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Simultaneous preparation of basolateral and brush-border membrane vesicles from sea bass intestinal epithelium

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A method is described for simultaneous preparation of brush-border and basolateral sea bass enterocyte membranes using simple differential centrifugation and discontinuous sucrose gradient density centrifugation techniques. Basolateral membranes were purified with a Na^+/K^+-ATPase yield of about 11% of the original activity, with an enrichment factor of 12. The yield of maltase-glucoamylase, a specific marker of brush-border membranes, was also about 11% of the original activity, with 15-fold enrichment. The characteristics of these membrane preparations were determined. Electron microscopy analysis showed that these two membrane preparations were uniform in size and vesicular in nature. Orientation studies revealed that the luminal membrane vesicles were right-side out and 43% of the antiluminal membrane vesicles were sealed inside out. Investigation of D-glucose and L-leucine uptake showed that these two plasma membrane preparations retained their transport properties.

Introduction

Euryhaline teleost fish intestine is of considerable interest and complexity because it is a major center of osmoregulation in addition to having nutritional functions. While many properties of monovalent ion transport across the epithelial enterocytes of this fish have already been clarified in connection with osmoregulation [1–3], only a few data have been published on the mechanism of intestinal absorption of organic solutes produced by digestion. Brush-border membrane vesicles prepared from the intestine of various species of fish have been used to study sugar [4–12] and amino acid transport processes [8–15]. Results suggest that the brush-border membrane of fish enterocytes possesses the basic hexose and amino acid transport mechanisms found in mammalian intestines [11,12]. Much less is known about the mechanisms of organic solute transport across the basolateral membrane. We found only three reports on D-glucose and L-amino acid transport by basolateral membrane vesicles prepared from fish intestines [15–17].

Physiological conditions such as environmental salinity [7] and quantitative modifications of the diet have recently been shown to modify the glucose transport properties of enterocyte brush-border membranes. It has been suggested that physiological modifications in the intestinal lumen, secondary to hyperglycemia or perfusion of the ileal lumen with glucose (which induces a rapid increase in intestinal glucose absorption), might be mediated by changes at the level of the basolateral membrane [18]. Consequently, analyses of the influence of factors such as environmental salinity, qualitative and quantitative modifications of the diet, and nutrient pollutants (hydrocarbon derivatives, heavy metals, etc.) on the mechanisms of absorption of organic solutes (hexoses and amino acids) across the epithelia of the small intestine are more significant when both membrane fractions used are prepared from the same material. Simultaneous preparation of both plasma membranes from fish intestine epithelial cells has not been reported to date. This paper describes a technique for preparation of brush-border and basolateral membrane vesicles from the sea bass (*Dicentrarchus labrax*) intestine. The method involves simple differential centrifugations, precipitation with divalent cations (Mg^{2+}), and discontinuous sucrose density centrifugation. Both membrane vesicles obtained in this manner were characterized by a satisfactory purification factor and conservation of their D-glucose and L-leucine transport properties.

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Material and Methods

Fish

Sea bass (*Dicentrarchus labrax*) weighing about 250 g, grown in seawater, were purchased from a fish farm (Aquamed, Théoules, France) and kept for a maximum of two weeks in reconstituted seawater aquariums under controlled salinity (20‰ NaCl), temperature (21° C), and nitrite concentration. Fish were fed to repletion daily with commercial sea bass food pellets (Aquasarb, SARB, France), except for the last two days prior to the experiments.

Isolation of basolateral and brush-border membrane fractions

Crude membrane fraction preparation. Sea bass were killed by decapitation. The intestines were immediately excised and divided into upper and lower segments. The upper portions (anterior intestine) were cut lengthwise, the mucosa was gently scraped with a microscope slide, and the scrapings were suspended under mild magnetic stirring in 50 times their weight of ice-cold hypotonic buffer composed of 50 mM mannitol, 2 mM Tris (pH 7.1) containing 0.1 mM phenylmethylsulfonyl fluoride to prevent any proteolytic degradation.

All subsequent steps were carried out at 4° C. After stirring, the suspension was homogenized using a Virtis Omnimixer at one half the maximum speed for two 30 s periods. The homogenate was then filtered through a Terylene filter (0.5 mm mesh) to remove cellular debris, fat and inhomogeneous material. 1 M $MgCl_2$ was added to the filtrate (final concentration 10 mM). After 20 min incubation with gentle stirring by backward and forward motion, the suspension was centrifuged for 15 min at $2500 \times g$ in a Sorvall SW 34 rotor. The supernatant was then centrifuged 45 min at $105\,000 \times g$. The pellet containing crude membrane fractions (essentially basolateral and brush-border membranes) was resuspended in 37% sucrose, and homogenized by 18 passages through a thin 21-gauge needle.

