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SOLUBILIZATION OF BRUSH BORDERS OF HAMSTER SMALL INTESTINE AND FRACTIONATION OF SOME OF THE COMPONENTS

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SUMMARY

About 90 % of the protein of hamster intestinal brush borders was solubilised in 0.25 % (w/v) sodium dodecyl sulphate without total loss of biological activity. Detergent-polyacrylamide gel electrophoresis of the solubilised protein separated 10–15 bands and partially resolved maltase, lactase, sucrase-maltase, trehalase and alkaline phosphatase activities. The disaccharidases, which were associated with the higher molecular weight proteins, were preferentially solubilised with 0.1 %. (w/v) Triton X-100, butanol or papain, whereas Tris and NaI extracted only the lower molecular weight proteins, possible derived from the core filaments.

Electrophoresis of brush border proteins metabolically labelled with [^{14}C] glucosamine suggested that many of the membrane-bound enzymes are glycoproteins. However, chromatography of a papain digest on Sephadex G-200 showed that the sucrase-maltase complex can be separated nearly free of carbohydrate without total loss of activity.

The importance of characterizing membrane proteins solubilised by a number of techniques is discussed.

INTRODUCTION

Many of the digestive hydrolytic enzymes have been shown to be integral parts of the brush border membrane [1, 2] and a number of transport systems for the end products of digestion have also been localised at this level [3–5]. Binding proteins for glucose [6, 7], calcium [8], iron [9] and vitamin B-12 [10] are also associated with the membrane, which is therefore thought of as a digestive absorptive surface [3, 11]. The membrane is covered on its luminal side by a prominent filamentous coat or glycocalyx, which has been shown by autoradiography to be synthesized by the epithelial cell and is strongly labelled by radioactive carbohydrate

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precursors such as glucosamine [12]. By negative staining, the surface of the membrane is covered with 60-Å particles [13] but it is not clear whether they are a separate structure from the filamentous coat. The large number of activities associated with the brush border, the prominent glycoprotein coat and the ease with which the membrane can be isolated [14] makes it ideal for study of membrane organization.

Some information about the organization of the membrane has already been obtained by studying the differential release of enzymes by incubation with papain [13, 15, 16]. In the present paper we report on methods for solubilization and fractionation of proteins of the brush border. A preliminary account of this work has been published elsewhere [17].

METHODS

Isolation and solubilization of brush border fractions. Brush borders and purified membrane fractions were isolated from the mucosa of the small intestine of 6- to 8-week-old male hamsters [15, 18].

Solubilization with detergents. Brush borders were resuspended in a 0.1–1 % (w/v) solution of the detergent in 5 mM EDTA, pH 7.0, to a final protein concentration of 2–5 mg/ml. The suspension was kept at 0 °C for 45 min except in the case of solubilization with sodium dodecyl sulphate which was performed at 20 °C. The extent of solubilization was followed by determining the distribution of protein between sediment and supernatant after centrifugation at 150 000 × *g* for 1 h.

Tris and NaI Extraction. Brush borders (3 mg protein/ml) were extracted with 1 M Tris, pH 7.0, at 0 °C for 3–12 h [14], then centrifuged at 20 000 × *g* for 1 h. The particulate material was washed with 5 mM EDTA, pH 7.2, and the combined supernatants dialysed against 0.05 M phosphate buffer, pH 7.2, for 24 h. Brush borders were also extracted with 6 M NaI/0.1 M Tris/0.01 M EDTA, pH 7.5, for 30 min at 0 °C, according to the method used by Fitzpatrick et al. [19] to solubilize kidney plasma membrane proteins.

Butanol extraction. The procedure for butanol extractions was based on that developed by Maddy [20] for red blood cells. Brush borders in 5 mM EDTA, pH 7.4, were mixed with 0.75 vols of *n*-butanol at 0 °C for approx. 30 min. The suspension was centrifuged at 3000 × *g* for 5 min, resulting in a butanol upper phase, a clear aqueous lower phase, and the accumulation of a large amount of protein at the interface. The interfacial and water-soluble proteins were collected for analysis of protein and enzymatic activities.

Polyacrylamide gel electrophoresis. Brush border proteins solubilized (5 mg/ml) in 0.25 % sodium dodecyl sulphate at room temperature were electrophoresed in 7.5 % polyacrylamide gels [21] using an upper buffer of 0.045 M Tris/glycine, pH 8.8, and a lower buffer of 0.12 M Tris, pH 8.0. The gel and upper buffer contained 0.25 % sodium dodecyl sulphate. Gels were fixed in 10 % trichloroacetic acid and stained with 0.05 % Coomassie Blue in 7 % acetic acid.

Enzyme analysis on gels. Unfixed gels were cut longitudinally and each half sliced into about 30 equal segments. The slices were incubated at 4 °C for 3 h with 0.1 ml of a buffer appropriate for subsequent assay of the enzyme under investigation. As the electrophoresis system contained Tris, a competitive inhibitor of disaccharidases, a 5-fold excess of substrate was used for the subsequent assay of these enzymes.

