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Intramembranous particles are clustered on microvillus membrane vesicles

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Many intramembranous particles in pig jejunal microvillus membranes cluster during cell disruption and membrane vesiculation with the $MgCl_2$ aggregation technique (Hauser, H., Howell, K., Dawson, R.M.C. and Bowyer, D.E. (1980) Biochim. Biophys. Acta 602, 567–577). Isolated brush borders and purified microvillus membrane vesicles were jet-frozen and examined by freeze-fracture electron microscopy. From 30 to 60% of purified vesicles exhibited no intramembranous particles on their fracture face and 22–39% exhibited clustered or aggregated intramembranous particles. Only 6–15% of the vesicles exhibited the random distribution of intramembranous particles that is characteristic of intact enterocytes. Aggregation was not reversed after dialysis to remove divalent cations. Prior freezing of tissue or vesicles (−70°C) gave the same results as fresh unfrozen material. Heterogeneity of microvillus vesicles may occur among the vesicles generated from a single microvillus.

Introduction

The intestinal microvillus membrane is one of the most viscous, high-melting-point membranes in the vertebrate body [1–3]. The lipids of this membrane are characterized by high concentrations of sphingolipids [1,4–7], which are thought to reside exclusively in the outer monolayer of the membrane [8,9] and to be tightly associated by a network of intermolecular hydrogen bonds [10]. The intestinal microvillus membrane contains numerous hydrolases and transport systems. Although there may be at least 150 different proteins in the intestinal microvillus membrane [11], some individual hydrolases such as sucrase-isomaltase [12] or aminopeptidase N (Semenza, G., personal communication) can comprise up to 10% of the total membrane protein. The classification of membrane proteins into extrinsic and intrinsic groups is particularly apt for this membrane [13], since many of the hydrolases are attached to the bilayer by small anchor peptides which make up less than 5% of the total enzyme protein. So, even though the protein/lipid weight ratio of intestinal microvillus membrane vesicles is approx. 2 [1,4–7], up to 80–95% of the total membrane protein can be removed by mechanical shaking and proteinase treatment [14]. A single major protein (165 kDa) left in the membrane following proteinase treatment with subtilisin and papain [15] has been tentatively identified as the sodium-dependent D-glucose transporter [16]. 'Arbitrary estimates' have suggested that the glucose transporter makes up no more than 1% of the total intestinal microvillus membrane protein [16]. The contribution to the total microvillus membrane protein of the 30–40 other transport systems that have been reported in the literature is unknown.

When biological membranes are freeze-fractured through the middle of their bilayer, small (6–12 nm) intramembranous particles appear on the fracture faces [17,18]. These particles are gen-

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erally thought to represent membrane proteins. Liposome-reconstitution studies with band 3 protein [19] and glycophorin [20,21] from red cell membranes, $(Na^+ + K^+)$-ATPase from outer renal medulla [22], hydrophobic protein (N-2) from brain myelin [23] and Ca^{2+}-ATPase from sarcoplasmic reticulum [24] all show that membrane proteins can produce intramembranous particles in otherwise smooth phospholipid vesicles. Although the lipid bilayer readily splits during fracture, transmembrane proteins may be pulled intact to one side or the other of the bilayer or peptide covalent bonds may be broken [26]. Under certain conditions, membrane lipids by themselves may also produce intramembranous particles [25].

Although the identities of the intramembranous particles in the small intestinal microvillus membrane are unknown, their random distribution, size, (6–12 nm) and densities are well known [27–30]. In mature human enterocyte microvilli there are approx. 4000 intramembranous particles/μm^2 in the P face (the membrane half in contact with the cytoplasm) and approx. 800 intramembranous particles/μm^2 in the E face (the membrane half in contact with the lumen) [27]. Similar densities and distributions have been seen in the monkey, rat and mouse [28–30]. Using the data from Ref. 27, intramembranous particles occupy roughly 17.5 and 3.8% of the P face and E face surfaces, respectively. The density of microvillus intramembranous particles increases (over 2-fold) and the specific activity of many microvillus hydrolases increases as enterocytes differentiate and migrate from crypt to villus tip [27]. In the disease celiac sprue, the specific activities of many microvillus hydrolases decrease and intramembranous particle densities decrease by 50% [28]. However, in experimental diabetes, where the specific activities of sucrase and maltase are more than doubled over controls, intramembranous particle density is unchanged [29]. Thus, it is unclear whether the stalked microvillus hydrolases form intramembranous particles.

Because high yields of microvillus membrane vesicles can be obtained with the Ca^{2+} or Mg^{2+} precipitation technique, they have become very popular for membrane studies [31]. Although vesicles appear to be oriented right-side-out [31], whole populations exhibit a high degree of heterogeneity with regard to their electrophoretic mobility and density [32,33] and subpopulations can show enrichment or depletion of enzyme activities [32]. Contamination by other membranes and the fact that vesicles are isolated from both young and old enterocytes have been suggested as possible causes of heterogeneity [32]. In this study, we show that heterogeneity may also arise directly during cell disruption which causes microvillar intramembranous particles to cluster during the vesiculation process.

