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A RAPID METHOD FOR THE ISOLATION OF KIDNEY BRUSH BORDER MEMBRANES

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Summary

A simple rapid method for the preparation of purified brush border membranes from rabbit kidney proximal tubules is described. The method is based on hypotonic lysis, Ca^{2+} aggregation of contaminants and differential centrifugation. In contrast to most other published methods, the brush border membranes are free of contamination by basolateral membranes.

The epithelial cells of the mammalian renal proximal tubule and small intestine are 'polarized' in that their plasma membrane is divided into apical 'brush border' and basal-lateral regions, which are morphologically quite distinct. The anatomical difference is paralleled by differences in the constitution and function of the two membrane regions with respect to enzyme localization, characteristics of the membrane transport processes, and lipid composition [1—4]. The study of these two membrane regions requires methods for their isolation free of each other and of cellular contaminants and organelles. In 1973, we published from this laboratory a relatively simple method involving the use of Ca^{2+} or Mg^{2+} to prepare purified intestinal brush border membranes [5]. Since that time others have applied the method with some differences varying from the simple to the elaborate to both the intestine and the mammalian kidney [6—8]. None of these variations have represented a marked improvement in terms of ease of manipulation or purity of product. In this laboratory we have worked steadily over the past six years to simplify the manipulations of the original method and to reduce the time required without loss of efficacy. What follows is our current method. The effectiveness of the procedure in non-mammalian species is demonstrated in the following paper [12].

The activities of the following enzymes were used as markers for estimating the degree of purity of the brush border membrane preparation; maltase, trehalase, leucylnaphthylamidase, γ-glutamyltransferase, and alkaline phosphatase for the brush border membrane, succinate dehydrogenase for mitochondria, NADPH-cytochrome *c* reductase for microsomes, $(Na^+ + K^+)$-ATPase for baso-lateral membranes and β-glucuronidase for lysosomes. All these enzymes were assayed as previously described [5]. Protein determination was by the method of Lowry et al. [9].

All reagents were obtained from standard commercial sources and were of the highest purity available.

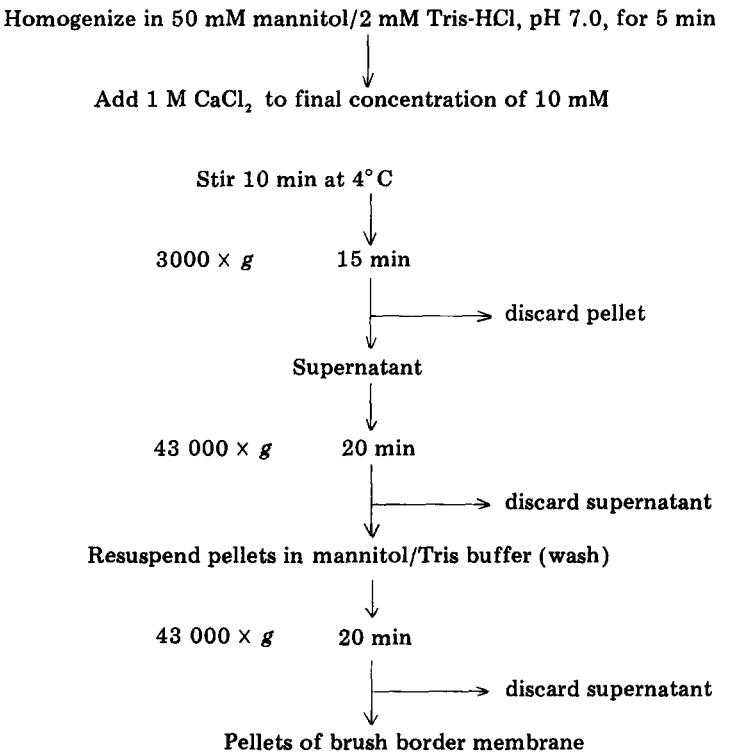
D-[U-^{14}C]Glucose (316 Ci/mol) and L-[U-^{14}C]alanine (171 Ci/mol) were purchased from Amersham Corporation.