In all experiments, one half of the gel was used to assay maltase and all other activities were compared to the distribution of this enzyme.

Assay methods. Disaccharidase activity was estimated by the method of Dahlqvist [22], alkaline phosphatase activity was measured by the rate of splitting of *p*-nitrophenyl phosphate at pH 10.5 [14] and leucyl naphthylamidase by the method of Goldbarg and Rutenberg [23]. Phospholipid was estimated by the method of Chen et al. [24] and cholesterol by the method of Zlatkis et al. [25].

Incorporation of [^{14}C]glucosamine into intestinal mucosal fractions. D-[U-^{14}C] glucosamine · HCl at 275 Ci/mol and D-[1-^{14}C] glucosamine · HCl, 45 Ci/mol were purchased from New England Nuclear Corp. From 5–40 μCi of [^{14}C]glucosamine were injected intraperitoneally into fed hamsters, the animals sacrificed 3–4 h later [26] and brush borders isolated along with the residual particulate and soluble protein fractions. In order to characterize the incorporated radioactivity, residues after trichloroacetic acid and chloroform/methanol extraction were incubated with 1.25 ml of 2 M HCl for 6–10 h at 100 °C. Acid-insoluble material was removed by centrifugation and free hexosamines and neutral sugars separated by the method of Boas [27].

RESULTS

Solubilization of brush borders in sodium dodecyl sulphate

A concentration of 0.25 % (w/v) sodium dodecyl sulphate reduced the turbidity (A_{530nm}) of a suspension of brush borders to 10 % of the original value, almost immediately after its addition. Above 0.25 % there was little change in absorbance, and this concentration of detergent was therefore chosen for further experiments. Centrifugation of a brush border fraction in 0.25 % sodium dodecyl sulphate at 150 000 $\times g_{av}$ for 1 h precipitated only 9 % of the total protein, approx. 90 % remaining in the supernatant although the amounts varied to some extent (85–95 %) possibly depending on the purity of the brush border preparation.

TABLE I

ULTRACENTRIFUGATION OF BRUSH BORDERS SOLUBILIZED IN 0.25 % SODIUM DODECYL SULPHATE

Brush borders were solubilized in 0.25 % (w/v) sodium dodecyl sulphate, centrifuged at 150 000 $\times g$ for 4 h and the distribution of protein and enzyme activities in the precipitate and supernatant determined. Specific activities are expressed as μmol/min per mg protein. Percent distribution of protein and enzyme activities in the fractions are expressed as a percentage of total recovery. The data presented are from a single experiment, although essentially similar results were obtained on at least four other occasions. Variability is recorded in the text.

Subcellular fraction	Protein (%)	Alkaline phosphatase		Sucrase		Trehalase	
		Spec. act.	Recovery (%)	Spec. act.	Recovery (%)	Spec. act.	Recovery (%)
Brush borders	100	1.72	100	1.4	100	0.34	100
Precipitate	24	1.53	86	3.6	99	0.33	56
Supernatant	76	0.08	14	1.6	1	0.08	44
Total recovery	111		28		70		47

Centrifugation for extended periods of time produced a larger sediment and after 4 h from 20 to 35 % of the protein was precipitated (Table I). Surprisingly, many of the enzyme activities survived these fairly low concentrations of detergent although leucyl naphthylamidase was totally inactivated. About 50–70 % of the disaccharidase activities was recovered, sucrase being more susceptible to inactivation than maltase and lactase. Alkaline phosphatase was apparently more sensitive to the detergent and only 20–30 % of the original activity remained. Inactivation depended on the concentration of detergent used, time and temperature. Variable amounts of disaccharidase and alkaline phosphatase activities were still detectable even when brush borders were solubilized in 2 % sodium dodecyl sulphate/0.1 M dithiothreitol and heated at 37 °C for 30 min, a standard procedure for dissociating proteins into their subunits. Interestingly, of the enzyme activities remaining after solubilization in 0.25 % sodium dodecyl sulphate, nearly all the sucrase, maltase, lactase and alkaline phosphatase (85–100 %) were recovered in the pellet after centrifugation at 150 00 × *g* for 4 h (Table I). Trehalase, however, distributed about evenly between pellet and supernatant.

Gel electrophoresis of proteins solubilized in sodium dodecyl sulphate

As brush borders were efficiently solubilized in sodium dodecyl sulphate, we attempted to separate the proteins in polyacrylamide gels and subsequently localize some of the enzyme activities. At least ten protein bands were resolved ater eletro-