Sucrose density gradient centrifugation. All sucrose solutions contained 10 mM Tris-HCl buffer (pH 7.4). The gradient set up in the 15 ml tubes of a Spinco SW 27-1 rotor was as follows: 47% (5 ml), 37% (6 ml) containing the crude membrane fractions, and 20% (3 ml). After centrifugation at $83\,000 \times g$ for 5 h, two main bands of turbidity were obtained (Scheme I). The basolateral membrane (fraction F_1) formed an upper band in the sucrose gradient, at the interface between 20% and 37%. The brush-border membrane (fraction F_2) formed the second band, at the interface between 37% and 47%. These two membrane fractions were aspirated, diluted with 300 mM mannitol, 10 mM Hepes-Tris (pH 7.4) buffer and centrifuged 45 min at $105\,000 \times g$. The pellets corresponding to the BBM and BLM vesicles were resuspended in the transport buffer by 18 passages through a thin 21-gauge needle. If not used immediately, the purified membrane vesicles (0.5 ml) were transferred to cryotubes, quickly frozen in liquid nitrogen, and stored in liquid nitrogen. The day of analysis, the frozen membrane vesicles were thawed rapidly at 40° C as described by Stevens et al. [19].

Protein and enzyme marker determinations

Proteins were measured by the Coomassie blue binding method [20], using lyophilized bovine plasma γ-globulin as standard.

Aminopeptidase N and maltase-glucoamylase, which are markers of brush-border membranes, were assayed respectively according to Roncari and Zuber [21] and De Burlet and Sudaka [22]. Na^+/K^+-ATPase, assayed to evaluate the basolateral membrane preparation, was measured according to Del Castillo and Robinson [23]. Cytochrome-*c* oxidase (a mitochondrial marker) and NADPH–cytochrome-*c* reductase (an endoplasmic reticulum marker) were investigated as described, respectively, by Cooperstein and Lazarow [24] and Sottocasa et al. [25]. Lactate dehydrogenase, a cytosolic marker, was assayed by the method of Hawkins et al. [26].

One enzyme unit is the amount of enzyme catalyzing hydrolysis of 1 μmol substrate per min.

Electron microscopy

Samples for electron microscopy study were fixed with 2.5% glutaraldehyde in 150 mM sodium cacodylate buffer (pH 7.4), then postfixed in 1% osmium tetroxide in the same buffer, dehydrated through graded alcohols and propylene oxide, and embedded in Epon. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and examined under a Philips CM 12 electron microscope.

Orientation of membrane vesicles

Brush-border membrane vesicle orientation was determined by evaluating hydrolysis of starch (an impermeant, high molecular weight polysaccharide) by maltase-glucoamylase, before and after dissociation of the membrane vesicles by treatment with 2% Triton X-100. Under these detergent conditions, all maltase-glucoamylase activity was solubilized without any enzyme activity activation [27–29].

Orientation of the basolateral membrane vesicles was determined as described by Del Castillo and Robinson [23]: the percentage of sealed, inside-out vesicles is related to the detergent activation of ouabain binding. The method used for [^{3}H]ouabain binding was a modification of that of Lane et al. [30]. The reaction was performed at room temperature in the presence of 70 mM NaCl, 1.5 mM $MgCl_2$, 1 mM Na_2-ATP, 10 mM Hepes-Tris (pH 7.4) in a total volume of 475 μl. The quantity of protein added was 0.1 mg. The reaction was initiated by addition of [^{3}H]ouabain (final concentra-

tion 0.8 μM); 60 min later, the reaction was stopped by filtration on a Sartorium 0.65 μm filter. After washing twice with 5 ml incubation medium containing 2 mM unlabelled ouabain, radioactivity was counted. In order to determine the nonspecific component of binding, membrane samples were first exposed for 30 min to 2 mM unlabelled ouabain. The difference between total binding and nonspecific binding provided a measure of specific binding.

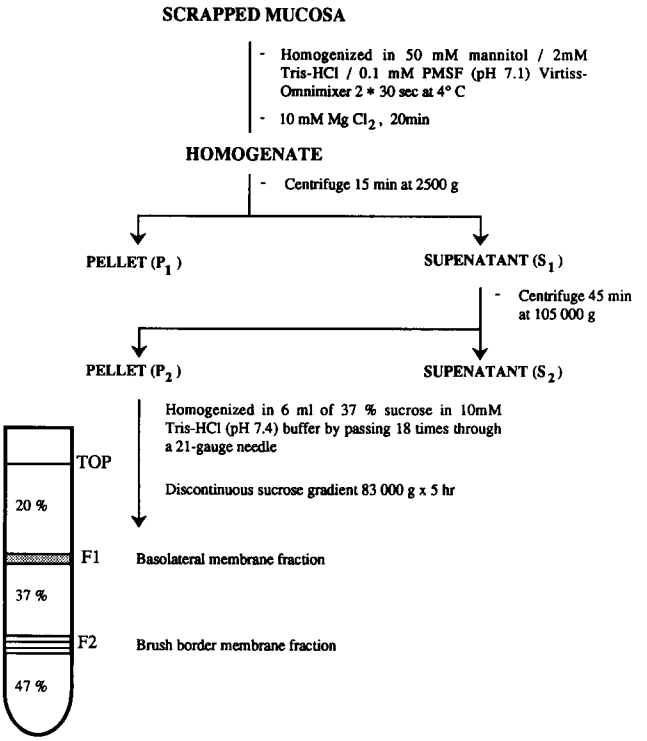
Membrane vesicles were opened by treatment with a mixture of deoxycholate/EDTA (final concentration 0.06% and 1 mM, respectively) for 30 min at room temperature. Preliminary research demonstrated that maximal ATPase activity was obtained with deoxycholate concentrations between 0.05 and 0.08% in the presence of 1 mM EDTA (concentrations of 0.003 to 0.15% of deoxycholate were tested in the presence of 1 mM EDTA). 0.06% deoxycholate was used in all subsequent experiments, as previously used by Del Castillo and Robinson [23] in their similar study on BLM vesicles purified from guinea-pig intestinal epithelium. After this treatment, the samples were tested for [^{3}H]ouabain binding.