Male New Zealand white rabbits were killed by cervical dislocation and the kidneys perfused with cold isotonic saline via the descending aorta, until cleared of blood. The kidneys were then excised, the cortices dissected from the medulla and either frozen at −20°C for later use or processed immediately.

Fresh or frozen cortices were homogenized in 30 volumes (v/w) of ice-cold 50 mM mannitol/2 mM Tris-HCl buffer, pH 7.0, for 5 min at top speed in a Sorvall Omnimixer. A Waring blender was on occasion used, with similar results. 1 M $CaCl_2$ solution was added to the homogenate to a final concentration of 10 mM and the mixture stirred in an ice bath for 10 min. The homogenate was then centrifuged at 3000 × *g* for 15 min in a Sorvall refrigerated centrifuge. The supernatant was carefully decanted and centrifuged again at 43 000 × *g* for 20 min. The pelleted material representing brush border membranes was resuspended in an equal volume of the same buffer using a 1 ml syringe and a 25 g needle and centrifuged again for 20 min at 43 000 × *g*. The brush border membrane pellets thus obtained were resuspended in an appropriate buffer depending upon the purpose for which the membranes were intended. Samples for enzyme assays were removed from the homogenate prior to addition of $CaCl_2$, and from the resuspended membranes. A scheme of the preparation procedure is given (Scheme 1).

Studies of the transport of D-glucose and L-alanine were carried out essentially as described by Hopfer et al. [10] with minor modifications which are described in the legend to Fig. 1.

The state of purity of the microvillus membrane fraction may be appreciated by a study of Table I. Contamination by nonbrush border marker enzymes was extremely small or undetectable for succinic dehydrogenase, β-glucuronidase and $(Na^+ + K^+)$-ATPase, indicating no significant contamination by mitochondria, lysosomes or basal-lateral membranes. While the activity of NADPH-cytochrome *c* reductase was significantly less than in the homogenate, it was still appreciable. Whether this means that the preparation is significantly contaminated with microsomes or that this enzyme is associated with the brush border membrane is uncertain [11]. Purification of brush border membrane of approximately nine fold is indicated by the specific activities of the brush border membrane enzymes maltase, trehalase, alkaline phosphatase, γ-glutamyltransferase and leucyl-



Scheme 1.
Flow diagram of procedure for preparation of brush border membranes.

TABLE I

ENZYME PROFILE OF ISOLATED BRUSH BORDER MEMBRANES

All activities are expressed as I.U/g protein ± S.D. except for NADPH-cytochrome *c* reductase which is expressed as $\Delta A \cdot min^{-1} \cdot g^{-1}$ protein. n, the number of tissues assayed. N.D., not detected.

Fraction	*n*	Homogenate (H)	Brush border membrane (M)	Relative activity (M/H)
Protein (mg)	9	1488.2 ± 341	60.2 ± 17.5	0.04
Succinate dehydrogenase	10	28.12 ± 7.2	N.D.	
NADPH-cytochrome *c* reductase	5	78.3 ± 12.4	24.8 ± 6.0	0.32
$(Na^+ + K^+)$-ATPase	8	12.35 ± 2.67	N.D.	
β-Glucuronidase	10	0.0604 ± 0.019	0.0025 ± 0.002	0.041
Maltase	5	87.00 ± 33.84	1028 ± 281.5	11.8
Trehalase	12	195.9 ± 95.6	1536 ± 625	7.8
Alkaline phosphatase	7	139.6 ± 41.75	947.0 ± 185.36	6.8
γ-Glutamyltransferase	6	1070 ± 145	8680 ± 142.0	8.1
Leucylnaphthylamidase	10	193.0 ± 42.18	1805.0 ± 630.0	9.4

