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ISOLATION OF BRUSH BORDER MEMBRANES IN VESICULAR FORM FROM THE INTESTINAL SPIRAL VALVE OF THE SMALL DOGFISH *(SCYLIORHINUS CANICULA)*

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Summary

A simple, rapid method for the preparation of purified brush border membranes in vesicular form from rabbit kidney proximal tubules has been applied with closely similar results to the intestinal spiral valve of the small dogfish (*Scyliorhinus canicula*). Since the dogfish belongs to one of the most ancient species of fish, it may be suggested that the method is generally applicable to all species later evolved which possess a brush border membrane at the mucosal surface of the cells of the intestine or kidney.

The preceding paper [1] describes a rapid method for the isolation of kidney brush border membranes in vesicular form which was found to be applicable to other mammalian species. It was our interest to find out whether this method might be more generally applicable even to species remote from mammals. Read [2] showed that the spiral valve of the smooth dogfish, *Mustelus canis* possessed Na^+-dependent active transport systems for sugars and amino acids with characteristics indicating that they operated according to the gradient hypothesis of Crane [3, 4]. Accordingly, we focussed our attention on the species of small dogfish, *Scyliorhinus canicula*, found in local waters off the coast of southern France where it bears the familiar name of La Petite Roussette. The epithelial cells of the spiral valve of the dogfish from American waters show, under the electron microscope, a brush border surface remarkably similar to the intestine of mammalian species (Doyle, Wm.L., per-

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sonal communication). We assume the same for la petite roussette.

Small dogfish captured in the coastal waters off Toulon, were kept in tanks supplied with circulating, aerated sea water until used. Animals were killed by spinal cord section. The spiral intestine was rapidly removed, opened and spread on a chilled glass plate. The mucosa of all surfaces was collected by scraping, weighed and either used directly for membrane preparation or frozen. The mucosal scrapings recovered from each animal weighed between 1.5—2.1 g.

No attempt was made to use elasmobranch saline containing urea or otherwise to account for any special characteristics of the cells and membranes of the dogfish intestine as compared to mammalian kidney. The method as described in the preceding paper [1] was applied without change. Briefly, the scrapings were homogenized in a hypotonic mannitol-Tris solution. The homogenate was treated with Ca^{2+} to aggregate the cellular membranes other than the brush border, and subjected to successive centrifugations of, first, low and then, moderately high speed to separate the membranes and recover the brush border membrane as pellet. The pelleted membrane was vesiculated by several passes through a 23 gauge needle and brought to the desired volume. The buffer used in this step was routinely 0.1 M KCl containing 1 mM dithiothreitol and 5 mM Hepes Tris, pH 7.5, when studies of membrane transport were to be carried out. For enzyme assays, samples of the homogenate were taken prior to the addition of Ca^{2+} and the pelleted brush border membranes were taken up in the same mannitol Tris buffer used for homogenization.

The enzyme assays were carried out by the same methods referred to by Schmitz et al. [5]. Studies of D-glucose transport were carried out essentially as described by Hopfer et al. [6] with the minor modifications described in the legend to Fig. 1 which again are the same as in the preceding paper.

The transport experiments were carried out in July, 1978: the enzyme assays in September—October, 1978.

The state of purity of the brush border membrane fraction recovered from the dogfish intestine (Table I), resembles closely the same fraction recovered from rabbit kidney [1] except that in the dogfish intestine, there is detectable contamination by membranes containing succinate dehydrogenase (mitochondria) and $(Na^+ + K^+)$-ATPase (basolateral membranes). However, the level of contamination seems acceptable for most purposes. For $(Na^+ + K^+)$-ATPase, it is about the same as found by Evers et al. [7] using a much more elaborate separation technique.

Taking the β-galactosidase activity detected in the homogenate using lactose as substrate as a measure of lysosomal membrane, there seems to be none in the brush border fraction. The degree of purification taking maltase, alkaline phosphatase and leucylnaphthylamidase, together, as markers of brush border membrane appears to be at least as great for the dogfish intestine as for the rabbit kidney (average of 10.5-fold vs. 9.3-fold, respectively)*.

All of the enzyme assays were carried out on scrapings that had been in the deep-freeze for two months; this because we had previously determined

* With some species of fish, trials with different centrifugal forces may be needed to achieve the same degree of purification.

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 detectable.

