Purification and Characterization of Guinea-Pig Intestinal Brush Borders

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The purification of guinea-pig intestinal brush borders by a rapid sucrose-gradient-centrifugation step is reported. A 29-fold increase in the maltase/DNA quotient indicates considerable purification of the brush borders from nuclei. The biological activity of the brush borders was well preserved, as demonstrated by a high recovery of human gastric-juice-mediated uptake of ^{57}Co -labelled vitamin B-12; homogeneity and purity were confirmed by scanning electron microscopy. Both the morphological appearance and biological activity were unchanged after prolonged storage in glycerol.

Miller & Crane (1961*a,b*) pioneered the technique of isolating brush borders from homogenates of the epithelial cells from the mammalian intestine, a technique involving several low-speed centrifugations and washings of the sediment and giving rise to a fraction containing brush borders as well as nuclei (Porteous, 1968, 1972). The extent of contamination seems to depend on the species used (Eichholz & Crane, 1974). Porteous (1972) reviewed fifteen publications on the isolation of intestinal brush borders from various species, but in most of these studies the extent of contamination by other organelles has been difficult to assess, since the quantitative analytical results reported were insufficient. Hübscher *et al.* (1965) used a modification of the subcellular fractionation procedure described by Porteous & Clark (1965), but failed to separate guinea-pig brush borders from nuclei. Eichholz & Crane (1974) described a rather pure preparation of guinea-pig brush borders, but in much decreased yield.

We report here a method, based on sedimentation in discontinuous sucrose gradients, for the rapid isolation of guinea-pig intestinal brush borders with high yield and low contamination by other subcellular organelles. Further, we report a method for preserving isolated brush borders for a period of 1 year.

Materials and methods

^{57}Co -labelled cyanocobalamin (10–20 $\mu\text{Ci}/\mu\text{g}$) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Unlabelled cyanocobalamin was a gift from Dr. H. M. Smalley, Glaxo Laboratories, Greenford, Middx., U.K. Human gastric juice was collected, processed and stored as described previously (Schjønby & Peters, 1971). The gastric juice had a total vitamin B-12-binding capacity of 68.5 ng/ml (Gottlieb *et al.*, 1965) and enhanced the absorption of a test dose of labelled vitamin B-12 in

patients with pernicious anaemia. All chemicals were of the highest purity commercially available.

Male guinea pigs (Hartley strain; 300–400 g body wt.) were starved for 24 h. Mucosal scrapings from three guinea pigs (approx. 3 g/animal) were obtained from the distal half of the small intestine, homogenized for 25 s in 5 mM-EDTA (pH 7.4; 10 ml/g of mucosa) at 3500 rev./min in an MSE top-drive macerator. The homogenate was dispensed in 35 ml portions into 50 ml polypropylene tubes (MSE 59635 1070) and centrifuged at 5500 g-min in an MSE High-Speed 18 refrigerated centrifuge (angle rotor 43114-106; $r_{\text{min.}}$ = 5.9 cm; $r_{\text{max.}}$ = 10.7 cm). The sediment was washed once (total volume 70 ml) and resedimented. The pellet thus obtained was resuspended in 5% (w/w) sucrose in 5 mM-EDTA (pH 7.4; total volume 32 ml), and 4 ml portions were layered above 15% (w/w) sucrose (3 ml). These discontinuous gradients were centrifuged at 1900 g-min ($r_{\text{av.}}$ = 13.6 cm) in an HL-4 rotor of the Sorvall GLC-1 centrifuge. The combined interfaces were diluted to 70 ml with 5 mM-EDTA and centrifuged at 5500 g-min as described above. The final sediment, containing purified brush borders, was resuspended in 50 mM-Tris-maleate buffer, pH 7.4, to a final protein concentration of 1.0 ± 0.1 mg/ml. If not used immediately the brush borders were stored at a temperature below -16°C in 50% (v/v) glycerol. All steps were carried out at 0 – 5°C . Centrifuge tubes and pipettes made of polypropylene were used throughout the purification to improve the recovery, since initial observations showed that brush borders together with nuclear aggregates tended to stick to glassware.

DNA was determined after extraction with trichloroacetic acid (Schneider, 1957) by the diphenylamine reaction modified by Burton (1956). Protein was determined by the method of Lowry *et al.* (1951). Maltase (EC 3.2.1.20) was determined by the method of Dahlqvist (1968). Leucine aminopeptidase (EC

3.4.11.1) was determined by the method of Nagel *et al.* (1964) with leucine *p*-nitroanilide as substrate. Malate dehydrogenase (EC 1.1.1.37) was determined as described by Bergmeyer (1970).