Transport experiments

D-Glucose and L-leucine uptake were measured by the rapid filtration technique of Hopfer et al. [31] using a 0.65 μm nitrocellulose membrane (Sartorius). All experiments were performed at 20 °C. The transport reaction was initiated by adding 10 μl vesicle suspension (about 80 μg) to 50 μl incubation medium containing radiolabelled 0.1 mM D-glucose or 0.2 mM L-leucine, when brush border membranes were tested, or 1 mM D-glucose or L-leucine, when basolateral membranes were used. The reaction was stopped by the addition of 1 ml ice-cold stop-solution consisting of 300 mM mannitol and 10 mM Hepes-Tris (pH 7.4). The suspension was immediately filtered, and the filter washed twice with 4 ml stop solution. Radioactivity was counted after addition of scintillation medium (Pico-fluor 30, Packard). Experiments in the presence of an inhibitor (phloretin and unlabelled L-leucine for glucose and L-leucine uptakes, respectively) were performed under the same conditions except that the inhibitor was added to the incubation medium. Experiments were performed in triplicate with three different membrane preparations. Radiolabelled L-[^{3}H]leucine was purchased from CEA (Saclay, France); D-[^{14}C]glucose and [^{3}H]ouabain were obtained from NEN (Biotechnology Systems, France). All other reagents were of the best available grade.

Results

Scheme I shows the procedure developed for simultaneous purification of the luminal and antiluminal membranes of sea bass enterocytes.



Scheme I. Flow diagram for the preparation of intestinal basolateral and brush-border membrane fractions. Further details of procedures are given in the text.

Table I shows the specific activity, relative specific activity, and recovery percentage for the enzyme markers at each stage of the preparation. The upper band (Fraction F_1), which contains basolateral membranes, exhibited 12-fold enrichment in Na^+/K^+-ATPase over the homogenate, with recovery of about 11% of the total activity of the original homogenate. The purity of this membrane preparation was also assayed using membrane enzyme markers. Cytochrome-*c* oxidase, lactate dehydrogenase, NADPH–cytochrome-*c* reductase (respectively, mitochondrial, cytosolic, and reticulum enzyme markers) were all impoverished. A certain degree of contamination, by luminal membrane was observed, as aminopeptidase N and maltase-glucoamylase were enriched 2.7- and 2.0-fold, respectively, in fraction F_1.

The lower band (Fraction F_2) contained brush border membrane vesicles, which showed 13- and 14.5-fold enrichment in aminopeptidase and maltase-glucoamylase activities. The purified membrane preparation contained about 11% of the total homogenate activity. Only Na^+/K^+-ATPase activity was also enriched in this preparation (about 1.7-fold). Except for this very low cross-contamination, the two membrane populations obtained by this method were devoid of any other membrane contamination, as proved by the lower yields for cytochrome-*c* reductase, cytochrome-*c* oxidase and lactate dehydrogenase.

TABLE I

Marker enzyme distributions during isolation of crude plasma membrane fractions and in the purified basolateral and brush-border membrane vesicles

S.A., specific activity (in mU/mg protein); R.S.A., relative specific activity; R%, percentage of total activity; n.d., not detectable. R.S.A. and R% refer to the homogenate fraction. Each value is expressed as the mean ± S.E. ($n = 6$).