Materials and Methods

Materials. Fresh pig small intestines were obtained from the Department of Food Science, University of Georgia. Adenosine deaminase was supplied by Boehringer Mannheim Biochemicals (Indianapolis, IN). Levamisole hydrochloride was obtained from Aldrich Chemical Co. (Milwaukee, WI). Sepharose 4B-200, L-alanine-*p*-nitroanilide, *p*-nitroaniline, AMP, bovine serum albumin, Folin-Ciocalteu reagent, sodium azide, phenylmethylsulfonyl fluoride (PMSF), dithiothreitol, ethylenediaminetetraacetic acid (EDTA), ethylene glycol bis (β-aminoethyl ether)-*N*,*N'*-tetraacetic acid (EGTA), Tris-HCl, and imidazole (grade I) were obtained from Sigma Chemical Co. (St. Louis, MO).

Isolation and purification of microvillus membrane vesicles. Microvillus membrane vesicles were isolated from fresh and frozen pig jejunum according to the method of Christiansen and Carlsen [5] in a 0.3 M mannitol buffer/12 mM Tris at pH 7.1. This procedure is a modification of the method of Kessler et al. [34] since $MgCl_2$ is substituted for $CaCl_2$ to prevent hydrolysis of membrane phospholipids by calcium-activated phospholipases [7]. Vesicles were then purified by the method of Carlsen et al. [35], on a 7 × 15 cm column of Sepharose 4B-200 at 4°C. Specifically, the vesicles were applied to the column in 20 ml (2 mg protein/ml) and eluted with 50 mM Tris-HCl (pH 7.4)/150 mM NaCl. Fractions of 4.0 ml were collected and assayed for protein and marker enzymes. With this method, purified microvillus membrane vesicles pass through the column unretarded while other membrane fractions remain on the column. Purified vesicles were then either frozen at −70°C,

used immediately or dialyzed at 4°C for 72 h against 50 mM Tris-HCl (pH 7.4)/150 mM NaCl or the same buffer containing 10 mM EDTA. Spectropore dialysis tubing (Fisher Scientific) (6 mm dry diameter, molecular weight cutoff 12 000–14 000) were used to dialyze 5 ml of vesicles in buffer (0.70 mg membrane protein) against 500 ml of buffer which was changed five times.

Marker enzyme determination. Aminopeptidase N (EC 3.4.11.2) was used as a marker enzyme for the microvillus membrane and 5′-nucleotidase (EC 3.1.3.5) for the basolateral membrane. Aminopeptidase activity was determined using L-alanine-*p*-nitroanilide as substrate [36]. For 5′-nucleotidase, AMP was used as substrate [37,38]. In both cases, the enzymatic reactions were monitored with a reaction rate program on an Apple IIe microcomputer coupled to an LKB Ultrospec 4050. Enzyme units were defined as the amount of enzyme necessary for catalyzing the disappearance of 1 nmol of substrate per min under the assay conditions. Protein was determined by the method of Lowry et al. [39] using crystalline bovine serum albumin as stardard.

Vesicle preparation and designation for freeze-fracture. Aliquots (1 ml) of purified vesicles (either fresh or prefrozen) obtained from the most turbid Sepharose 4B fractions were centrifuged at 15 000 $\times g$ in an Eppendorf microcentrifuge at 4°C for 30–45 min. The resulting pellet was resuspended in 1 drop of buffer containing 10% glycerol/50 mM Tris-HCl/150 mM NaCl at pH 7.0. Samples were held at either 4 or 40°C for 1 h prior to jet-freezing. Vesicle preparations were designated as: (1) prefrozen - vesicles stored at −70°C after being purified from tissue which had been frozen at −70°C, (2) non-frozen - purified vesicles which had never been stored at −70°C but were isolated from frozen tissue, (3) fresh - purified vesicles isolated from fresh tissue and (4) dialyzed - purified vesicles isolated from fresh tissue then stored at −70°C prior to dialysis.