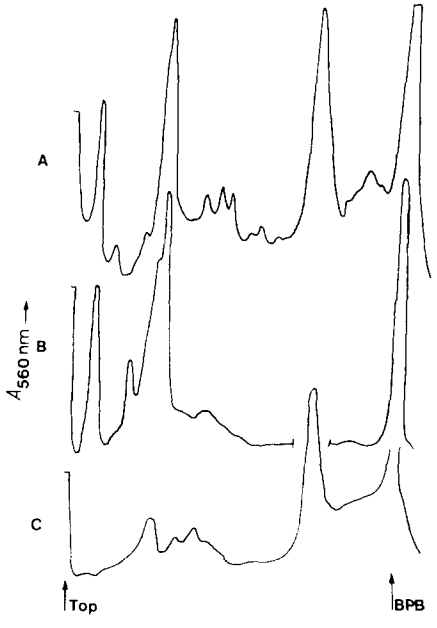

Fig. 1. Sodium dodecyl sulphate/polyacrylamide gels of (a) intact brush borders, (b) the pellet produced by centrifuging sodium dodecyl sulphate-solubilized brush borders at 150 000 · *g* for 4 h and (c) the supernatant from (b). Gels were stained with Coomassie Blue and scanned at 560 nm with a Hilger-Gilford spectrophotometer. BPB represents the position of the bromophenol blue marker dye.

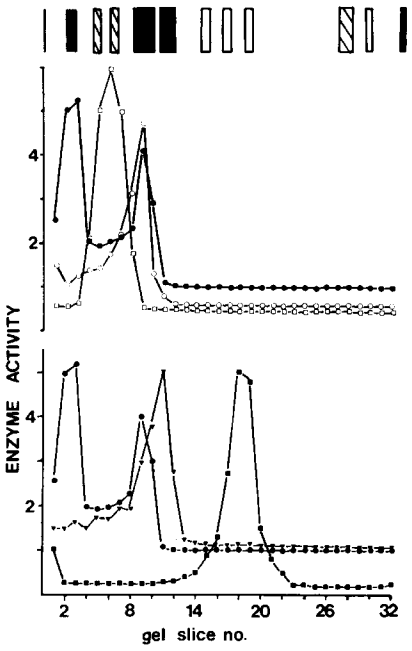

Fig. 2. Distribution of brush border enzyme activities on sodium dodecyl sulphate/polyacrylamide gels. ● — ●, maltase; ○ — ○, sucrase; □ — □, lactase; ▼ — ▼, alkaline phosphatase; ■ — ■, trehalase. Enzyme activities are in arbitrary units. The distribution of brush border protein bands is shown at the top of the figure.

phoresis of a whole brush border preparation and little material failed to penetrate the gel (Fig. 1a). The major bands stained by Coomassie Blue were a low molecular weight component (mol. wt approx. 45 000), running just behind the bromophenol blue, and a broad band of high molecular weight (mol. wt approx 150 000), which frequently separated as a doublet. Many less intensely stained bands were consistently seen between these two components along with two or three higher molecular weight bands, one of which only just penetrated the gel. The reproducibility of the gel profile suggested that proteolysis of solubilized proteins was not a major problem.

Analysis for maltase in the gel slices showed that there were two peaks of activity in the high molecular weight region of the gel. Sucrase activity was coincident with one of the peaks (Fig. 2). There was a distinct separation of lactase and trehalase activities from the two maltase peaks, although there was some overlap between alkaline phosphatase and the maltase-sucrase peak. Comparison of the enzyme distribution with gels simultaneously run, fixed and stained have enabled identification of certain protein bands with enzyme activities. The position of the alkaline phosphatase band was confirmed by a histochemical lead conversion technique [28].

Electrophoresis of the fractions obtained by centrifugation of solubilized brush borders at 150 000 $\times g$ for 4 h showed two distinct protein profiles (Figs 1b and 1c). As expected from quantitative enzyme analysis (Table I), the sediment contained those proteins migrating only one-third of the way into the gel and shown to contain disaccharidase and alkaline phosphatase activities. The supernatant

contained those proteins, of lower molecular weight, which penetrate over half way into the gel, although there was some overlap between the fractions, possibly in the region of trehalase activity.

Solubilization of brush border proteins with other detergents

None of the other detergents tested was as effective as sodium dodecyl sulphate in solubilizing brush border proteins even when used at elevated concentrations, i.e. 1 % (w/v)(Table II). In contrast to sodium dodecyl sulphate they did allow better than 70 % recovery of all enzymes assayed.

Whereas sodium deoxycholate solubilized sucrase, alkaline phosphatase and total protein to about the same extent, Triton X-100 solubilized 86 % of the sucrase activity but only 47 % of the protein. Lubrol WX and Tween 20 were slightly less effective in this respect. Examination of the effect of lower concentrations of Triton X-100 showed that 0.01 % solubilized little material but 0.1 % released 85–95 % of all the disaccharidase activities over 60 % of the leucyl naphthylamidase activity and 45–55 % of the protein. Most of the alkaline phosphatase (> 60 %) remained with the sediment until the concentration of the detergent was increased to 1.0 %.

Centrifugation of material extracted with 0.1 % (w/v) Triton X-100 at 150 000 × *g* for 1 h produced little or no sediment and even after 4 h, only 10–20 % of the protein was precipitated. Dialysis of the preparation against 0.05 M Tris/0.01 M $MgCl_2$, pH 7.5, for 24 h caused an increase in turbidity and ultracentrifugation sedimented 60–70 % of the protein along with 60–80 % of the sucrase activity.