Fractions:		H	S_1	C_1	S_2	C_2	F_1	F_2
Proteins	R%	1.9 ± 0.6	1.04 ± 0.2	1.35 ± 0.47	0.74 ± 0.17	0.93 ± 0.35	2.26 ± 1	1.83 ± 0.6
Amino-peptidase N	S.A.	228 ± 16	365 ± 58	268 ± 59	50 ± 0.7	1375 ± 200	565 ± 156	3055 ± 392
	R.S.A.	1	1.60	1.18	0.22	6.03	2.48	13.40
	R%	100	50	49	5	41	3.2	10.8
Maltase-glucoamylase	S.A.	134 ± 33	221 ± 13	173 ± 12	17 ± 2	738 ± 231	271 ± 80	2035 ± 290
	R.S.A.	1	1.65	1.29	0.13	5.50	2.0	15.2
	R%	100	52	40	6	43	3.2	9.7
NADPH-cytochrome-*c* reductase	S.A.	0.67 ± 0.06	0.25 ± 0.17	1.02 ± 0.16	0.16 ± 0.06	0.43 ± 0.15	0.15 ± 0.10	n.d.
	R.S.A.	1	0.37	1.52	0.24	0.64	0.2	
	R%	100	7	96				
Cytochrome-*c* oxidase	S.A.	0.95 ± 0.04	0.13 ± 0.07	1.9 ± 0.07	n.d.	0.29 ± 0.08	n.d.	n.d.
	R.S.A.	1	0.11	2.0		0.31		
	R%	100	5	102		2		
Na^+/K^+-ATPase	S.A.	0.78 ± 0.16	0.93 ± 0.3	0.83 ± 0.18	0.095 ± 0.013	2.5 ± 2.5	1.3 ± 0.08	
	R.S.A.	1	1.19	1.06	0.30	3.33	12.0	1.7
	R%	100	62	32	27	65	10.5	–
Lactate dehydrogenase	S.A.	0.32 ± 0.06	0.56 ± 0.15	0.065 ± 0.07	0.63 ± 0.15	n.d.	n.d.	n.d.
	R.S.A.	1	1.75	0.2	1.97			
	R%	100	108	11	106			

Structural aspect of the two membrane preparations

Electron microscopy observations

Ultrathin sections of the brush border (Fig. 1) and basolateral (Fig. 2) membrane preparations were examined under an electron microscope. Both purified membranes appeared homogeneous and mostly vesicular; the essential structural difference was the appearance of their external surface. The brush-border membranes were covered by an abundant, fuzzy coat (inset, Fig. 1), whereas the external surface of the basolateral membrane was smooth (inset, Fig. 2). Similar differences between these two membrane preparations have previously been reported for dog kidney cortex [32] and rabbit intestine [33].

Interestingly, the brush-border membrane vesicles were partially filled with electron-dense fibrous material. This material was also observed in the basolateral membrane vesicles, but in much smaller amounts. All of the vesicles in both preparations were under 0.5 μm in diameter. Luminal membranes were more homogeneous in size and usually shorter than antiluminal membranes.

TABLE II

Orientation of brush-border and basolateral membrane vesicles

The values are means ± S.D. obtained from three different preparations.

A. Brush-border vesicle orientations

Incubation time (min)	% of enzyme activity [a]	
	without Triton X-100	with Triton X-100
10	100	101 ± 1.0
20	100	105 ± 3.0
30	100	105 ± 2.5
60	100	104 ± 1.5

B. Basolateral vesicle orientations

	[^{3}H]Ouabain binding (pmol/mg protein)	
	+ DOC/EDTA	− DOC/EDTA
Total binding	20.804 ± 0.27	12.168 ± 0.279
Nonspecific binding	1.202 ± 0.04	0.896 ± 0.206
Specific binding	19.602 ± 0.355	11.273 ± 0.283

[a] Enzyme activity without Triton X-100 was considered as 100%.