Isolation of brush borders. Brush borders from pig intestines were isolated by the modified method of Mooseker and Howe [40,41]. Briefly, a segment (20 cm) of jejunum was removed, flushed with Tris buffer (50 mM Tris/150 mM NaCl (pH 7.0)) and loaded with 50 ml of cell-dissociation medium (0.20 M sucrose/76 mM Na_2HPO_4/12 mM EDTA/19 mM K_2HPO_4/4 mM NaN_3/0.1 mM dithiothreitol/0.1 mM PMSF). The segment was massaged at 15-s intervals for 30 min at 4°C, then the cells were isolated from the contents by a low-speed centrifugation (2150 $\times g$) for 5 min in a clinical centrifuge. The resulting mass of cells was resuspended in homogenization buffer (10 mM imidazole-HCl (pH 7.0)/4 mM EDTA/1 mM EGTA/4 mM NaN_3 or 0.1 mM dithiothreitol/0.1 mM PMSF) then homogenized at top speed in a blender with 1–2 s bursts for 10–20 s. The mixture was then pelleted and washed two more times and dounced 10–20 strokes with a hand tissue dounce (Wheaton Scientific) and pelleted again as above. The washed pellet was resuspended and washed twice in stabilization buffer (10 mM imidazole (pH 7.0)/7.5 mM KCl/5 mM $MgSO_4$/1 mM EGTA/4 mM NaN_3/0.1 mM dithiothreitol/0.1 mM PMSF) then layered onto 50% sucrose. This preparation was centrifuged at 26 000 rpm in an SW-27 rotor (Beckman Instruments, Palo Alto, CA) for 30–45 min. Brush borders were taken from the band at the 40%/50% sucrose interface and repelleted in 1.5 ml conical centrifuge tubes (Ulster Scientific) 15 700 $\times g$ for 5 min in an Eppendorf microfuge. The sedimented brush borders were resuspended in 1 ml of stabilization buffer containing 10% glycerol, repelleted, then resuspended in 1 drop of the same buffer. Brush borders were then processed for freeze-fracture microscopy.

In addition to brush borders and microvillus membrane vesicles, segments of intact jejunum were also examined by freeze-fracture. Fresh segments of jejunum (30 cm in length) were flushed with 100 ml of buffer containing 50 mM Tris-HCl/150 mM NaCl (pH 7.0 at 4°C) and 2-cm segments were removed and immersed in ice-cold 3% glutaraldehyde/0.1 M cacodylate at pH 7.0. Small cubes (1 mm) were cut and then fixed for 1.5 h at 4°C. These pieces were washed with one change of 0.1 M cacodylate buffer at 4°C prior to jet-freezing.

Jet-freezing and freeze-fracture. Tissue pieces, brush borders or aliquots (0.5 μl) of purified microvillus membrane vesicles were frozen in a jet of liquid propane in a Balzers QFD 101 quick-freezing device. Samples were sandwiched between two copper planchets prior to freezing, then fractured

at −120°C and 10^{-7} Torr in a Balzers 360M freeze-etch plant. Replicas were made by evaporating 2.8 nm of platinum and 20.0 nm of carbon onto the fractured surface at −140°C followed by etching for 3 min at −95°C.

Replicas were cleaned in 40% Clorox solution for 1–3 h, washed once in water, then cleaned in a solution of Chromerge (Fisher Scientific): H_2SO_4 (conc.)/H_2O (1:1:1) for 30 min at 60°C. Five washes in water preceded mounting the replicas on 200 × 75 mesh copper grids (Ernest F. Fullam, Schenectady, NY). Replicas were viewed at 100 KV in a Philips 400 electron microscope. From ten randomly selected 30.75 μm^2 fields, at least 250 microvillus membrane vesicles were analyzed for intramembranous particle distribution, fracture face type, and vesicle diameter. Only vesicles presenting the P or E fracture face were chosen to be measured across their diameter. Statistical analyses were performed according to Bailey [42] using an Apple IIe microcomputer.

Results

Aminopeptidase activity was used as a marker for the microvillus membrane and 5′-nucleotidase for the basolateral membrane [5,35]. Table I shows the activities of these two enzymes in the standard microvillus membrane vesicle preparation and after Sepharose purification. The ratio of activities of the purified vesicles versus the standard preparation were 1.10 ± 0.04 for aminopeptidase and 0.32 ± 0.08 for 5′-nucleotidase. This indicates that the final vesicles were obtained primarily from the microvillus region [3,35].

Fig. 1 shows a typical field of freeze-fracture microvillus membrane vesicles after purification on a Sepharose 4B column. The size distribution of vesicles from a variety of preparations are shown in Fig. 2. Vesicles that were isolated from frozen tissue and then stored at −70°C (termed 'prefrozen') appeared more heterogeneous in size (Fig. 2A, B) when compared to other treatments. The vesicle size range was broad for prefrozen vesicles (Fig. 2A) isolated from frozen tissue and for all other vesicles incubated at low temperature (4°C) prior to jet-freezing (Fig. 2A and C). For all preparations, the major size class peak occurred between 80 and 160 nm with 42.8% (40°C non-frozen vesicles) to 78.2% (40°C dialyzed vesicles without EDTA) of the vesicles in this size range. The most homogeneous group of vesicles were obtained following dialysis (Fig. 2D) with 75.2% of the dialyzed vesicles occurring in the 80–160 nm diameter size class compared to 59.5% for all other treatments.