Electrophoresis of material solubilized by Triton X-100 showed, as expected from direct analysis, that the detergent preferentially extracted those proteins of higher molecular weight associated with enzyme activities (Figs 3a and 3b). Only trace amounts of the smaller molecular weight proteins were solubilized. These proteins were found to be concentrated in the insoluble residue. A small band in

TABLE II

EXTRACTION OF BRUSH BORDERS WITH DETERGENTS, BUTANOL AND TRIS

Brush borders were incubated with 1 % (w/v) solutions of the detergents in 5 mM EDTA, pH 7, and a residue and supernatant obtained by centrifugation at 150 000 × *g* for 1 h. Extraction procedures with butanol and Tris are described in Methods. The distribution of protein and enzyme activities is expressed as a percentage of total recovery. The results presented are from single experiments, although the essential observations were confirmed in at least four other separate experiments. The variability of results is recorded in the text.

Extraction procedure	Protein			Sucrase			Alkaline phosphatase		
	Residue	Supernatant	Recovery (%)	Residue	Supernatant	Recovery (%)	Residue	Supernatant	Recovery (%)
Deoxycholate	42	58	(87)	43	57	(95)	47	53	(98)
Triton X-100	53	47	(104)	14	86	(103)	51	49	(98)
Lubrol WX	54	46	(108)	32	68	(78)			
Tris	63	37	(98)	100	0	(77)	100	0	(98)
Butanol	91	9	(77)	57	43	(72)	85	15	(83)

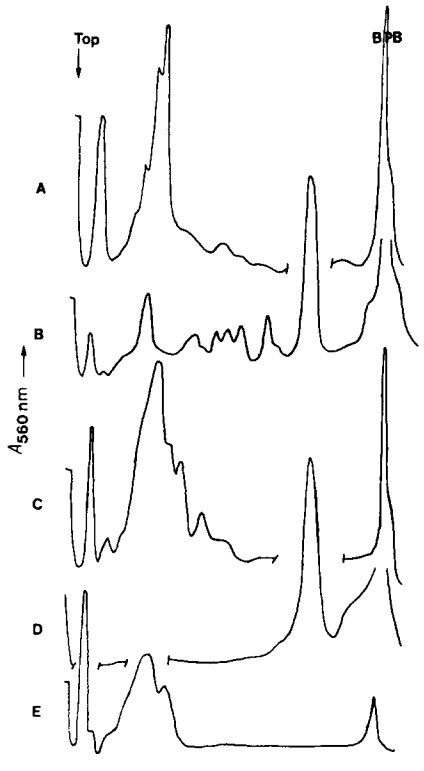

Fig. 3. Sodium dodecyl sulphate/polyacrylamide gels of brush border proteins. a, solubilized by 0.1 % (w/v) Triton X-100; b, the insoluble residue after Triton X-100 extraction; c, the particulate fraction after disruption of brush borders with 1 M Tris, pH 7.0; d, the proteins solubilized by 1 M Tris, pH 7.0; e, the proteins solubilized by butanol extraction.

the higher molecular weight region of the gel may represent that alkaline phosphatase which did not enter the Triton phase.

No detailed study of the effect of Triton X-100 on lipid composition was made, but a comparison of the phospholipid:cholesterol molar ratios of intact brush border (0.82) with those of the residue (0.57) and soluble fraction (2.4) showed that cholesterol was poorly solubilized by this detergent.

Examination of the insoluble residue in the electron microscope showed it to contain much amorphous material, possible derived from the core filaments and terminal web (Fig. 4B). Open-ended trilaminar structures lacking a distinct glycocalyx were associated with this material and may represent the partially solubilized membrane.

Extraction with Tris and NaI

Brush borders were rapidly disrupted on exposure to 1 M Tris, pH 7.0, or NaI and a particulate fraction containing all of the measured enzyme activities of the brush border was sedimented at 20 000 $\times g$ for 45 min (Table II). The mechanism of disruption is unknown, but 35–50 % of the brush border protein remained in the clear supernatant after sedimenting the particulate fraction. Electron microscopy