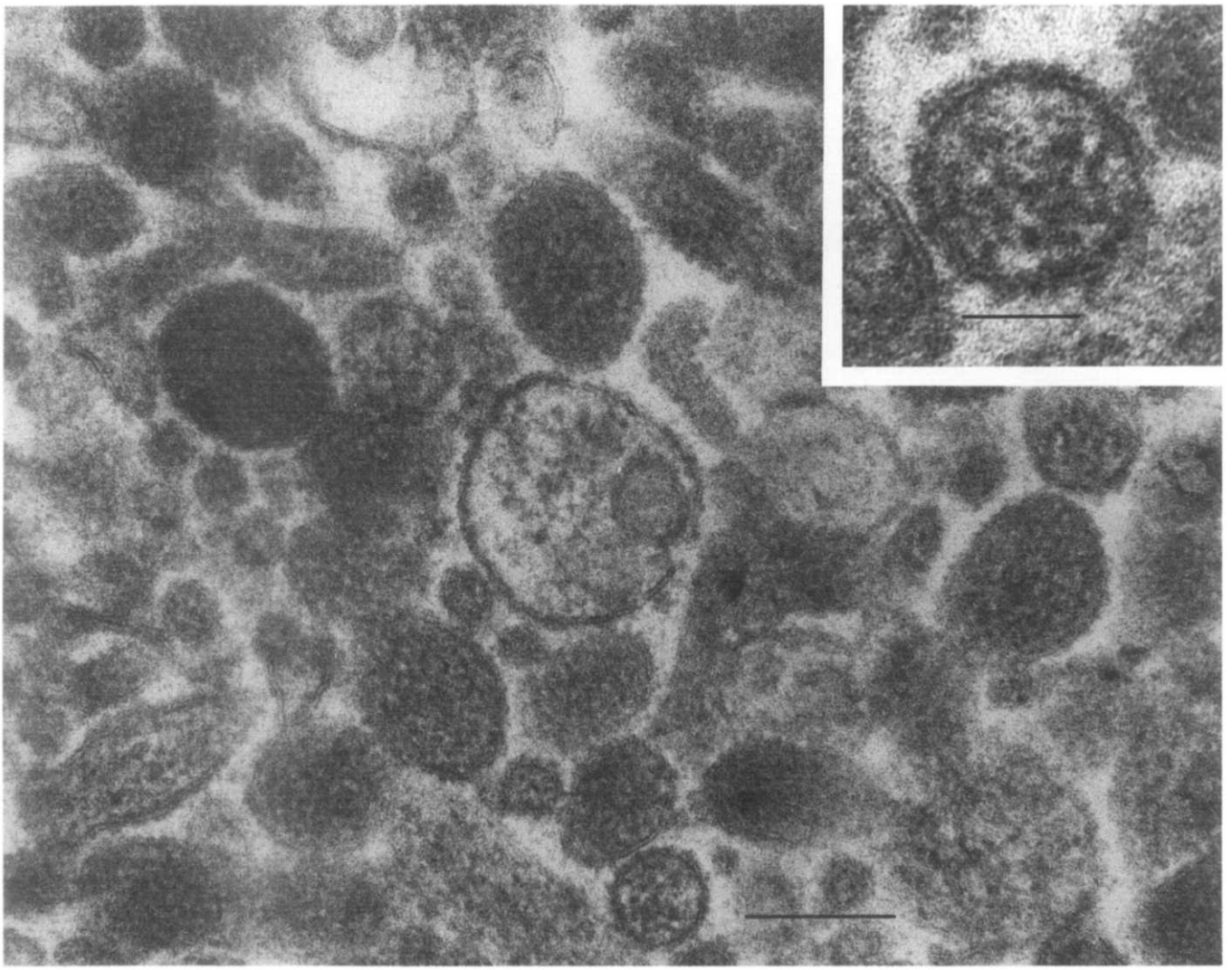


Fig. 1. Electron micrograph of intestinal brush-border membrane vesicles from teleost fish, sea bass. Length of bar corresponds to 0.2 μm (0.06 μm in inset).

Sidedness of plasma membrane vesicles

The orientation of brush-border membrane vesicles can be investigated by evaluating hydrolysis of starch (an impermeant, high molecular weight polysaccharide) by maltase-glucoamylase after pretreatment with Triton X-100 during 120 min [28,29]. The absence of any significant modification (expressed as the percentage of starch hydrolysis) after detergent pretreatment (Table II) is consistent with a sealed membrane vesicle preparation and 'right-side-out' orientation of the vesicles.

Estimation of the sidedness of basolateral membrane vesicles is based on the fact that ouabain diffuses poorly across this membrane, and the only binding site is located on the external surface. The percentage of 'inside-out' membrane vesicles can be estimated from ouabain binding before and after deoxycholate/EDTA treatment, which transforms these vesicles into sheets [23]. Specific [^{3}H]ouabain binding was increased 1.73-fold after treatment with deoxycholate/EDTA (Table II). The preparation is thus composed of approximately 43% sealed, inside-out vesicles and 57% sealed, right-side-out and leaky vesicles.

Investigation of transport properties

In order to further characterize the two membrane preparations, and to confirm the differences in monosaccharide and amino acid uptake by the luminal and antiluminal membranes, the time courses of D-glucose and L-leucine transport were measured. Brush-border membrane vesicles exhibited Na^+ dependence for D-glucose (0.1 mM) and L-leucine (0.2 mM) uptake (Fig. 3). Compared with a K^+ gradient, presence of this sodium gradient between the extravesicular and intravesicular media enhanced uptake of both solutes by the brush-border membrane vesicles. The initial rates of D-glucose and L-leucine uptake by the brush-border membrane in the presence of the Na^+ gradient were, respectively, around 15- and 12-fold higher than in the presence of a K^+ gradient. The 'overshoot' observed between 1 and 2