The most striking feature of freeze-fractured brush borders and microvillus membrane vesicles was the aggregation of the intramembranous particles on both the P and E fracture faces. In intact cells, intramembranous particles were randomly distributed over the entire surface of the microvillus shaft (Fig. 3A). In isolated brush borders, large

TABLE I

ISOLATION AND PURIFICATION OF MICROVILLUS MEMBRANE VESICLES

Stage	Vol. (ml)	Protein ($mg \cdot ml^{-1}$)	Aminopeptidase			5′-Nucleotidase		
			$nmol \cdot min^{-1} \cdot mg^{-1}$	yield (%)	purification	$nmol \cdot min^{-1} \cdot mg^{-1}$	yield (%)	purification
Homogenate	1050	7.91	72.9	100.0	1.0	11.4	100.0	1.0
Vesicle preparation (before chromatography	40 [a]	2.00	4084.0	54.0	56.0	70.5	6.0	6.2
Pure vesicles (after chromatography)	70	0.14	4507.0	14.2 [b]	61.8	22.7	0.5	2.0

[a] Only 20 ml of vesicle preparation were chromatographed.
[b] Calculated to the original volume.

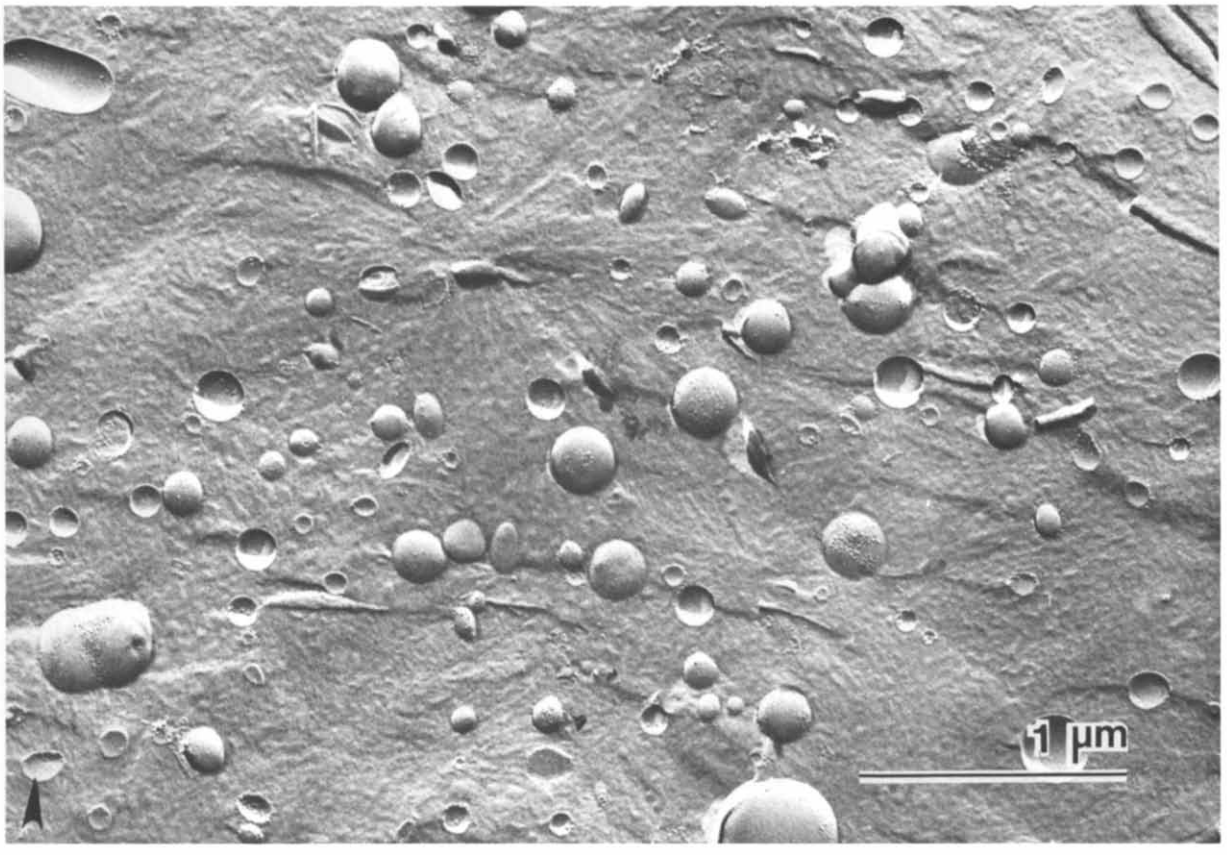

Fig. 1. Field of jet-frozen pig intestinal microvillus membrane vesicles purified on a Sepharose 4B column.

