins with the cytoskeleton. The role of these proteins in the structure of the microvillus core remains to be investigated.

Cytovillin was first detected by antibodies against a synthetic peptide based on a cloned human endogenous retrovirus nucleotide sequence [41]. Later it was purified [25] and shown to be localized to the microvillus membranes of the JEG-3 cells [26] and in related cell surface protrusions of several other cell lines [27]. In previous experiments cytovillin behaved like a monomeric protein. However, this result was based solely on preparative gel filtration, in which the resolution was limited [25]. In the present study the analytical gel filtration of the Triton X-100-solubilized microvillar proteins and the native polyacrylamide gel electrophoresis of purified cytovillin indicated that the protein exists, at least partially, in an oligomeric form. The molecular weight of the complex suggests that it was a trimer. However, dimerization cannot be excluded, if it caused changes in the conformation of cytovillin molecules. The interaction between the cytovillin molecules was weak. The protein migrated mainly in monomer form and the oligomers were dissociated in SDS-PAGE. This explains why we have not observed this oligomeric form in our earlier studies [17,25–27]. So far, we have not been able to show any interaction of cytovillin with other cellular components.

Ezrin is a M_r 80,000 microvillus protein, which was initially purified from chicken intestinal brush border microvilli [24]. It has properties, such as relative molecular weight and microvillar localization, which pointed to a possible identity with cytovillin. In order to determine whether cytovillin and ezrin are related, two approaches were used. First, antiserum to human ezrin gave a strong positive reaction with our purified cytovillin in immunoblotting. Second, in immunoprecipitation of the radiolabeled JEG-3 proteins both anti-cytovillin and anti-ezrin antisera precipitated a polypeptide that co-migrated in the SDS-PAGE. The results suggest that cytovillin and ezrin are indeed closely related. Recently, a tyrosine kinase substrate p81 was shown to be homologous to ezrin, and located in the microvilli of a human A 431 epidermoid carcinoma cell line [42]. It should be noted, however, that ezrin [24] and p81 [42] are at least partially associated with cytoskeletons, while cytovillin is practically completely soluble in conditions that left cytoskeletons intact [26]. In addition, no oligomeric forms of ezrin were observed [24]. Therefore, sequencing data will finally reveal whether these three proteins are truly identical and encoded by the same gene.

The appearance of microvilli and related structures on cultured cells is associated with many important biological phenomena such as transformation [18], virus infection [12–17], and mitosis [8–11] in addition to their apparent role in mediating cell-cell and cell-substratum contacts [19–21]. However, it is not known whether different types of microvilli are involved in each case. According to previous reports [28,29] and our results, the basic structural components of the microvilli of cultured cells are apparently actin and α-actinin. On the other hand, our results also indicate that the microvilli of different cultured cells are not structurally identical. Whether this reflects differences in the biological functions of the microvilli remains to be investigated.

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