Fraction	*n*	Homogenate (H)	Brush border membrane (M)	Relative activity (M/H)
Protein (mg)	8	131 ± 5	3.0 ± 0.2	0.023
Succinate dehydrogenase	4	98.5 ± 10.9	31.4 ± 3.7	0.32
NADPH-cytochrome *C* reductase	4	93.8 ± 3.2	53.5*	0.57
(Na^+-K^+)-ATPase	6	10.0 ± 0.9	21.2 ± 5.5	2.2
Maltase	4	53.9 ± 3.0	591.5 ± 47.7	11.0
Trehalase	4	7.9 ± 1.8	58.3 ± 15.1	7.4
Sucrase	4	8.5 ± 1.0	40.2 ± 8.0	4.7
β-galactosidase (lactase)		4.5 ± 0.5	N.D.	—
Alkaline phosphatase	4	20.3 ± 1.7	189.3 ± 36.5	9.3
Leucylnaphthylamidase	4	633.5 ± 43.6	7011 ± 7.29	11.1

*Statistics not determined.

that similar freezing of the tissue had no detectable effect on the formation of vesicles or their ability to carry out Na^+-dependent active transport (see below). However, assays of alkaline phosphatase carried out in July on brush border membranes prepared from tissue that had not been frozen gave somewhat better indications. Unfortunately, the method used for the determination of alkaline phosphatase at that time was different. We used then, for want of the usual reagents, the solutions and directions of the Boehringer-Mannheim automated analysis and took the difference in readings between 1 and 3 min in a spectrophotometer as a measure of enzyme activity. The units of activity recovered were 5—10 times higher than those shown in Table I, but the meaning of this difference is not clear without a direct comparison of the two assay methods. More to the point, however, and difficult not to take seriously is the specific activity ratio M/H, found in July; namely, 17.6 ± 3.3. At the present time, we can realistically go no further in conclusions about the efficacy for the dogfish intestine of the method of preparing brush border than that allowed in Table I. However, from the results in July, one may reasonably be aware of a possible greater efficacy of the method when tissue that has not been frozen is used. This can only be decided after a new fishing season for the local dogfish which occurs during the winter. In the summer months the dogfish seek the cooler water of greater depths and are out of reach of the gear available.

The brush border membrane vesicles exhibit Na^+-gradient coupled transport (Fig. 1) with the characteristics to be expected from prior studies with vesicles from mammalian intestine [8] and kidney [9]. Uptake of D-glucose is rapid in the presence of Na^+ and exceeds the final equilibrium position when a membrane potential (inside −) is induced by the liposoluble anion, SCN^-. This uptake of glucose is strongly inhibited in the presence of appropriate concentrations of α-methylglucoside, an alternative substrate for the process in the dogfish intestine [2] as well as in mammalian intestine and kidney, or of phlorizin, a particularly effective specific inhibitor of glucose transport in the dogfish intestine [2] as well as in mammalian intestine and kidney.

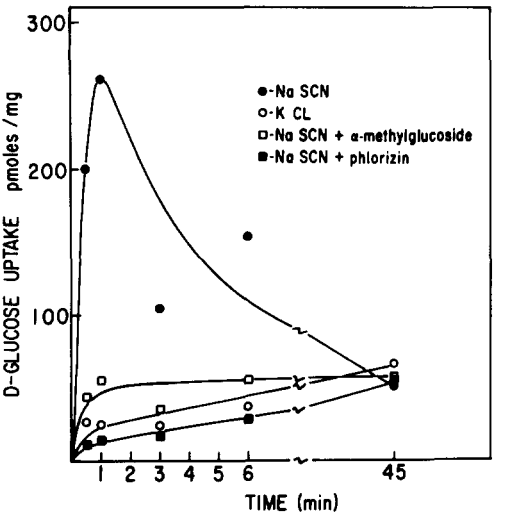

Fig. 1. Na^+-dependent active transport in brush border membrane vesicles from the spiral valve of the dogfish: membrane vesicles were loaded with 0.1 M KCl as described in the text. Uptake of D-glucose was measured by incubating 50 μl of membrane vesicles (0.05 mg protein) in a reaction mixture containing 0.2 mM D-[U-^{14}C] glucose, 5 mM Hepes Tris, pH 7.5 and as indicated, 0.1 M KCl or 0.1 M NaSCN with or without 5 mM β-methyl-D-glucoside or 0.2 mM phlorizin. 50-μl aliquots were removed at the time intervals indicated, 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 a modified Bray's solution and counted. Parallel results were obtained using 0.45 μm millipore filters. The points in the figure are the averages of 4 experiments.

A potential use of these findings may be in the field of environmental research, inasmuch as the intestine is a major entry point or target for noxious environmental agents. Some of the effect of these agents may be on the content of brush border membrane enzymes or the activity of brush border membrane transport system. The membranes prepared as described would be a suitable test material.

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