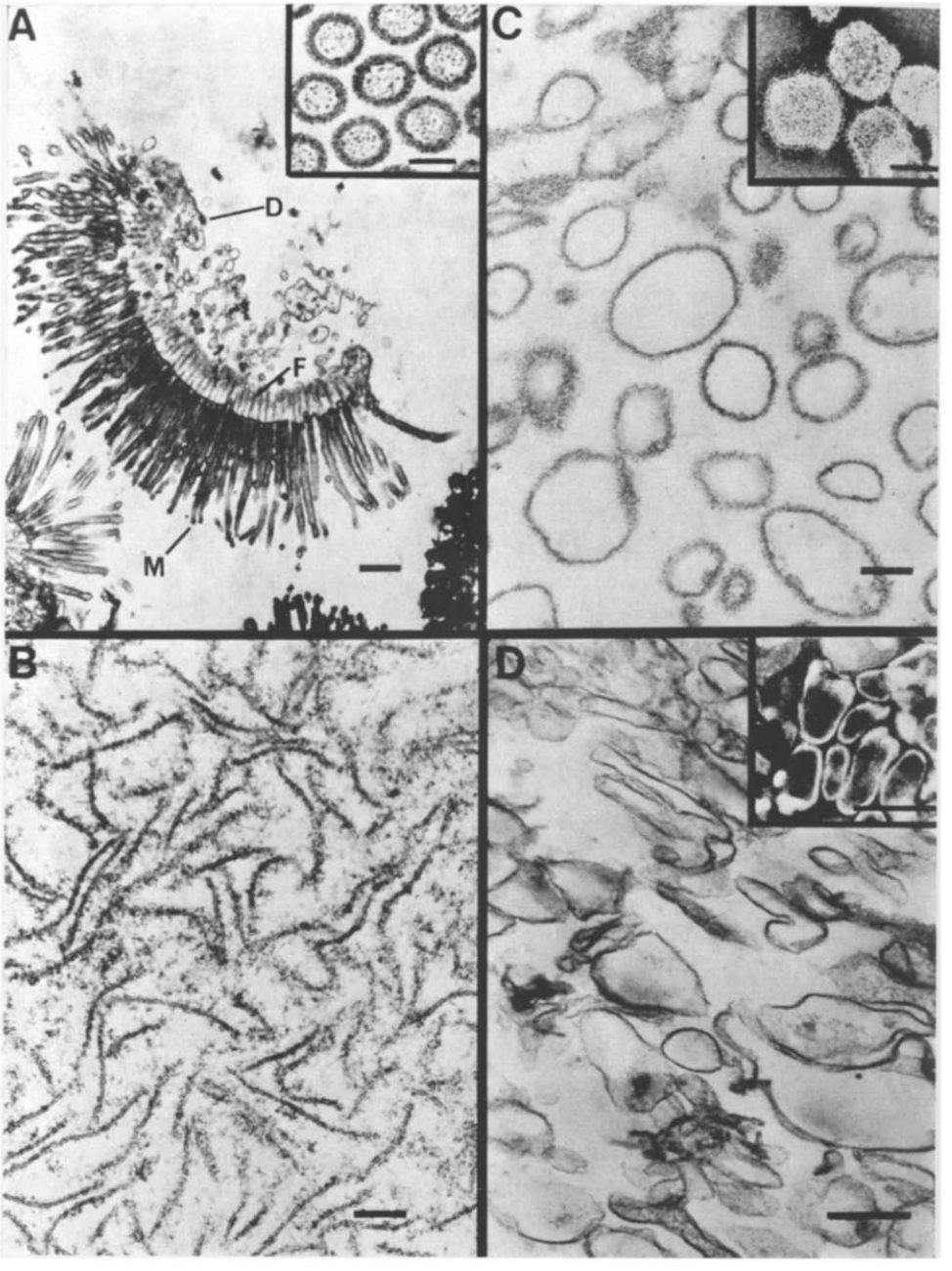

Fig. 4. Electron micrographs of brush border fractions. (A) An isolated brush border fraction showing microvilli (M), core filaments (F) and apical desmosomes (D) (magnification bar 1 μm). Insert, a cross-section through the microvilli showing core filaments, trilaminar membrane and prominent surface glycocalyx (magnification bar 0.1 μm). (B) Residue after extraction of brush borders with 0.1 % (w/v) Triton X-100 (magnification bar 0.1 μm). (C) Particulate fraction produced by disruption of brush borders with 1 M Tris, pH 7.0 (magnification bar 0.1 μm). Insert, negative staining of the same fraction (magnification bar 0.1 μm). (D) Particulate fraction remaining after digestion

showed the particulate fraction to consist mainly of empty membrane vesicles with a distinct glycocalyx on the outside, suggesting that only right-side-out vesicles are formed (Fig. 4C). The width of the glycocalyx was considerably reduced when compared with intact brush borders (see insert Fig. 4a) suggesting loss of material during membrane isolation. By negative staining, surface particles were clearly visible.

Electrophoresis showed the particulate fraction to contain all the high molecular weight components associated with the membrane enzyme activities, but to be completely lacking the lower molecular weight proteins (Fig. 3c). In contrast, the Tris extract contained a number of proteins running in the region of the major low molecular weight protein of whole brush borders (Fig. 3d). There was a complete absence of proteins in the high molecular weight region of the gel.

Extraction with butanol

In contrast to studies on the red cell membrane [20], only 9 % of brush border protein was present in the aqueous phase after butanol extraction, the majority precipitating at the butanol-water interface. However, 43 % of the sucrase activity was recovered in the aqueous fraction at a specific activity 7.5-fold greater than in the brush border (Table II). Only 15 % of the alkaline phosphatase was extracted at a specific activity 1.8-fold that in the initial brush border preparation.

Electrophoresis of the water-soluble material showed four protein bands travelling in the high molecular weight region of the gel (Fig. 3e). A broad band with a shoulder on the frontal side probably represents the partial separation of the sucrase-maltase peak from the little alkaline phosphatase activity contained in the extract. The two bands nearer the origin are in the region of isolated maltase and lactase activities. Only a single protein of smaller molecular weight was detected, even under heavy loading of the gel.