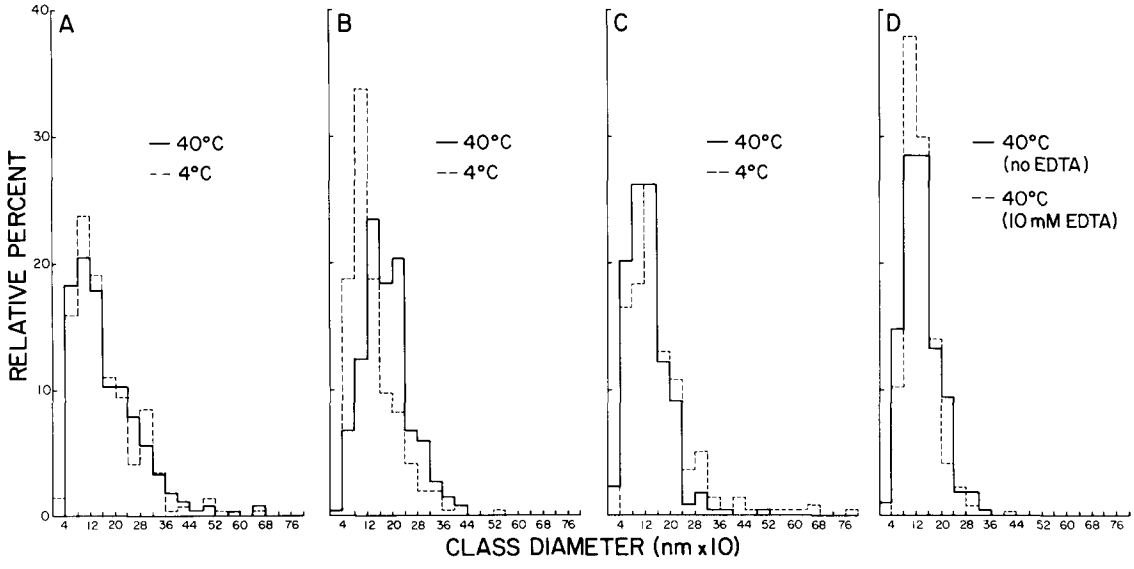

Fig. 2. Size distribution of vesicles as a function of temperature and preparation method. (A) Frozen vesicles from frozen tissue. (B) Nonfrozen vesicles from frozen tissue. (C) Fresh vesicles. (D) Fresh vesicles which had been frozen prior to dialysis. (See text for details.)

