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ASYMMETRIC DISTRIBUTION OF OUABAIN-SENSITIVE ATPase ACTIVITY IN RAT INTESTINAL MUCOSA

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SUMMARY

A carefully washed preparation of intestinal brush border membranes from rat contained two-thirds of the sucrase activity of a starting homogenate and 2–4 % of the ouabain-sensitive ATPase activity.

It has been reported that ouabain-sensitive ATPase activity of intestinal mucosal cell is more densely distributed in brush borders than in basal and lateral plasma membranes[3–5]. These earlier studies, however, seemed to be done with crude brush border preparations possibly contaminated by basal and lateral membranes.

In the present work, crude brush borders were exhaustively washed to remove other membranous components. More than half of the total cellular sucrase activity was recovered in washed brush borders, whereas the activity of ouabain-sensitive ATPase detected in the same fraction was only a small fraction of the total activity.

METHODS

Cell fractionation

Two young albino rats (about 100 g each) were sacrificed in each experiment. The inside of an excised jejunum was flushed with cold saline from one end using a 50-ml syringe without a needle. The rinsed jejunum was cut into 10-cm segments and immediately placed in cold saline. Each segment was cut open, and the mucosal surface was blotted by pressing between two sheets of filter paper, then scraped softly with a slide glass. The scrapings from segments were collected in 5–10 ml of cold sucrose–EDTA medium (1 vol. of 0.25 M sucrose, 0.01 vol. of 10 mM EDTA adjusted to pH 7 with NaOH and 0.05 vol. of 50 mM histidine–imidazole buffer, pH 6.8) and transferred to a loosely fitting Dounce-type homogenizer (glass-Teflon; the diameter of the pestle, 18 mm) using another 10–15 ml of the same medium. The homogenization was performed applying 50 strokes without rotation of the pestle. After the volume was adjusted to 50 ml, the homogenate (Fraction 1) was centrifuged at 100 × *g* for 5 min. The supernatant (Fraction 2) was removed carefully with a large Pasteur pipette. The pellet was suspended in 20 ml sucrose–EDTA and homogenized for 30 strokes. The light microscopy showed that this fraction was mainly composed of individual and

aggregated brush borders and a large number of fine granules in the back-ground. After the volume was made to 50 ml, the crude brush border fraction was centrifuged at 250 × *g* for 5 min. The supernatant (Fraction 3) was recovered. The similar homogenization and centrifugation were repeated with decreasing numbers of strokes applied: 20, 10, 10, yielding supernatant Fractions 4, 5, 6, respectively (see Fig. 1). The pellet after Fraction 6 was suspended in 5 ml of sucrose–EDTA with a 10-stroke homogenization, then diluted with cold water to 50 ml. After 30 min at 0° the suspension was spun at 350 ×*g* for 5 min. The supernatant (Fraction 7) was removed and the pellet suspended in water (Fraction 8). The final suspension was assayed immediately with regard to ATPase and sucrase activities.

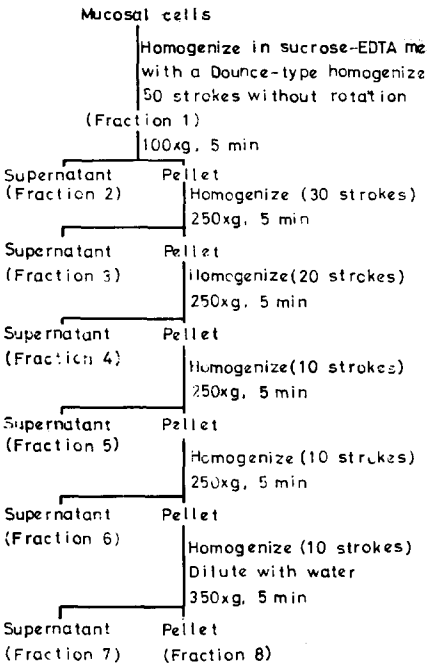

Fig. 1. Fractionation of rat jejunal mocosa.