In vivo labelling of membrane glycoproteins

4 h after intraperitoneal injection of [^{14}C]glucosamine, the specific activity of the brush border fraction was about five times higher than the homogenate, residual particulate or soluble protein fractions. About 20 % of the label was extracted by lipid solvents and presumably represents incorporation into glycolipid. At least 80 % of the radioactivity incorporated into protein was as hexosamine and none was associated with neutral sugars or with the acid-insoluble protein.

Brush borders labelled with [^{14}C]glucosamine were solubilized in sodium dodecyl sulphate and the proteins separated on polyacrylamide gels (Fig. 5). A peak of radioactivity equivalent to 34 % of the counts recovered was found in gel slices 7–10, corresponding with protein bands known to contain alkaline phosphatase, sucrase and maltase activites. The peak at slices 3–6 was in the region of lactase and represented 17 % of the counts, and about 15 % of the counts at or near the origin in the region of isolated maltase activity. Approx. 33 % of the counts was in gel slices 11–19, which corresponds with many small bands, one of which

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of brush borders with soluble papain (magnification bar 0.1 μm). Insert, negative staining of the same fraction (magnification bar 0.1 μm). Thin sections were stained with uranyl acetate and lead citrate [63]. Samples were negatively stained by suspension in 2 % phosphotungstic acid, pH 7.

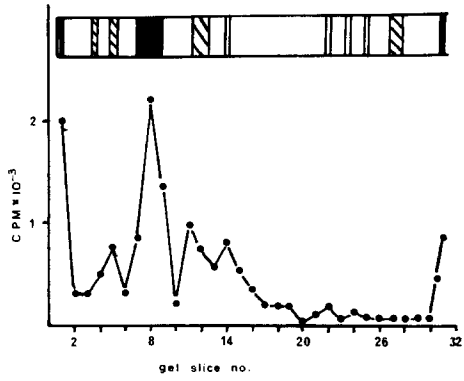

Fig. 5. Sodium dodecyl sulphate/polyacrylamide gels of brush border proteins metabolically labelled with [^{14}C]glucosamine. The distribution of radioactivity is related to gels simultaneously run and stained for protein.

may be trehalase, and 6.4 % of the counts ran with the bromophenol blue marker dye.

Brush borders labelled with [^{14}C]glucosamine and solubilized in sodium dodecyl sulphate were also centrifuged at 150 000 × *g* for 4 h. Approx. 35% of the protein precipitated along with 97 % of the sucrase, 89 % of the alkaline phosphatase and 59 % of the radioactivity. Gel slices 1–10 represent those proteins precipitated by ultracentrifugation. Addition of the radioactivity in this region of the gel accounts for 65 % of the label recovered, in good agreement with the 59 % obtained by ultracentrigugation.

Papain treatment of [^{14}C]glucosamine-labelled brush borders

Treatment of [^{14}C]glucosamine-labelled brush borders with soluble papain for 10 min released all of the sucrase activity from the particulate fraction (125 % recovery). Although alkaline phosphatase was grossly inactivated (16 % recovery), that which remained after 10 min was mainly associated with the particulate material (66 %), in agreement with previous work from this laboratory [15]. About 50 % of the radioactivity was released during a 5–10-min incubation, and the figure only increased to 60 % after 35–45 min incubation.

Brush borders were rapidly disrupted by soluble papain, leaving membrane vesicles lacking a glycocalyx, and the surface particles seen by negative staining (Fig. 4d).

Chromatography of a [^{14}C]glucosamine-labelled papain supernatant on Sephadex G-200 resolved two maltase peaks, one of which was coincidental with sucrase activity (Fig. 6). Approx. 50 % of the radioactivity recovered appeared in a peak after the void volume. It was slightly displaced from the first maltase peak, but exactly corresponded with the leucyl naphthylamidase activity. The second radioactive peak was distinctly separated from the sucrase-maltase peak, although there was some considerable overlap. The data indicate that much of the radioactivity which has closely paralleled sucrase activity can be separated from it under certain conditions.