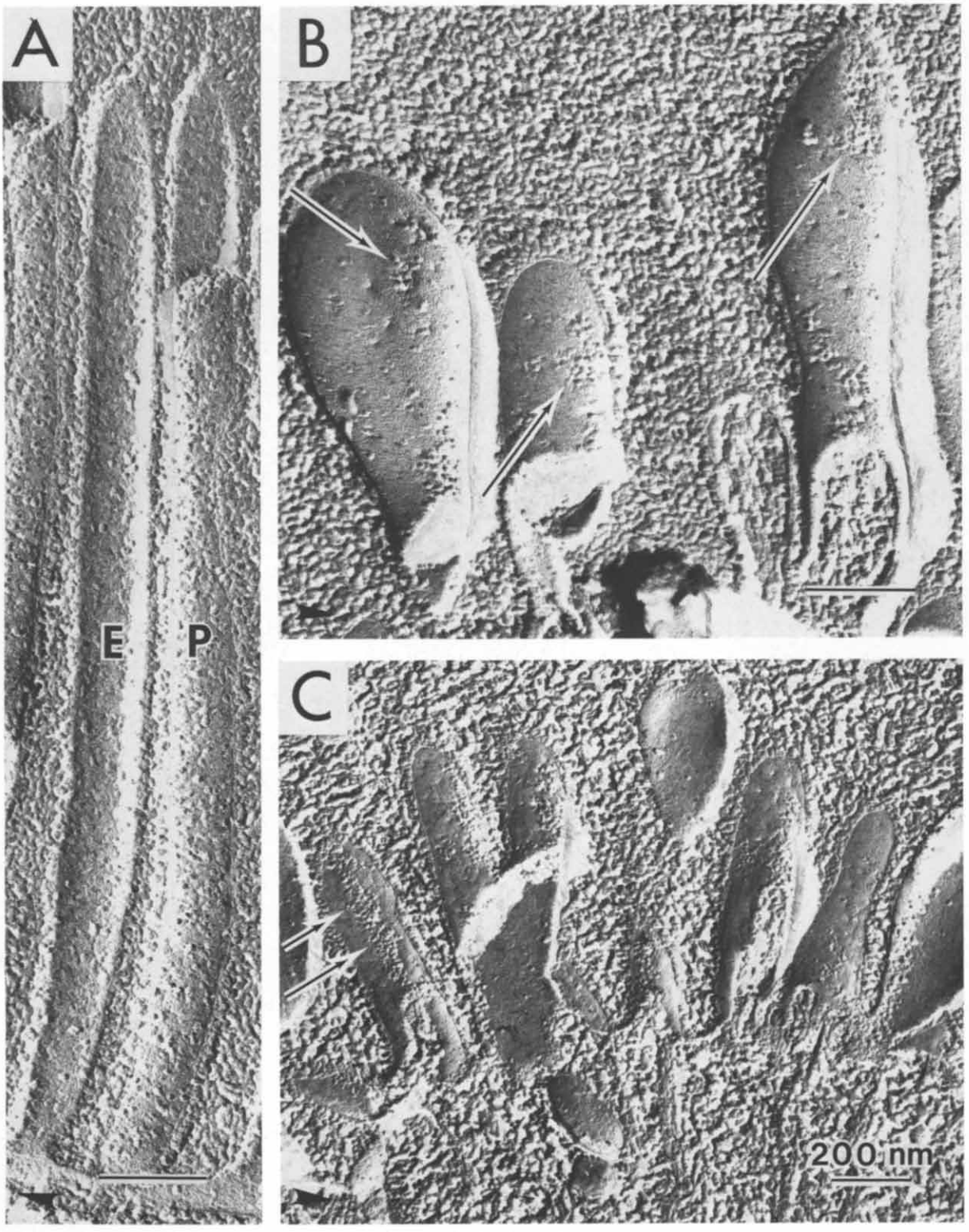

Fig. 3. (Legend opposite.)

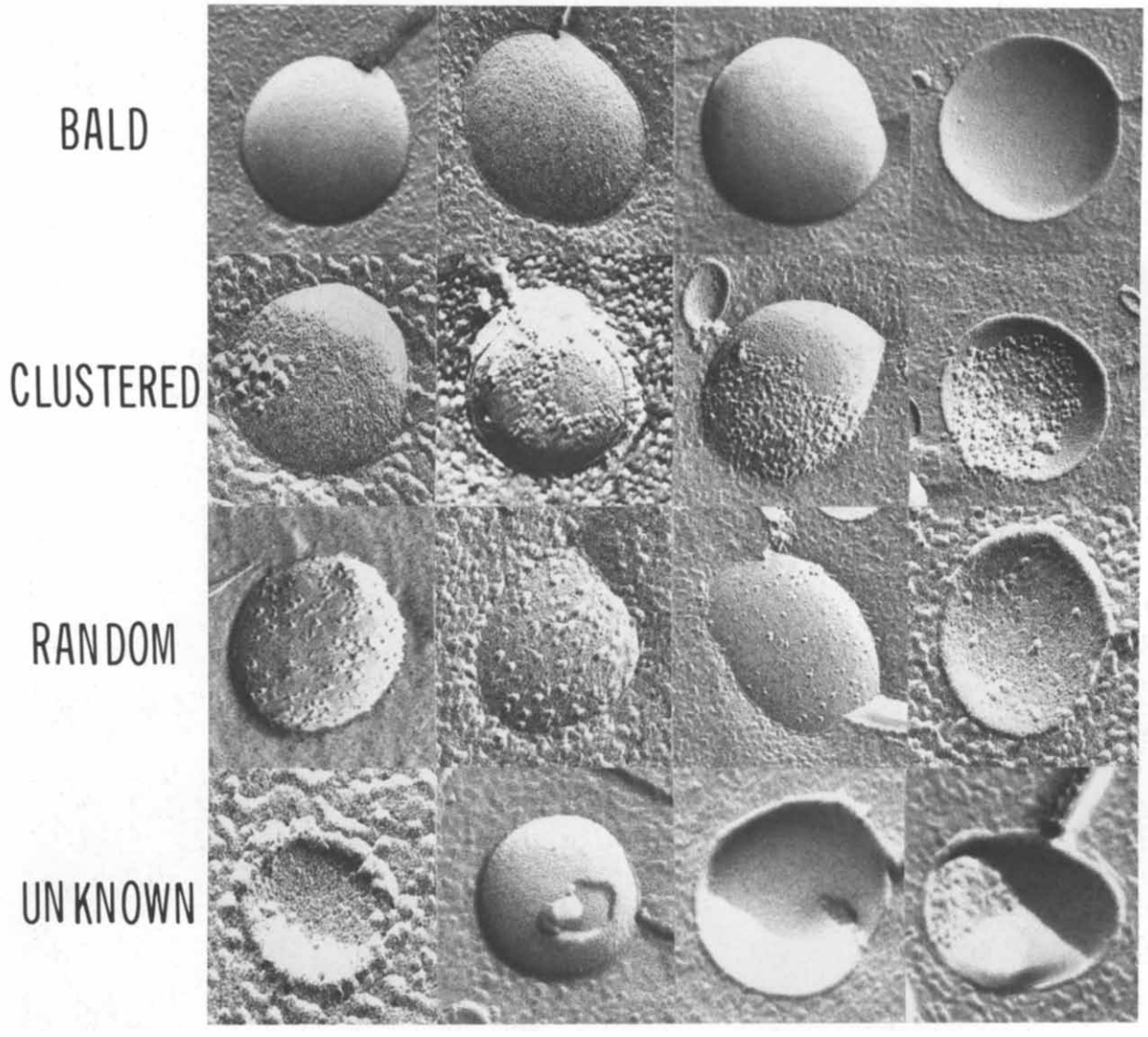

Fig. 4. Representative examples of microvillus membrane vesicles from the four intramembranous particle classifications. Magnification from left to right is: bald: 65000, 95300, 80500, 66300; clustered: 120000, 80000, 77700, 76200; random: 64800, 120000, 60000, 111400; unknown: 184000, 102100, 93900, 92400. Platinum shadowing was from the lower left.