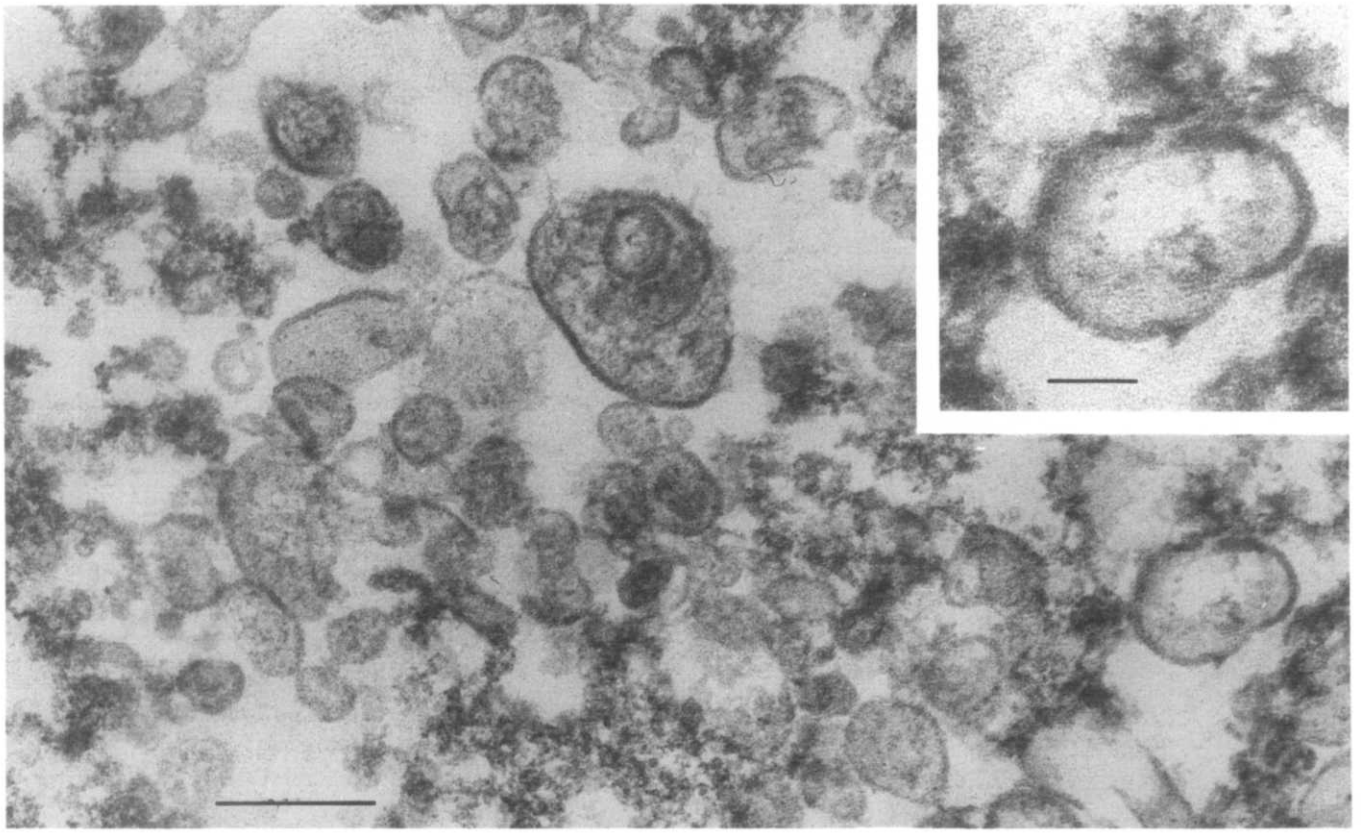


Fig. 2. Electron micrograph of intestinal basolateral membrane vesicles from teleost fish, sea bass. Length of bar corresponds to 0.3 μm (0.1 μm in inset).

min corresponded to accumulations 2.6- and 5-times higher than the equilibrium values determined for amino acid and hexose, respectively.

Additional evidence of the basolateral origin of the membrane vesicles purified in fraction F_1 was obtained by measuring the cation dependence and the effect of various inhibitors on the D-glucose and L-[^{3}H]leucine uptakes by these membrane vesicles. As precised in Fig. 4A although the initial rate of D-glucose uptake in the presence of the Na^+ gradient was minimally higher 1.25-fold) than that in presence of the K^+ gradient, both hexose uptakes were always significantly higher than that observed in the presence of 100 mM NaCl + 0.1 mM phloretin, an inhibitor of the D-glucose uptake, in the incubation medium. Results for L-leucine uptake were analogous to those described for D-glucose (Fig. 4B). Only a minimally difference was noted in the uptake of the amino acid between the Na^+ and K^+ gradients and L-[^{3}H]leucine uptake was inhibited by addition of high concentration (10 mM) of this unlabelled amino acid.

Discussion

Studies comparing the influence of such factors as environmental salinity, qualitative and quantitative dietary modifications, and the presence of pollutants such as hydrocarbon derivatives and heavy metals on hexose and L-amino acid transport across the luminal and antiluminal membranes of intestinal epithelial cells are more significant when carried out on plasma membrane fractions prepared from the same initial material. The present study investigated simultaneous preparation of brush-border and basolateral membrane vesicles from sea bass intestine. These two plasma membrane fractions were obtained by centrifugation of crude membrane preparations on a discontinuous sucrose gradient.