Similar results were obtained by quantitative analysis for carbohydrate using

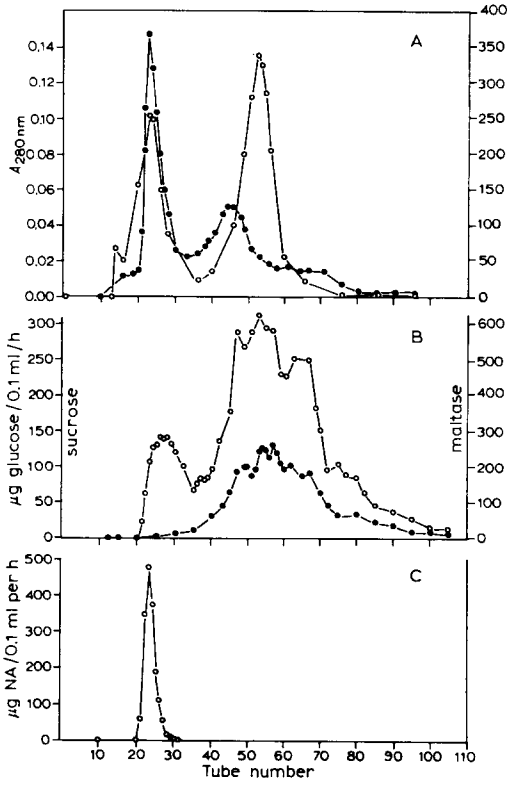

Fig. 6. Sephadex G-200 column chromatography of proteins solubilized by digestion of [^{14}C]-glucosamine-labelled brush borders with complex papain [15]. (A) ●—●, radioactivity; ○—○, $A_{280\,nm}$. (B) ○—○, maltase; ●—●, sucrase; (C) ○—○, leucyl naphthylamidase. Columns (3 cm internal diameter × 45 cm) were packed and eluted with 0.15 M NaCl, 0.001 M imidazole buffer, pH 7.0, at a flow rate of 16 ml/h.

the gas chromatographic method of Lenhardt and Winzler [29]. By comparing the data obtained from whole brush borders with those of the membrane prior to digestion, it is apparent that, in terms of carbohydrate content per dry weight, there is an enrichment during the preparation of the microvillus membranes (Table III). This is consistent with the notion of an intimate association of the carbohydrates with the membrane. Upon treatment with papain, which releases all of the disaccharidase activity, less than 50 % of the carbohydrate is released from the membrane. The sucrase-maltase fraction purified from the papain supernatant on Sephadex G-200 columns (Fig. 6) contained little carbohydrate.

TABLE III

DISTRIBUTION OF CARBOHYDRATES AMONG SUBFRACTIONS OF THE BRUSH BORDER

Results are expressed as nmol of carbohydrate/mg of dry weight of a fraction and represent the mean value from analysis on duplicate samples. Sialic acids were not determined.

	Whole brush borders	Isolated microvillous membranes	Membranes after papain digestion	Soluble fraction after papain digestion	Dissaccharidase particles after Sephadex
Fucose	20.1	36.4	27.2	13.1	1.9
Mannose	30.4	59.4	33.0	27.1	13.2
Galactose	89.8	181.8	167.6	38.3	8.7
Glucose	35.2	57.0	59.3	65.4	502.9*
Idose	8.8	11.7	12.6		
Glucosamine	51.3	102.0	44.5	170	8.2
Galactosamine	9.7	10.0	4.4	5.7	0
Total carbohydrate as percentage of dry weight	4.9	9.2	7.0	6.4	0.62

* The high glucose in this fraction results from enzymatic breakdown of the Sephadex column.

DISCUSSION

A large number of different methods have previously been employed to solubilize membrane structures [30, 31]. Many studies have confirmed the efficiency of sodium dodecyl sulphate in solubilizing membrane proteins, but it has the disadvantage that it causes loss of biological activity. We were surprised to find that many enzyme activities of the brush border were still detectable if low concentrations of the detergent were used, although solubilization of the structure was still virtually complete. The degree of inactivation may reflect the oligomeric nature of an enzyme [32, 33] or the amount of detergent bound to the protein. However, large conformational changes in proteins have been found to occur before they are saturated with sodium dodecyl sulphate [34] and, besides, in the case of maltase and alkaline phosphatase, activities were detectable even in 2 % detergent plus a reducing agent. The reason for their resistance to inactivation remains unexplained.

Sodium deoxycholate and Triton X-100 were much less efficient in solubilizing brush border proteins but resulted in much less inactivation of enzymes, in agreement with studies in other systems [30, 31]. Whereas deoxycholate solubilized total protein, alkaline phosphatase and disaccharidase activities to about the same extent, Triton X-100 extracted disaccharidase activities more readily than alkaline phosphatase or leucyl naphthylamidase. Similar differential solubilization of membrane proteins with detergents have been reported elsewhere [35-39]. It seems likely that different detergents solubilize membrane proteins by different mechanisms [36], and Triton X-100 is thought to act by binding to hydrophobic residues in proteins [40, 41]. Helenius and Soderlund [41] found disruption of viral membranes by Triton X-100

proceeded though protein-lipid-detergent complexes finally resulting in delipidation of membrane proteins. Our failure to resolve the enzyme activities extracted from brush borders in 0.1 % (w/v) Triton X-100 on Sepharose 4B-detergent columns (unpublished) may be due to the formation of detergent-lipid complexes containing a mixture of proteins.

The ease with which disaccharidases can be solubilized with Triton X-100 suggests that they are attached to the membrane in a different way from the leucyl naphthylamidase and alkaline phosphatase activities and are presumably bound by hydrophobic interactions, possibly with lipids. The same enzymes were also readily solubilized by butanol extraction, substantiating the idea that their primary interaction is with membrane lipids. Butanol has previously been used with varying degrees of success to solubilize membrane proteins [19, 20, 42], and both butanol and Triton X-100 have been used to extract sucrase from intact intestinal mucosa [43, 44].

