THE PREPARATION OF RAT JEJUNAL BRUSH BORDER MEMBRANE

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Received 31 December 1980; revised version received 2 February 1981

1. Introduction

The intestinal epithelium, composed of two functionally distinct regions, the crypts and the villi, is a tissue which undergoes rapid renewal [1,2]. The undifferentiated, mitotically active, crypt cells, which do not participate in the digestive process, differentiate, while moving up the villus toward the lumen, into the absorptive villous cells to form the brush border. To determine the mechanism of this maturation process, which is accompanied by the acquisition of a cellular polarity such that the brush border membrane in contact with the lumen bears distinct functional characteristics (digestive process and transport), it would seem appropriate to describe as precisely as possible the cell surface chemical changes involved in this process. This study requires purification methods for the plasma membrane of the different cell types.

We have devised [3] a procedure for the preparation of the plasma membrane from rat undifferentiated crypt cells which can also serve to obtain the plasma membrane from cells at different stages of maturation, including the basal–lateral membrane from the villous cells. The enzymic and protein content of these membranes has been reported [3].

Purification of the brush border membrane from the villous cells can, in principle, easily be assessed due to the presence of specific hydrolases such as aminopeptidase, alkaline phosphatase and saccharidases [4]. Several procedures have been proposed over the past years to prepare this membrane from the intestine of different species as well as from mammalian kidney [5–7]. When applied to rat mucosa, these procedures and especially that outlined in the case of hog brush border membrane [5], did not lead to good yields of purified membrane. To carry out further chemical analyses on reasonable amounts of material, it seemed thus important to provide a modified method more suitably adapted to the specific problem of rat mucosa. Such a method is presented here.

2. Materials and methods

2.1. *Enzyme activity determinations*

Standard methods were used for the assay of aminopeptidase (EC 3.4.11.2) on Ala-*p*-nitroanilide [8], alkaline phosphatase (EC 3.1.3.1) on *p*-nitrophenyl phosphate [5], cytochrome *c* oxidase (EC 1.9.3.1) [9] and NADPH cytochrome *c* reductase (rotenone-insensitive) on cytochrome *c* [10]. 5′-Nucleotidase (EC 3.1.3.5) was assayed as in [3] on 5′-AMP as substrate. The substrates and reagents were of the best available grade and purchased from Bachem, Sigma and Merck.

Enzyme units were defined in each case as that amount of enzyme catalyzing the disappearance of 1 nmol substrate/min under the assay conditions. Specific activities were expressed in units/mg protein as evaluated by the method in [11] using bovine serum albumin as reference.

2.2. *Centrifugations*

A Sorvall superspeed refrigerated centrifuge Model RC 2B equipped with a GSA (6 × 250 ml) or SS 34 (8 × 50 ml) angle rotor was employed for low-speed centrifugations. High-speed centrifugations were carried out in a Spinco Beckman preparative ultracentrifuge Model L5 65 equipped with a 35 angle rotor.

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Gradient centrifugations were also performed in a Spinco Beckman ultracentrifuge equipped in this case with a swinging bucket rotor SW 27. The *g*-values mentioned in the text correspond to those calculated for the bottom (Sorvall) or the middle (Spinco Beckman) of the tubes.

2.3. *Buffers*

The following buffers were used: (A) 10 mM Tris–HCl buffer (pH 7.3) containing 10 mM $MgCl_2$ and 0.15 M NaCl; (B) 10 mM Tris–HCl buffer (pH 7.3) containing 10 mM $MgCl_2$, 1 mM $CaCl_2$ and 0.25 M sucrose; (C) 1.5 M Tris–HCl buffer (pH 7.8) containing 10 mM $MgCl_2$ and 0.53 M NaCl.

2.4. *Mucosa homogenization*

Ten male Wistar rats (from Lagrace, Marseille or Evic Ceba, Bordeaux) of ~200 g body wt were killed by decapitation. Their small intestines were excised after removal of the duodenum and ileum, thoroughly washed with ice-cold buffer A, placed on cold glass plates and opened longitudinally with scissors. The mucosa was gently scraped off with a microscope slide and the collected scrapings were suspended by magnetic stirring in 8 times their weight buffer B. The suspension was homogenized by 2 strokes of a motor-driven Teflon–glass homogenizer operated at 600 rev./min, filtered through gauze and further homogenized by one additional up-and-down stroke of the pestle.

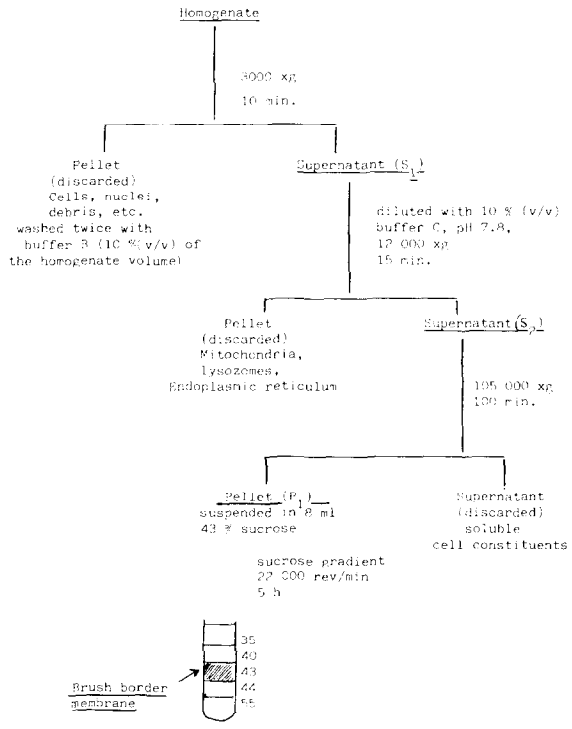
3. Results and discussion

When the preparation of brush border membrane vesicles in the case of hog mucosa [5] was applied as such to rat mucosa, an overall yield of aminopeptidase activity used as marker, ≤5%, was obtained. Important losses were observed at each step including the last one which is a centrifugation on a stepped sucrose gradient.