Biological activity was assessed by the ability of the brush-border preparations to take up gastric-juice-bound ^{57}Co -labelled vitamin B-12 in preference to the unbound ^{57}Co -labelled vitamin (Donaldson *et al.*, 1967). The assay was performed essentially as described previously (Schjønby & Peters, 1971) at 25°C for 40 min, with 5 ng of ^{57}Co -labelled vitamin B-12 and 0.3 ml of human gastric juice.

Scanning-electron-microscopy studies were performed after fixation in 1% (followed by 2.5%) glutaraldehyde, post-fixation in 1% OsO_4 and critical-point drying of the specimens in CO_2 (Nemanic, 1972).

Results and discussion

To achieve a separation of the brush borders from nuclei, the crude brush-border fraction obtained by low-speed sedimentation was subjected to centrifugation on discontinuous sucrose gradients. Pelletting nuclei and nuclear aggregates through a cushion of 15% (w/w) sucrose was efficient, leaving brush borders banded in the interface. Prolonged centrifugation decreased the yield dramatically, and no further purification of brush borders from nuclei was observed. All steps were carefully examined by phase-contrast microscopy to ensure recovery of morphologically intact brush borders devoid of nuclear contamination.

As assessed by scanning electron microscopy, the brush-border preparations were homogeneous and apparently free of other subcellular organelles, and the brush-border luminal surface was covered by numerous microvilli (Plate 1). The purity was further confirmed by biochemical tests (Table 1), with a recovery of 74% (leucine aminopeptidase) and 80% (maltase) of brush-border marker enzymes (8.3–9-fold increase in sp. activity). The recovery of 2.6% of DNA (nuclei) and 1% of malate dehydrogenase (mitochondria and cell sap) may be due to non-specific redistribution during the homogenization procedure and the following centrifugations.

According to Porteous (1968), the best assessment of the purification of brush borders from nuclei is the increase of the invertase-activity/DNA quotient during the purification. From the data in Table 1, the maltase/DNA quotient can be seen to increase from 1.7 in the homogenate to 49.3 in the purified fraction, giving rise to a 29-fold purification of the brush borders from the nuclei originally present in the homogenate. Results published previously for the guinea pig by Hübscher *et al.* (1965) demonstrated a preferential purification of nuclei rather than brush borders, whereas other workers have prepared brush-border fractions containing nucleic acids (Porteous, 1972) or contaminated with other subcellular organ-

Table 1. Purification and storage of brush borders isolated from guinea-pig small intestine

	Protein (mg/fraction)	DNA (mg/fraction)	Malate dehydrogenase (units/g of protein)	Leucine aminopeptidase (units/g of protein)	Maltase (units/g of protein)	Maltase/DNA (units/mg)	Uptake of vitamin B-12 (pg of ^{57}Co -labelled vitamin B-12/mg of protein)	
							–HGJ	+HGJ
Homogenate	662.4	27.2	398.1	18.0	70.0	1.7	11.2	33.3
Purified brush borders	58.6	0.7	4.0	149.5	630.1	49.3	15.8	346.7
Brush borders stored in glycerol for 1 year	—	—	0	183.6	—	—	26.5	332.8

Purification, storage and analysis were performed as described in the text. One unit of each enzyme is the activity required to convert 1 μmol of substrate/min. The brush-border uptake was expressed as pg of ^{57}Co -labelled vitamin B-12 bound per mg of protein of the fraction analysed. The values shown are the means from three separate determinations. HGJ, human gastric juice.

EXPLANATION OF PLATE I

Scanning electron micrographs of the purified brush-border fraction

Specimens prepared as described in the text were examined in a JEOL scanning electron microscope, JSM-US, at 25kV and tilted at an angle of 45°.

elles (Peters, 1970). In the publications reviewed by Porteous (1972) measurements reflecting biological activity of the brush-border fractions are scarce. To assess the biological activity of our preparation, the effect of human gastric juice on the uptake of ^{57}Co -labelled vitamin B-12 by mucosal homogenates and purified brush borders was compared (Table 1), human gastric juice giving rise to a threefold stimulation of the uptake of ^{57}Co -labelled vitamin B-12 by the homogenate and a 22-fold stimulation by the brush-border fraction. The high recovery (92%) of the intrinsic-factor-mediated uptake compared with the rather poor recovery (12.5%) of non-specific uptake confirms the purity of the brush-border fraction obtained. The brush-border functions measured were well preserved when the purified fraction was stored in glycerol at low temperature (-16°C) (Table 1). Electron-microscopic examination after storage for 1 year showed that the luminal surface of these brush borders was covered by well-preserved microvilli identical with those seen in fresh preparations of brush borders (Plate 1). Large-scale purification of guinea-pig intestinal brush borders followed by storage in glycerol at low temperature should then be possible.

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