The exact number of proteins in the isolated brush border or in membrane fractions is not clear from this study, although many of them are of high molecular weight. In a detailed study of the proteins of human intestinal brush borders, Maestracci et al. [45] found about 23 protein bands, many of which had a molecular weight greater than 200 000 when gels were run under non-reducing conditions. A number of these high molecular proteins were converted to subunits under reducing conditions. We have found that the high molecular weight proteins are associated with the enzymatic activities of the brush border, some of which were well resolved on sodium dodecyl sulphate/polyacrylamide gels.

By direct carbohydrate analysis, we have confirmed that much of the glycoprotein of the brush border is associated with the membrane. The concentration of carbohydrate present and the individual sugar composition are similar to those described elsewhere for rat brush borders [46, 47]. It is noteworthy that the glycoproteins contain only low levels of sialic acid [46, 47], in contrast to those isolated from kidney brush borders [48].

Using [^{14}C]glucosamine-labelled brush borders, we have found that about 60 % of the carbohydrate is associated with high molecular weight proteins, as shown by ultracentrifugation of sodium dodecyl sulphate-solubilized material, gel electrophoresis and papain digestion. No label was associated with the major low molecular weight component, although 30 % of the [^{14}C]glucosamine label electrophoresed with a number of proteins in the region of trehalase activity. This suggests that many of the enzyme activities of the membrane are glycoproteins, in agreement with previous studies [49–52]. We have found, however, that the sucrase released from the membrane by a 40-min digestion with papain can be separated from the bulk of the [^{14}C]glucosamine label on Sephadex G-200. Direct carbohydrate analysis of the sucrase fraction from the Sephadex G-200 column confirmed this result. In contrast to these studies, Forstner [51] found that a [^{14}C]glucosamine label closely parallelled the similar enzyme activities of rat intestinal brush borders when a 5-min papain digest was chromatographed on Sepharose 4B and polyacrylamide gels.

The sucrase-maltase complex released by papain from rabbit intestine was found by Cogoli et al. [53] to be a glycoprotein containing 15 % carbohydrate. The

complex contained no lipid and the hydrophobicity was not considered to be greater than some non-membrane proteins. Comparison of these results raises the possibility that, during the papain digestion, we released a carbohydrate-rich fragment from the enzyme without loss of activity. In a study of the aminopeptidase from pig kidney, which is thought to be a glycoprotein [54], Wacker [55] has shown that all the sialic acid, over 60 % of neutral and 45 % of the amino-sugars can be removed by glycosidases with no effect on the catalytic properties of the protein, although its water solubility was reduced.

Our results emphasize the need to characterize the sucrase-maltase complex isolated by several different procedures. The molecular weight, carbohydrate composition and hydrophobicity of the enzyme isolated by papain digestion may be quite different from that isolated by Triton X-100 or butanol extraction. In fact, papain has already been shown to affect the sedimentation characteristics of the enzyme extracted with Triton X-100 [44] and we also noted that, whereas the enzyme isolated by papain digestion is water soluble, that isolated by Triton X-100 extraction comes out of solution in the absence of detergent. Indeed, the ease of solubilization of the enzyme by Triton X-100 or butanol suggests that it may well have a hydrophobic region, although whether it would be analogous to that in the major red cell membrane glycoproteins remains to be seen [56, 57].

It is interesting that these high molecular weight glycoproteins are those preferentially extracted by Triton X-100, leaving a residue enriched in lower molecular weight proteins. Butters and Hughes [37] recently reported that the glycoproteins of KB cell membranes were also relatively easy to extract with low concentrations of Triton X-100, whereas certain non-glycosylated proteins were extremely resistant to extraction.

Comparison of the proteins extracted by Triton X-100 and Tris or NaI with electron microscope data suggests that the core filaments and terminal web are associated with the lower molecular weight proteins of the brush border, the most predominent of which has a molecular weight of about 45 000. Tilney and Mooseker [58] demonstrated that a similar low molecular weight protein of chick intestinal brush borders was actin derived from the core filaments. The fact that trilaminar structures can be seen in the Triton X-100-insoluble residue even after repeated extractions suggests that some proteins and lipids integral to membrane structure are insoluble under these conditions. Proteins [36, 37, 59, 60] and lipids [36, 37] resistant to detergent extraction have been reported in a number of other membrane systems.

The heterogeneity in the membrane carbohydrate indicates that the fuzz associated with the luminal surface of the brush border is not composed of one predominant molecular species. However, much of the fuzz is clearly lost during isolation of the membrane as seen in the electron micrographs in this paper. Similarly, although the binding protein for calcium is present in the fuzz of the intact intestinal epithelium [61], the protein appears in the soluble fraction in a mucosal homogenate [8]. It is therefore necessary to remember the possibility that we have selectively lost a major group of surface glycoproteins during our isolation procedures. Labelling the proteins of intact epithelium using cell surface labelling techniques [62] may provide an answer to this and the question of how the enzymes are inserted in the membrane.

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