areas of the P and E faces appeared 'naked' and intramembranous particles clustered on the microvilli (Fig. 3B and C). The microvilli on the isolated brush borders appeared to be in the process of vesiculating as indicated by their swollen, ballooned appearance and shortened length (Fig. 3B and C). Clustering of intramembranous particles on microvillus membrane vesicles was also common and a classification scheme describing vesicle type based upon intramembranous particle distribution was made. Vesicles were classified as follows: (1) bald (very few or no intramembranous particles), (2) clustered, (3) random or (4) unknown (Fig. 4).

Fig. 3. (A) Microvilli from an intact enterocyte showing randomly distributed intramembranous particles on both E and P faces. (B and C) Isolated brush borders demonstrating aggregated intramembranous particles (arrows) and swollen microvilli.

TABLE II

CLASSIFICATION OF MICROVILLUS MEMBRANE VESICLES ACCORDING TO THEIR PREPARATION HISTORY AND THEIR INTRAMEMBRANOUS PARTICLE (IMP) DISTRIBUTION

Preparation history:	Prefozen[a] tissue Prefrozen[a] vesicles				Prefrozen tissue Nonfrozen vesicles				Fresh tissue Fresh vesicles				Fresh tissue Dialyzed prefrozen vesicles			
													− EDTA		+ 10 mM EDTA	
Incubation temp. prior to jet-freezing:	4°C		40°C		4°C		40°C		4°C		40°C		40°C		40°C	
Total counted	283		266		266		264		267		263		261		263	
P faces (%)	60.8		60.9		56.2		58.3		63.7		65.0		55.2		62.2	
E faces (%)	39.2		39.1		43.8		41.7		36.3		35.0		44.8		37.8	
Mean diameter (nm)	166.4 ± 99.2		177.0 ± 144.7		143.5 ± 91.9		181.5 ± 71.2		168.2 ± 104.4		136.3 ± 80.4		133.1 ± 47.7		134.2 ± 50.31	
IMP class totals (%)																
1 bald	50.6		51.0		45.0		60.2		36.7		52.3		34.9		30.9	
2 clustered	30.0		22.0		37.8		28.4		38.6		30.0		28.4		28.6	
3 random	8.1		15.2		9.0		7.2		9.0		6.4		21.4		25.1	
4 unknown	11.3		11.8		8.2		4.2		15.7		11.3		15.3		15.4	
Fracture face: IMP class per face (%)	P	E	P	E	P	E	P	E	P	E	P	E	P	E	P	E
1 bald	52.9	46.8	61.1	33.6	44.7	45.3	66.2	51.8	42.4	26.8	51.3	29.0	43.1	24.8	33.5	26.5
2 clustered	29.1	31.5	20.3	26.9	34.0	42.8	22.7	36.3	32.9	48.5	23.4	25.2	21.5	36.8	32.3	22.4
3 random	5.8	11.7	9.3	23.1	13.3	3.4	8.4	5.5	8.8	9.3	16.4	29.0	21.5	21.4	21.7	30.5
4 unknown	12.2	9.9	9.3	16.4	8.0	8.5	2.6	6.4	15.9	15.4	8.9	16.8	13.9	17.0	12.5	20.4

[a] −70°C.

Table II shows the effects of temperature and preparation history on characteristics of Sepharose-purified microvillus membrane vesicles. An average of 60.3 ± 3.5% of the vesicles exhibited the P face when fractured regardless of preparation method. For comparison, 279 intact microvilli from 20 intact pig enterocytes exhibited the P face when fractured 67.5% of the time. Among nondialyzed vesicles, the mean vesicle diameter varied considerably (162.2 ± 98.6 nm) and no dependence on either temperature or method of preparation was noted. In contrast, the mean size of dialyzed vesicles was smaller and exhibited greater homogeneity (133.7 ± 49.0 nm) than nondialyzed vesicles. The percentage of Sepharose-purified vesicles that exhibited random distribution of intramembranous particles ranged from 6 to 15% among nondialyzed preparations (Table II). Vesicles that had not passed through the Sepharose column also appeared to have clustered intramembranous particles. The most common vesicle in all preparations (30–60%) appeared to be free of intramembranous particles (bald). The second most common one exhibited clustered intramembranous particles (22–39%). Freezing of tissue or vesicles at −70°C and preincubation of vesicles at 4 or 40°C had no significant effect on intramembranous particle distributions. Dialysis to remove traces of magnesium, however, appeared to slightly increase the number of vesicles with randomly distributed intramembranous particles from 6–15% to 21–25% of the total. Rarely, two microvillus membrane vesicles were observed in what appeared to be fusion, and at their junction intramembranous particles were clustered. Both E (outer monolayer) and P (inner monolayer) faces exhibited intramembranous particle clustering with no obvious dominance of clustering in either fracture face (Table II).