The brush-border membrane (Fraction F_2) was enriched about 15-fold in the luminal marker maltase-glucoamylase. This fraction was only slightly contaminated by basolateral membrane, as revealed by the variation in the relative specific activity of Na^+/K^+-ATPase (about 1.7-times). No other significant enzyme marker activities were detected in this membrane fraction. Bogé et al. [10] proposed another procedure for purification of brush-border membrane vesicles from sea bass intestine. However, their method only allows preparation of the brush-border membrane, with respective enrichments in maltase-glucoamylase and amino-peptidase activites of just 9.54 and 8.13. Another advantage of our preparation technique is the use of Mg^{2+} rather than Ca^{2+}, which is known to induce

phospholipase activation and membrane phospholipid breakdown.

Specific Na^+/K^+-ATPase activity in the basolateral membrane (Fraction F_1) was enriched about 12-fold. This result is in the same range as previously reported enrichments of this enzyme marker for fish intestine basolateral membrane preparations: 14.9 for *Anguilla anguilla* [15] and 11.3 for *Oreochromis mossambicus* [16]. Our results are comparable to those obtained for the simultaneous preparation of both membrane vesicles from mammal intestines by other laboratories, which obtained enzyme marker enrichments of 18 and about 11 in BBM and BLM vesicles, respectively [12,33]. However, they are lower than those obtained by Maenz and Cheeseman [35] for purification of both membrane vesicles from rat small intestine; in particular these authors reported 18-fold purification of BLM vesicles as determined by the increase in specific activity of ouabain-sensitive Na^+/K^+-ATPase compared to the initial homogenate. Enhancement of aminopeptidase N and maltase-glucoamylase activities in the BLM vesicles could be explained by cross-contamination of the basolateral membrane by the brush-border membranes. Another hypothesis is transit of these brush-border enzymes into the basolateral membrane during processing,

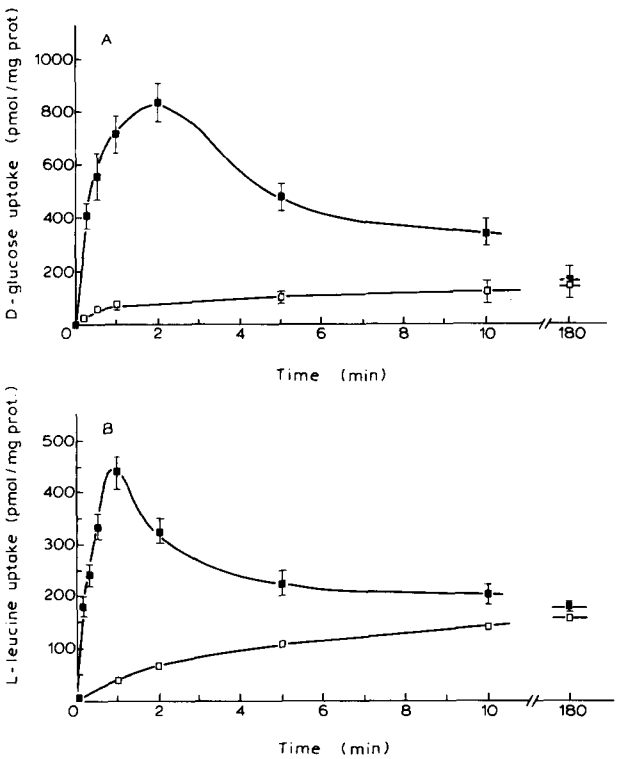


Fig. 3. Uptakes of 0.1 mM D-[^{14}C]glucose (A) and 0.2 mM L-[^{3}H]leucine (B) in brush-border membrane vesicles as a function of time. Assays were performed at 20 °C in 100 mM mannitol. 10 mM Hepes-Tris (pH 7.4) with 100 mM NaCl (■) or 100 mM KCl (□). Membrane vesicles were suspended in 300 mM mannitol, 10 mM Hepes-Tris (pH 7.4).