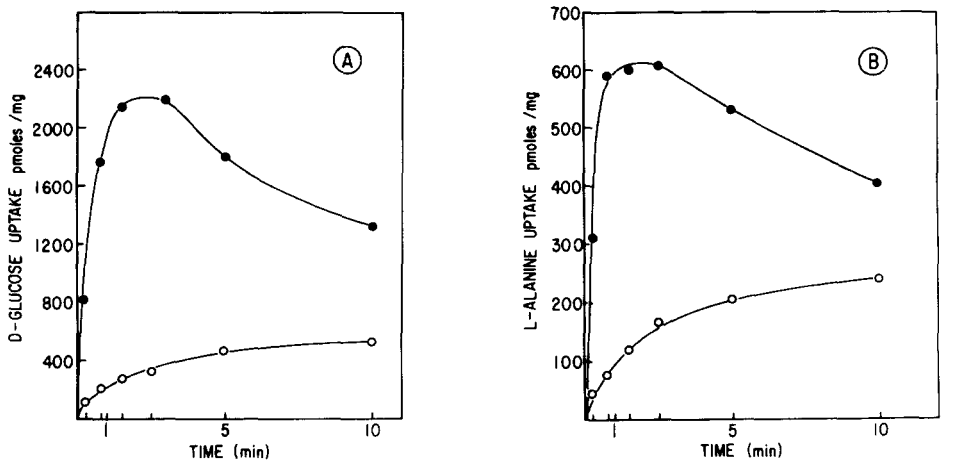


Fig. 1. (A) Membrane vesicles were loaded with 0.1 M KCl containing 1 mM dithiothreitol and 5 mM HEPES/Tris, pH 7.5, by taking up the final membrane pellet in the buffer through a 25 gauge needle. Uptake of D-glucose was measured by incubating 50 μl of membrane vesicles (approx. 0.5 mg protein) in a reaction mixture containing 0.2 mM D-[U-^{14}C] glucose, 5 mM HEPES/Tris pH 7.5, and 0.1 M KCl (○—○) or 0.1 M NaCl (●—●). 50-μl aliquots were removed at appropriate time intervals, diluted into 1 ml of cold 0.15 M NaCl, rapidly filtered through 0.22 μm millipore filters, and washed with 5 ml of cold 0.15 M NaCl. The filters were then dissolved in scintillation fluid containing Triton X-100 and counted. (B) L-Alanine uptake was measured in an identical manner using 0.2 mM L-[U-^{14}C] alanine in the presence of 0.1 M KCl (○—○) or 0.1 M NaCl (●—●).

naphthylamidase. These results are similar to those of others [2,7] in the degree of purification of the brush border member enzymes, but the total lack of contamination by (Na^++K^+)-ATPase is in marked contrast to the more usual enrichment of this marker of the basal lateral membrane reported [2,7]. Booth and Kenny [7] have described a similar method for the preparation of kidney brush border membranes using $MgCl_2$ instead of $CaCl_2$ to aggregate and remove other contaminant cell membranes. Their preparation however shows considerable contamination with both (Na^++K^+)-ATPase (basolateral membranes) and enzymes of lysosomal origin. They argue that the latter may be due to rupture of lysosomes caused by freeze-thawing and hypotonic lysis of the tissue, leading to a subsequent binding of released lysosomal enzymes to the brush border membrane. This seems unlikely since we have found no differences in the enzyme profiles of membranes prepared from fresh and frozen-thawed kidney cortices. The brush border membranes isolated by the present method are therefore more suitable for the study of brush border membrane structure and function than those previously reported.

The brush border membrane vesicles also exhibit Na^+-gradient coupled transport phenomena, for example for D-glucose and L-alanine (Fig. 1). The lack of contamination by basal lateral membranes should make it easier to interpret transport data in terms of events at the brush border membrane alone.

The method as described is simple and rapid; the purified brush border membrane fraction may be used in transport studies in less than 2 h from sacrificing the animal. In addition, the yield of membranes is relatively high; about 60 mg brush border membrane protein from one pair of rabbit kidney

cortices (6 mg brush border membrane protein/gm kidney cortex). We have also prepared functional brush border membrane vesicles from the kidneys of beef cattle, goat and hamster with essentially similar results.

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