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A SIMPLE METHOD FOR THE ISOLATION OF BASOLATERAL PLASMA MEMBRANE VESICLES FROM RAT KIDNEY CORTEX

ENZYME ACTIVITIES AND SOME PROPERTIES OF GLUCOSE TRANSPORT

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A procedure for preparing basolateral membrane vesicles from rat renal cortex was developed by differential centrifugation and Percoll density gradient centrifugation, and the uptake of D-[^{3}H]glucose into these vesicles was studied by a rapid filtration technique. ($Na^+ + K^+$)-ATPase, the marker enzyme for basolateral membranes, was enriched 22-fold compared with that found in the homogenate. The rate of D-glucose uptake was almost unaffected by Na^+ gradient (no overshoot).

The basolateral plasma membranes from renal epithelial cells have been isolated by the procedures of differential centrifugation with sucrose density gradient centrifugation [1–3] or free-flow electrophoresis [4]. Both methods are time-consuming, and the latter, in addition, is limited for general applicability because of the special instrumentation required. Recently Scalera et al. [5] reported a simple method for the isolation of basolateral membranes from intestinal epithelial cells based on a density gradient centrifugation with Percoll. The present paper describes the adaptation of Percoll gradient method to the isolation of basolateral membranes from rat renal cortex. A preliminary report in abstract form has been published elsewhere [6].

The basolateral plasma membranes were isolated from renal cortex of male Wistar albino rats (190–230 g). The minced tissue was homogenized (1 : 5, w/v) in ice-cold buffer comprising 0.25 M sucrose/1 mM EDTA/and 10 mM Tris-HCl, pH 7.5 (buffer S) with ten strokes of Dounce homogenizer. The homogenate was centrifuged rapidly and briefly by setting the centrifuge to attain 2 400 $\times g$, and when this force was reached the centrifuge was stopped immediately. The supernatant was recentrifuged in the same manner, and repeated a third time (discard pellet 1). The supernatant was centrifuged at 2 400 $\times g$ for 15 min (discard pellet 2). The supernatant and the fluffy upper portion of the sediment were centrifuged at 20 500 $\times g$ for 20 min (discard supernatant and pellet 3). The fluffy layer of the pellet was resuspended carefully in buffer S, and homogenized by a glass/Teflon Potter homogenizer with ten strokes at 1 000 rev./min (crude plasma membranes). The membrane suspension was mixed with Percoll. obtained from Pharmacia Fine Chemicals (10%, v/v) in buffer S using a glass/Teflon Potter homogenizer with two strokes at 1 000 rev./min. The membrane-Percoll mixture (total volume, 30 ml) was centrifuged in a Hitachi RP50T rotor at 48 000 $\times g$ for 30 min. The

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid.

top 8 ml of the gradient were collected as fraction I, then 5 ml as fraction II, 6 ml as fraction III and 11 ml as fraction IV were collected using a Hitachi DGF-U Density Gradient Fractionator. In order to remove the Percoll particles, four pooled fractions were diluted with buffer S up to 30 ml, and then centrifuged in a Hitachi RPS27 rotor at 100 000 × *g* for 60 min. The membranous material was resuspended in 30 ml of the medium containing 100 mM mannitol and 20 mM Tris-Hepes, pH 7.5 (buffer A). The membrane suspension was homogenized by a glass/Teflon Potter homogenizer with ten strokes at 1 000 rev./min, and centrifuged again in the same manner. The final pellet was resuspended in buffer A by sucking the suspension ten times through a fine needle (0.4 × 20 mm) with a plastic syringe.

As the main difficulty in the isolation of basolateral membranes is the overlap in density of brush borders, the separation of basolateral from brush border membranes was assessed by the activities of marker enzymes. The distributions of aminopeptidase, a marker enzyme for brush border membranes, and $(Na^+ + K^+)$-ATPase, a marker enzyme for basolateral membranes, after centrifuging the crude plasma membrane preparation in 10% (v/v) Percoll medium are illustrated in Fig. 1. The distribution pattern of alkaline phosphatase, a marker enzyme for brush border membranes, was similar to that of aminopeptidase (data not shown). The peak fraction