Sucrase

The assay mixture for sucrase activity consisted of 50 mM histidine–imidazole buffer, pH 7.0, 30 mM sucrose, 50 mM NaCl and a suitable amount of a subcellular fraction in a total volume of 1.0 ml. The incubation was performed at 37° for 30 min, then terminated by placing the test tubes quickly in a boiling water bath and heating for 3 min. The liberated glucose was assayed with glucose oxidase–peroxidase system[1].

ATPase

ATPase activity was assayed with 5 mM $MgCl_2$, 100 mM NaCl, 10 mM KCl, 3 mM ATP (pH 7.4 with NaOH), 0.1 M Tris–HCl, pH 7.4, 0.5 mM ouabain when added, and a suitable amount of a subcellular fraction in the total volume of 1.0 ml. The incubation was carried out at 37° for 30 min, and the liberated P_i was determined by the method of Fiske and SubbaRow. Protein was determined by the method of LOWRY *et al.*

RESULTS

Rat jejunal mucosa was homogenized and fractionated as described in METHODS (see also Fig. 1). A minor sucrase activity was detected in each supernatant (Table I). However, 70 % of the total sucrase activity was recovered in the final pellet.

The ATPase activity resistant to ouabain was uniformly distributed in all fractions, while the sensitive activity showed a markedly different distribution pattern. Only 2 % of the total sensitive activity was found in the final pellet.

A separate experiment was carried out with slight modifications. The water treatment at the final step was omitted. Instead the last pellet was homogenized for 70 strokes and centrifuged at 1000 × *g* for 5 min. All the supernatants were combined as one fraction. An aliquot of the homogenate, the combined supernatant and the final pellet were subsequently centrifuged at 100000 × *g* for 30 min to remove soluble

TABLE I

DISTRIBUTION OF SUCRASE AND ATPASE ACTIVITIES IN SUBCELLULAR FRACTIONS OF RAT JEJUNAL MUCOSA

See METHODS for details. Also refer to Fig. 1 for the numbers of fractions.

Fraction	*Protein (mg)*	*Sucrase (μmoles substrates hydrolyzed/h)*	*ATPase (μmoles substrates hydrolyzed/h)*			*A/S**
			− Ouabain	*+ Ouabain*	*Difference*	
1	340	—	2070	1570	500	—
2	110	66	640	410	230	3.5
3	67	45	410	300	110	2.4
4	32	41	310	250	60	1.5
5	18	10	113	96	17	1.7
6	12	22	64	50	14	0.6
7	11	43	45	45	0	0
8	38	470	640	630	10	0.02
Recovery	288	697	2222	1781	441	

* *A/S*: The ratio of ouabain-sensitive ATPase to sucrase activity.

TABLE II

DISTRIBUTION OF SUCRASE AND OUABAIN-SENSITIVE ATPASE

An experiment similar to that of Table I. See the text for details.

Fraction	*Protein (mg)*	*Sucrase (μmoles substrates/h)*	*Ouabain-sensitive ATPase (μmoles substrates/h)*	*A/S**
Starting homogenate	100	520 (100%)	580 (100%)	1.1
Combined supernatant	85**	187 (36%)	580 (100%)	3.1
Final pellet	15	320 (62%)	25 (4%)	0.08
Recovery	100	507 (98%)	605 (104%)	

* See Table I

** The value was obtained by subtracting the protein content of the final pellet from that of the starting homogenate.

components. By this procedure the total sucrase and ouabain-sensitive ATPase activities were recovered in the pellets. The results are presented in Table II. In accord with the experiment of Table I, 60 % of the total sucrase activity was found in the final pellet. The ouabain-sensitive ATPase activity of the same fraction, however, was only 4 % of the total cellular activity.

When the final pellets in the present experiments were examined with a light microscope, single and paired brush borders formed the overwhelming portion of the visible particles, other components such as nuclei and cell debris being very scarce. The background was free of fine granular components such as those profusely present in the early supernatants.