Discussion

This study suggests that many intramembranous particles in pig jejunal microvillus membrane cluster during cell disruption and membrane vesiculation. Freshly isolated and prefrozen vesicles, vesicles heated above their phase transition temperature (approx. 35°C) [43], and vesicles isolated from prefrozen tissue all exhibited similar degrees of intramembranous particle clustering. Evidence that intramembranous particle clustering occurred before vesiculation is provided by micrographs of isolated brush borders that show large patches of intramembranous particles on swollen microvilli about to vesiculate (Fig. 3B and C). Prevesiculation clustering on microvilli should produce a heterogeneous population of vesicles in which some are almost completely devoid of intramembranous particles and others are heavily adorned.

From this study it was not possible to determine if the bald vesicles contained an intramembranous particle cluster on the missing portion of the vesicle or if some of them were, in fact, completely devoid of intramembranous particles. Steiger and Murer [32], however, also used the magnesium precipitation technique and found by electrophoresis two distinct populations of rat jejunal microvillus membrane vesicles – one depleted and one enriched with protein and enzyme activity (maltase, aminopeptidase N and alkaline phosphatase). Their results are thus in accord with ours and support the idea that an individual microvilli may vesiculate into intramembranous particle-depleted and intramembranous particle-enriched vesicles.

The mechanism of intramembranous particle clustering in the disrupted microvillus membrane is unknown. Tilney and Mooseker [44] showed that in the presence of 15 mM $MgCl_2$, intramembranous particles clustered into bands on microvilli of isolated chicken brush borders. The bands appeared in a regular array (approx. 850 Å between bands) that did not correlate with the periodicity (approx. 330 Å) of the cytoskeletal cross-bridges. They suggested that the intramembranous particle clustering might be caused by magnesium and that the banding was induced by a "damming effect brought about by structural components within the membrane that are not resolved by the freeze-fracture technique" [44]. The microvillus membrane shown by Tilney and Mooseker appeared to be uniformly attached to the cytoskeleton of the isolated brush border as it is in intact cells. Our brush borders, however, isolated by the same technique possessed swollen 'ballooned' microvilli whose cytoskeletal attachments appear to have been disrupted. Although we

did not see banding, we still observed clustering in the presence of magnesium. This suggests that banding may be related to the presence of an intact cytoskeleton.

At least three major events happen to the microvillus membrane during its isolation. It is chilled, exposed to high concentrations of cations, and it's cytoskeletal association is disrupted. Each of these three factors can lead to intramembranous particle clustering in the red cell membrane [45–47]. The presence of added calcium, high ionic strength, low pH or low temperature can cause intramembranous particle clustering in red cell membrane ghosts [46]. Both acridine orange (a membrane-penetrating cationic dye) and concentrations of Triton X-100 that do not lyse the cell can also cause particle aggregation in intact red cell membranes [46,48]. In all of these cases with the red cell membrane, particle aggregation is thought ultimately to arise from lateral separation of lipid components in the inner monolayer of the membrane (i.e., low temperatures cause phase separation of liquid and solid lipid domains, which segregate proteins into domains; Triton X-100 preferentially interacts with certain membrane lipids causing phase separations; calcium causes phase separation of phosphatidylserine, etc.). Presumably, in the native membrane, the cytoskeletal protein, spectrin, prevents lipid phase separations in the inner monolayer [46]. Since the red blood cell membrane is unique, comparisons between it and the brush-border membrane must be made with caution.

In this study, two treatments (warming and dialysis) were used in an attempt to reverse intramembranous particle aggregation on the isolated microvillus membrane vesicles. Although jet-freezing of vesicles heated at 40°C for 30 min should have melted the bilayer frozen during isolation at 4°C, no intramembranous particle reversal occurred with this treatment. Exhaustive dialysis of vesicles in the absence of divalent cations, however, appeared to partially reverse intramembranous particle clustering (Table II). Since some vesicles contained high concentrations of intramembranous particles before dialysis, it is arguable that even if clustering were reversed by dialysis, local crowding could still make intramembranous particles appear to be aggregated and those vesicles without intramembranous particles would not be effected by dialysis. Alternatively, aggregation of disrupted cytoskeletal proteins inside the vesicle may direct clustering of intramembranous particles in a nonreversible fashion or the dialysis treatment may not have been sufficient to disrupt divalent cation-lipid-protein aggregates. Whatever the cause, microvillus membrane vesicles with aggregated membrane particles are less than ideal for transport studies. Gains and Hauser [49] have recently shown that only one in four to six of these vesicles is sealed when isolated by the magnesium precipitation technique. Perhaps a microvillus membrane vesicle with clustered intramembranous particles is also a leaky vesicle.

Acknowledgments

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