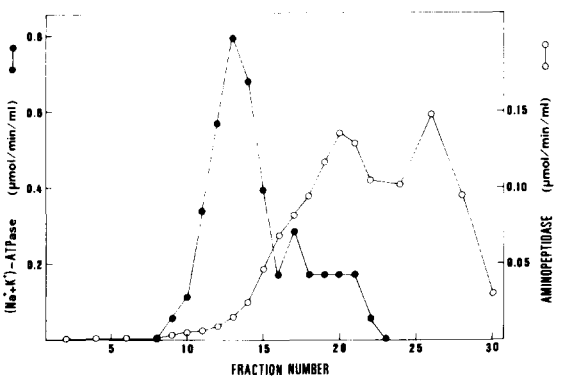

Fig. 1. Distribution of marker enzymes for basolateral and brush border membranes on Percoll gradient. The crude plasma membranes, suspended in 0.25 M sucrose/1 mM EDTA/10 mM Tris-HCl (pH 7.5)/10% (v/v) Percoll, were centrifuged in a Hitachi RP50T rotor at 48 000 × *g* for 30 min. The Percoll gradient was collected from the top into 30 fractions of 1 ml. •, $(Na^+ + K^+)$-ATPase; ○, aminopeptidase.

of $(Na^+ + K^+)$-ATPase had the density of 1.037 g/ml by Density Marker Beads (Pharmacia Fine Chemicals). The fractions 25–26 had the density of 1.049 g/ml. On the basis of the profile in Fig. 1, fractions 9–13 were combined as the basolateral membranes (Fraction II).

In Table I are compiled the recoveries and specific activities of marker enzymes during the isolation of crude plasma membranes and the purification of basolateral membranes by Percoll gradient centrifugation. The crude plasma membrane fraction comprised approx. 6–7% of the total protein of the homogenate and had an approx. 3–5-fold increase in specific activities for the marker enzymes (alkaline phosphatase, aminopeptidase, $(Na^+ + K^+)$-ATPase) relative to those in the homogenate. After the removal of Percoll particles by centrifugation, the enzyme recoveries through the gradient were about 80%. The specific activity of $(Na^+ + K^+)$-ATPase in the basolateral membranes was approx. 22-fold relative to that in the homogenate. In contrast, the specific activities of alkaline phosphatase and aminopeptidase were enriched by a factor of 2.1 and 0.7, respectively. Contamination by glucose-6-phosphatase derived from endoplasmic reticulum and by acid phosphatase from lysosomes was small. Furthermore, mitochondria and cytoplasm were not present. The final basolateral membrane preparation contained approx. 7% of the $(Na^+ + K^+)$-ATPase activity present in the starting homogenate. The yield of basolateral membranes was 550 μg protein/g renal cortex. So far, free-flow electrophoresis [4] or sucrose gradient centrifugation [1–3] procedures have proved useful for the separation of brush border and basolateral membranes from renal epithelial cells. In this study, shallow gradients of Percoll easily achieved the high degree of resolution necessary to distinguish the populations as defined by the marker enzymes. Basolateral membranes isolated by the present procedure meet or exceed other preparations in quality and quantity. Electron micrographs revealed the membrane fraction consisting of mostly closed vesicles (not shown).

As shown in Fig. 2, the characteristics of glucose transport by basolateral membrane vesicles were tested by a Millipore filtration technique [13] in the uptake media containing 100 mM NaCl or 100 mM KCl, and were compared with those by brush border membrane vesicles prepared according to Evers et al.

TABLE I

MARKER ENZYME DISTRIBUTIONS DURING ISOLATION OF CRUDE PLASMA MEMBRANE FRACTION AND PURIFICATION OF BASOLATERAL MEMBRANES BY PERCOLL GRADIENT CENTRIFUGATION

Protein was determined, after precipitation with 10% (w/v) trichloroacetic acid, by the method of Lowry et al. [7]. The following marker enzymes were assayed: alkaline phosphatase (EC 3.1.3.1) [8]; aminopeptidase (EC 3.4.11.2) [9]; ($Na^+ + K^+$)-ATPase (EC 3.6.1.3) [10]; glucose-6-phosphatase (EC 3.1.3.9) [11]; acid phosphatase (EC 3.1.3.2) [5], cytochrome *c* oxidase (EC 1.9.3.1.) [12]. Lactate dehydrogenase (EC 1.1.1.27) was measured with lactate as substrate by recording the formation of NADH from NAD in 30 mM Tris-HCl buffer, pH 7.5. S.A., specific activity (nmol/min per mg protein) (ΔA_{550}/min per mg protein for cytochrome *c* oxidase). % represents the percentage of the enzyme activity found initially in the homogenate. n.d., not detectable. Enzyme activities in each Percoll fraction were determined after removal of Percoll. Each value represents the mean ± S.E. for 6–7 experiments.