DISCUSSION

The cellular sucrase activity was largely present in the brush border fraction. This finding suggests that the activity is associated with brush borders in agreement with the current conclusion[2]. This assumption is further supported by the highest specific activity shown by the brush border fraction (Table I). If we presume that sucrase is a marker enzyme of a brush border, we could say that 70 % of the total brush borders was recovered in the final pellet (Table I).

Earlier workers have concluded that a comparatively large portion of ouabain-sensitive ATPase activity was present in the brush border[3–5]. However, they used crude brush borders in their studies. We also obtained similar findings with our crude brush border fractions (Table III). Repeated homogenization, however, removed most of the activity as shown in Tables I and II, suggesting the increasing release of the basolateral membrane fragments to the supernatant. A high (Na^+,K^+)-ATPase activity was found in a membrane fraction differing from brush borders by density gradient centrifugation[9].

As suggested by the presence of minor sucrase activity in supernatants (Table I), it was likely that some brush borders were fragmented during repeated homogenization, although they seemed to be generally more resistant to mechanical forces than the other part of the surface membrane, presumably due to the fibrillar network beneath the microvilli.

TABLE III

APPARENT PRESENCE OF OUABAIN-SENSITIVE ATPASE ACTIVITY IN CRUDE BRUSH BORDER FRACTIONS

To obtain crude brush borders, rat jejunal mucosa was homogenized with a Dounce-type homogenizer for 50 strokes without rotation of the pestle, then centrifuged at 100 × *g* for 5 min. The concentration of ouabain was 0.5 mM. See METHODS for details. The figures in parentheses show the total ATPase activity of the fraction in the absence of ouabain.

Fraction	*Ouabain-sensitive ATPase activity (μmoles P_i released/h)*	
	Expt. 1	*Expt. 2*
Starting homogenate	580 (1450)	430 (1910)
Crude brush borders	190 (500)	260 (770)
Purified brush borders	25 (230)	6 (210)

One of the essential histological differences between epithelial and non-epithelial cells is that the former form a continuous boundary layer. In intestinal mucosa the apical surface of the cell faces a different environment from the one its basal and lateral surfaces do. The latter membranes may be regarded as forming one common surface or a functional unit as was discussed by PARSONS[6]. As a corollary the surface of an intestinal mucosal cell must be regarded as geometrically asymmetric. This is a situation not met in non-epithelial cells such as erythrocytes. This spatial asymmetry can have an important implication in cellular transport of solutes.

It seems evident from physiological works[7] that there is an active transport of Na^+ from mucosal to serosal medium irrespective of animal species. The measurements of transmural potential differences and short-circuit currents, their increases following luminal additions of amino acids or hexoses, and their inhibitions by serosal addition of ouabain, all favor the assumption that a coupled-transport system for Na^+ and nutrients is situated at the mucosal border, whereas an Na^+ pump is situated at the serosal border of the cell. The asymmetric distribution of an Na^+ pump such as this was first proposed by KOEFOED-JOHNSEN AND USSING[8].

Spatial asymmetry of Na^+ pumps does not necessarily require the absence of them in the brush border, provided that more pump activity is in the serosal part of the cell membrane than in the brush border. However, in our present work only 3–6 % of the total ouabain-sensitive ATPase activity was present in the brush border which occupies about 60 % of the cell surface by calculation (unpublished). This implies that if all the (Na^+,K^+)-ATPase activity is in the plasma membrane, then its relative density in the brush border is 2 % of its average density elsewhere. The asymmetry like this might well suggest that the regional specialization of the mucosal-cell surface membrane is fairly extensive.

The present work is restricted by the paucity of ouabain-sensitive ATPase in the brush border. Further work is necessary to present a conclusive evidence for the location of (Na^+,K^+)-ATPase in basal and lateral plasma membranes.

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