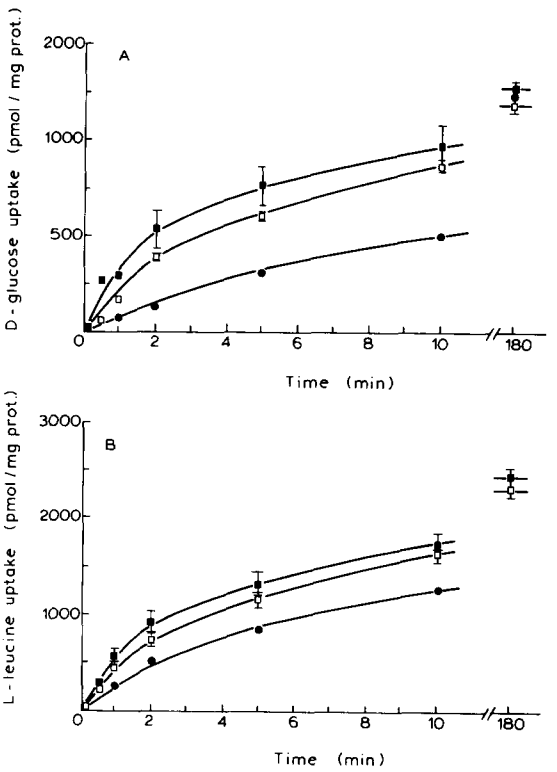


Fig. 4. Uptakes of 1 mM D-[^{14}C]glucose (A) and L-[^{3}H]leucine (B) in basolateral membrane vesicles as a function of time. Assays were performed at 20 °C in 100 mM mannitol, 10 mM Hepes-Tris (pH 7.4) with 100 mM NaCl (■); 100 mM KCl (□) or 100 mM NaCl + inhibitor (0.1 mM phloretin in Fig. 4A and 10 mM unlabelled L-leucine in Fig. 4B) (●). Membrane vesicles were suspended in 300 mM mannitol, 10 mM Hepes-Tris (pH 7.4).

as suggested by Colas and Maroux [33]. These authors estimated that the rabbit enterocyte basolateral membrane contains about 7% of the cellular aminopeptidase N and alkaline phosphatase activities.

In terms of enzyme recovery, the similar luminal and antiluminal membrane yields (average 11%) could be explained by equal proportions of these two membrane types in the total plasma membrane of sea bass enterocytes. Similar observations have been reported for the plasma membrane of rabbit enterocytes [33].

Study of the orientation of BBM vesicles was possible because of the asymmetric distribution of maltase-glucoamylase on the external side of the luminal membrane. When this enzyme activity towards an impermeant, high molecular weight substrate (starch) was measured before and after treatment with 2% Triton X-100 for 120 min, to solubilize this membrane enzyme [27–29], treatment was not found to induce a significative enhancement of the glucoamylase activity. This result is compatible with existence of 'right-side-out' vesicles in the brush-border membrane fractions. Kinsella et al. [32] used a technique to label the exposed sugar moieties of glycoproteins to determine the orientation of brush-border membrane vesicles prepared from

dog kidney cortex; for these authors, 86% of the luminal membranes were closed 'right-side-out'.

Basolateral membrane orientation can be determined because of the asymmetric distribution of the [^{3}H]ouabain binding sites [23]. In our experiments, specific [^{3}H]ouabain binding was increased 1.73-fold when the preparation was treated with deoxycholate/EDTA. This enhanced binding (43%) permits estimation of the percentage of sealed inside-out vesicles. About 57% of the BLM vesicles were thus sealed 'right-side-out' or were leaky. Only one of the two teams that previously prepared basolateral membranes from fish intestine studied the orientation of their membrane preparations. The basolateral membrane preparation obtained by Reshkin and Ahearn [16] from *Oreochromis mossambicus* intestine contained significant percentages of unsealed (70%) and 'inside-out' vesicles (20%).

To test whether brush-border and basolateral membrane vesicles purified in this manner retained their transport properties, D-glucose and L-leucine uptake were measured. Results demonstrate that both membrane preparations retained their transport properties [4–15]. BBM vesicles showed the characteristic Na^+-driven overshoot of both organic solutes. These results are similar to those obtained with other types of fish [4–12] and mammal intestines (for review, see Ref. 36). No overshoot was observed in the initial rates of both organic solutes in the presence of a Na^+ gradient. In addition, the uptake rates were always significantly higher, regardless of the cation used, than that observed in the presence of a transporter inhibitor. Indeed, these membrane vesicles transported D-glucose and L-leucine by transmembrane sodium-independent facilitated diffusion and apparent simple diffusion. These results concur with data obtained using BLM vesicle preparations of other types of fish intestines [13–15] and mammal intestines [35,37].

The difference between the initial uptake rates of both solutes by the basolateral membrane vesicles (about 1.25-fold stimulation in the presence of Na^+ compared to results with a K^+ gradient) could be accounted for by contamination of this membrane preparation by brush-border membrane. Similar observations have been made previously [34]. Another possible explanation is slower equilibrium of K^+ than Na^+. Indeed, it has recently been demonstrated that neither BBM vesicles nor BLM vesicles of rat renal cortical are equilibrated in 100 mM KCl, even after prolonged incubation, and that BLM vesicles are markedly less permeable to K^+ [38,39] than to Na^+. When compared to results obtained in presence of NaCl, KCl induces an inwardly directed gradient which produces a different equilibrium space and thereby could induce a lower membrane permeability for the solute.

Our purification process produced pure brush-border and basolateral membrane vesicles from the same initial material, and is thus a valuable experimental model for analysis of the influence of various factors (environmental salinity, qualitative and quantitative dietary modifications, presence of pollutants such as hydrocarbon derivatives and heavy metals) on the mechanisms of organic solute absorption across the epithelial cells of the small intestine.

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