The flow sheet for the purification of rat brush border membrane as revised herein is outlined in scheme 1.

The homogenate, prepared as in section 2 was first fractionated by a low-speed centrifugation. Washing of the pellet twice with buffer B led to a higher recovery of aminopeptidase activity in the supernatant and was performed routinely. Supernatant S_1 was then diluted with 10% (v/v) buffer C. This treatment, by raising the pH to 7.8, resulted in an aggregation of the contaminating endoplasmic reticulum which could be removed by the following centrifugations at 12 000 × *g* and 105 000 × *g*.

Scheme 1
Flow sheet of the purification of rat brush border membrane



To define more efficient conditions of centrifugation of the rat brush border membrane on a sucrose gradient, the pellet P_1 was submitted to an analytical centrifugation on a 30–50% linear sucrose gradient. The results obtained showed:

(i) That most of the contaminating basal–lateral membranes, mitochondria and endoplasmic reticulum were removed since they sedimented at lower sucrose density.

(ii) That the mean density of the brush border membrane appeared to correspond to 42.5% sucrose. This was in good agreement with previous observations that brush border membranes from porcine and rat origin may differ slightly in their densities. Indeed, on a stepped sucrose gradient constituted by a 43% layer and a 42% layer, the rat membrane was distributed almost equally amongst the 2 layers while the porcine membrane was all recovered in the 43% layer. This

Table 1
Composition and enzyme content of various fractions from rat jejunal mucosa

Fraction	Aminopeptidase	Alkaline phosphatase	Protein	5'-Nucleotidase	Cytochrome oxidase	Cytochrome reductase
Homogenate	100 (75) (1)	100 (240) (1)	100	100 (6)	100 (14)	100 (6)
Supernatant S_1	81 (95) (1.3)	80 (300) (1.25)	63	75 (7)	29 (6)	82 (8)
Supernatant S_2	66 (85) (1.1)	80 (330) (1.4)	58	75 (8)	29 (7)	70 (8)
Pellet P_1	61 (1000) (13)	60 (3200) (13)	4.5	19 (25)	8 (23)	2.5 (3)
Brush border membrane	31 (2300) (30)	26 (5000) (21)	1.0	2 (12)	<1 (10)	<0.5 (1)

The separation of the fractions is described in scheme 1. For each enzyme, the first figure indicates the number of units found in the fraction/100 units in the homogenate. The figure in the first parentheses gives the specific activity in units/mg protein and in the second parentheses the increase in specific activity relative to the homogenate. The figures for aminopeptidase and proteins were S.M. values from 25 assays and the figures for alkaline phosphatase, 5'-nucleotidase, cytochrome oxidase and cytochrome reductase were S.M. values from two assays

was also confirmed by their different lipid:protein ratios (B. Entressangles, personal communication).

Various combinations of sucrose layers in stepped gradients were tested and finally the following one was regarded as most satisfactory: 4 ml (55%); 8 ml (44%); 8 ml (43%); 8 ml (40%); 10 ml (35%). After a 5 h centrifugation at 22 000 rev./min and 1°C, the bulk of the material was found in the 43% layer.

As shown in table 1, the first step not only removed from supernatant S_1 a high percentage of the cytochrome oxidase activity but left in it the largest part of aminopeptidase and alkaline phosphatase used as specific markers for the brush border membrane. The remainder of the contaminants, including the basal–lateral membrane as judged from the 5'-nucleotidase activity used as marker for this membrane [3], were eliminated by the other conventional and gradient centrifugations leading to the brush border membrane. The two markers of the brush border membrane were purified ~25-fold when compared with the crude homogenate with an overall yield of 30%. The figures in table 1 compare well with those obtained in [5] in the case of porcine brush border membrane and strongly suggest that the degree of homogeneity of the two preparations is comparable.

As in the case of the porcine membrane, the rat brush border membrane is probably obtained as closed right-side out vesicles although no electron micrographs of this preparation have been taken.

The rat membrane prepared as described herein as well as that from porcine origin have lost their active transport activity at the level of the microvilli. This is not surprising since this property is abolished very rapidly after the tissue has been disrupted (M. Knibiehler and M. Sémériva, personal communication). By contrast, when the bruh border membrane is purified according to [6] or [7], its active transport function is maintained, due to the fact that the overall procedure is accomplished in <3 h. However, from a chemical point of view and due to its very high level of purification the rat brush border membrane (this paper) appears well suited to perform lipid and protein analyses.

Acknowledgements

Our sincere thanks are due to Dr M. Sémériva for his fruitful suggestions. This work was supported by grant 1551 from DGRST 'Membranes Biologiques'.

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