	($Na^+ + K^+$)-ATPase		Alkaline phosphatase		Aminopeptidase	
	S.A.	%	S.A.	%	S.A.	%
Homogenate	44 ± 4	100	24 ± 1	100	18 ± 1	100
Pellet 1	39 ± 10	40	21 ± 2	52	18 ± 1	52
Pellet 2	66 ± 7	16	22 ± 2	10	16 ± 1	9
Supernatant	n.d.		14 ± 1	16	7 ± 1	8
Pellet 3	86 ± 13	7	65 ± 6	11	33 ± 4	7
Crude plasma membranes	130 ± 28	14	129 ± 4	34	63 ± 9	21
Fraction I	75 ± 38	0.1	32 ± 6	0.1	n.d.	
Fraction II (basolateral membranes)	943 ± 30	6.5	53 ± 4	0.7	13 ± 1	0.2
Fraction III	302 ± 64	2.5	285 ± 23	5.7	78 ± 6	1.9
Fraction IV	57 ± 19	4.1	164 ± 9	22.9	76 ± 9	15.6

[14]. The simultaneous addition of D-glucose and NaCl to brush border membrane vesicles produced a transient accumulation above the equilibrium level (overshoot phenomenon) (Fig. 2A). The initial rate of D-glucose uptake in the presence of Na^+ gradient was approx. 20-fold that in the presence of K^+ gradient. On the other hand, as shown in Fig. 2B, the uptake of D-glucose by basolateral membrane vesicles was significantly less responsive to Na^+ (no overshoot) compared with brush border membrane vesicles, although the initial rate of D-glucose uptake was higher than that of L-glucose. The initial rate of D-glucose uptake in the presence of Na^+ gradient was only 1.5-fold that in the presence of K^+. The small stimulation could be accounted for by contamination of the basolateral membrane preparation with brush border membranes. The D-glucose accumulated by the vesicles in 20 min was found to be inversely proportional to the mannitol concentration of incubation medium (data not shown). Therefore, D-glucose uptake represents transport into an intravesicular space. The above results of D-glucose transport by basolateral membrane vesicles are essentially in agreement with the data obtained by Kinne et al. [15] and Slack et al. [16]. Furthermore, in respect to the Na^+ dependency of D-glucose uptake, our results were less sensitive to Na^+ than those of Slack et al. [16]. During the preparation of our revised manuscript, Scalera et al. [17] reported an isolation method for renal basolateral membranes by Percoll gradient.

In conclusion, Percoll density gradient centrifugation is a simple and rapid method for the isolation of basolateral membranes from cortical slices of rat kidney. Basolateral membrane vesicles obtained by this method can be used for the analysis of the transport properties.

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Glucose-6-phosphatase		Acid phosphatase		Cytochrome *c* oxidase		Lactate dehydrogenase		Protein (%)
S.A.	%	S.A.	%	S.A.	%	S.A.	%	
154 ± 6	100	126 ± 3	100	15.1 ± 0.5	100	86 ± 18	100	100
150 ± 10	59	115 ± 6	54	13.7 ± 0.9	55	26 ± 2	19	62
135 ± 4	10	118 ± 4	10	27.1 ± 0.7	9	61 ± 20	1	11
43 ± 3	7	67 ± 2	13	n.d.		253 ± 36	75	24
217 ± 14	5	143 ± 10	4	28.9 ± 2.9	7	n.d.		4
235 ± 15	10	145 ± 4	7	6.2 ± 0.3	3	39 ± 7	3	6
79 ± 30	0.1	151 ± 19	0.1	n.d.		n.d.		0.04
231 ± 56	0.5	162 ± 11	0.4	n.d.		n.d.		0.3
478 ± 31	1.2	172 ± 8	0.6	1.4 ± 0.2	0.1	n.d.		0.4
246 ± 15	5.0	150 ± 9	3.4	13.6 ± 1.2	2.7	n.d.		3.5

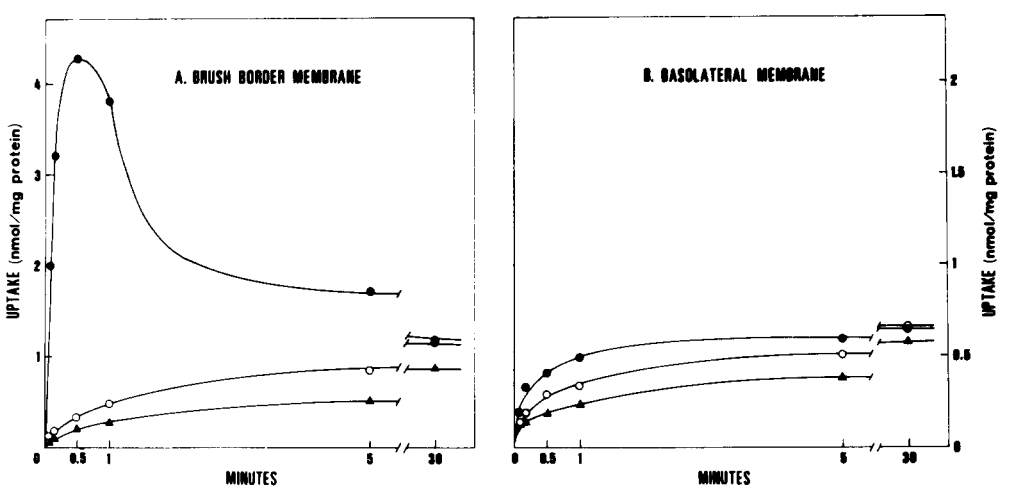

Fig. 2. Time course of D- and L-glucose uptake by brush border (A) and basolateral membrane vesicles (B) from rat kidney cortex. Membrane vesicles were preincubated at 25°C in 100 mM mannitol and 20 mM Tris-Hepes, pH 7.5 (buffer A) for 10 min. The vesicles (20 μl. A, 61 μg protein; B, 45 μg protein) were incubated with substrate medium (20 μl) of buffer A containing either D-[^{3}H]glucose (50 mCi/mmol, ●,○) or L-[^{3}H]glucose (▲), and with NaCl (●,▲) or KCl (○) (final concentration; 1 mM glucose; 100 mM NaCl; 100 mM KCl). Each point represents the mean value of three experiments.

References

1 Ebel, H., Aulbert, E. and Merker, H.J. (1976) Biochim. Biophys. Acta 433, 531–546
2 Liang, C.T. and Sacktor, B. (1977) Biochim. Biophys. Acta 466, 474–487
3 Kinsella, J.L., Holohan, P.D., Pessah, N.I. and Ross, C.R. (1979) Biochim. Biophys. Acta 552, 468–477
4 Heidrich, H.G., Kinne, R., Kinne-Saffran, E. and Hannig, K. (1972) J. Cell Biol. 54, 232–245
5 Scalera, V., Storelli, C., Storelli-Joss, C., Haase, W. and Murer, H. (1980) Biochem. J. 186, 177–181
6 Inui, K., Okano, T., Takano, M., Kitazawa, S. and Hori, R. (1981) J. Pharm. Dyn. 4, S-20 (abstrt.)
7 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
8 Fujita, M., Matsui, H., Nagano, K. and Nakao, M. (1971) Biochim. Biophys. Acta 233, 404–408
9 Kramers, M.T.C. and Robinson, G.B. (1979) Eur. J. Biochem. 99, 345–351
10 Jørgensen, P.L. (1974) Methods Enzymol. 32, 277–290
11 Aronson, N.N.Jr. and Touster, O. (1974) Methods Enzymol. 31, 90–102
12 Peters, T.J., Müller, M. and de Duve, C. (1972) J. Exp. Med. 136, 1117–1139
13 Inui, K., Moller, D.E., Tillotson, L.G. and Isselbacher, K.J. (1979) Proc. Natl. Acad. Sci. USA 76, 3972–3976
14 Evers, C., Hasse, W., Murer, H. and Kinne, R. (1978) Membrane Biochem. 1, 203–219
15 Kinne, R., Murer, H., Kinne-Saffran, E., Thees, M. and Sachs, G. (1975) J. Membrane Biol. 21, 375–395
16 Slack, E.N., Liang, C.T. and Sacktor, B. (1977) Biochem. Biophys. Res. Commun. 77, 891–897
17 Scalera, V., Huang, Y.K., Hildmann, B. and Murer, H. (1981) Membrane Biochem. 4, 49–61