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A COMPARISON OF THE (Na^+–K^+)-ATPase ACTIVITIES FOUND IN ISOLATED BRUSH BORDER AND PLASMA MEMBRANE OF THE RAT INTESTINAL MUCOSA

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SUMMARY

A quantitative comparison of the properties of the (Na^+–K^+)-stimulated ATPase activities of isolated rat intestinal mucosal brush borders and plasma membranes is described. Differences in K_m for ATP, apparent K_i for ouabain and phloridzin, pH optima and other properties are presented.

INTRODUCTION

QUIGLEY AND GOTTERER[1,2] isolated a membrane fraction from rat intestinal epithelial cells which contained a majority of the total cellular (Na^+–K^+)-ATPase in high specific activity. This fraction was free of brush border membrane, nuclei, mitochondria and microsomal membrane and it was suggested that it represented a part of the lateral or basal plasma membrane. Additional evidence for this based on the lipid composition of this fraction will be reported[3]. A homogeneous brush border fraction was also isolated from the same cells. The brush border fraction consistently was found to contain about 15 % of the total cellular (Na^+–K^+)-ATPase. It was not possible to determine whether this activity in the brush border fraction was part of the microvillus membrane proper, separate and distinct from the plasma membrane (Na^+–K^+)-ATPase, or whether the ATPase activity represented tags of plasma membrane which remained attached to the brush border during fragmentation of the cell. This distinction is important in relating the properties of the (Na^+–K^+)-ATPase to their cellular location and their physiological role in the active transport of sodium and potassium across the intestinal epithelial cell. In an attempt to clarify this point the properties of the (Na^+–K^+)-ATPase in isolated brush borders were compared with those of the enzyme in the plasma membrane fraction.

MATERIALS AND METHODS

Preparation and purity of isolated fractions

Purified brush borders and plasma membrane were isolated by differential centrifugation and sucrose gradient centrifugation as described previously[1]. The pu-

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rity of the preparations was estimated by (1) phase contrast and electron microscopy (2) marker enzyme analysis and (3) lipid analysis. Phase contrast microscopy indicated that the brush border fraction was a homogeneous preparation, free of nuclei and whole cells. Electron microscopy revealed (Fig. 4, ref. 1) that the isolated brush borders contain the entire apical pole of the cell, including the microvillus membrane, core and terminal web. The micrographs of purified brush borders resembled those published by other laboratories[4,5] and indicated the presence of lateral tags on some of the brush border fragments. The plasma membrane fraction appeared in the electron micrographs as a heterogeneous population of membrane vesicles, free of brush border and mitochondria. Invertase and alkaline phosphatase were used as marker enzymes for the brush border and showed no contamination of the plasma membrane fraction by brush border. The (Na^+–K^+)-ATPase was employed to monitor the purification of the plasma membrane. An indeterminant amount of plasma membrane fraction may contaminate the brush border fraction. Glucose-6-phosphatase and cytochrome oxidase were also used to demonstrate that each preparation was free of microsomal and mitochondrial contamination, respectively. Lipid analysis[2] indicated that the brush borders and plasma membranes possessed characteristic ratios of cholesterol to phospholipid (1.1 and 0.5 μmoles cholesterol per μmole phospholipid, respectively) which are similar to other reported values for purified brush border[6] and plasma membrane[7].

Enzyme assays

Invertase, alkaline phosphatase, glucose-6-phosphatase, cytochrome oxidase and the (Na^+–K^+)-ATPase were measured as described previously[1].

RESULTS

The (Na^+–K^+)-ATPase activity of both the brush borders and the plasma membrane fraction is linear with respect to enzyme concentration, up to 400 μg of protein, and is linear with respect to time, up to 30 min, at 37°. Assay conditions were chosen within these limits so that the activities in the following studies represent true maximum initial velocities.

Fig. 1 shows that the activity as a function of pH of the two fractions showed slight differences. The plasma membrane (Na^+–K^+)-ATPase exhibited maximum activity between pH 6.5 and 7.0 while the brush border activity had a peak between pH 7.0 and 7.5.

The two enzyme systems behaved similarly with respect to activation by Na^+ and K^+. In the presence of optimal concentrations of K^+ (20 mM) the activation by Na^+ of both the plasma membrane and the brush border ATPase was nearly identical (Fig. 2). Both activities were optimal at 120 mM Na^+ and the brush border activity was only slightly inhibited at Na^+ concentrations as high as 300 mM. Similar activation by Na^+ was also obtained when the K^+ concentration was lowered to 2 mM. At a constant Na^+ concentration the activation by K^+ of both activities was similar, but not identical (Fig. 3). The plasma membrane enzyme appeared to be more sensitive to inhibition by higher concentrations of K^+, especially at low (20 mM) Na^+ concentrations.

The enzyme systems of the two preparations differed in their affinity for their

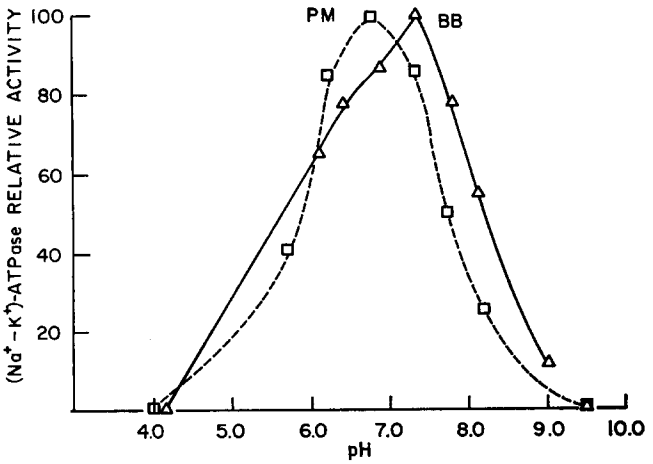

Fig. 1. Effect of pH on (Na^+–K^+)-ATPase activity. Each fraction was assayed in the presence of 5.0 mM ATP, 10 mM $MgCl_2$, 120 mM NaCl, 20 mM KCl, Tris (25 mM)–imidizole (25 mM)–glycine (25 mM) buffer at the indicated pH with about 50 μg plasma membrane (PM) protein or about 200 μg of brush border (BB) protein. Total assay volume was 1.0 ml and incubation was for 10 min at 37°. At each pH ouabain (1 mM)-insensitive activity was determined and subtracted from the total activity. The specific activity of the plasma membrane (Na^+–K^+)-ATPase at optimum conditions was 120 μmoles P_i liberated per h per mg, that of the isolated brush borders was 20 μmoles P_i liberated per h per mg. □ -----□, plasma membrane (Na^+–K^+)-ATPase; △—△, isolated brush border (Na^+–K^+)-ATPase.

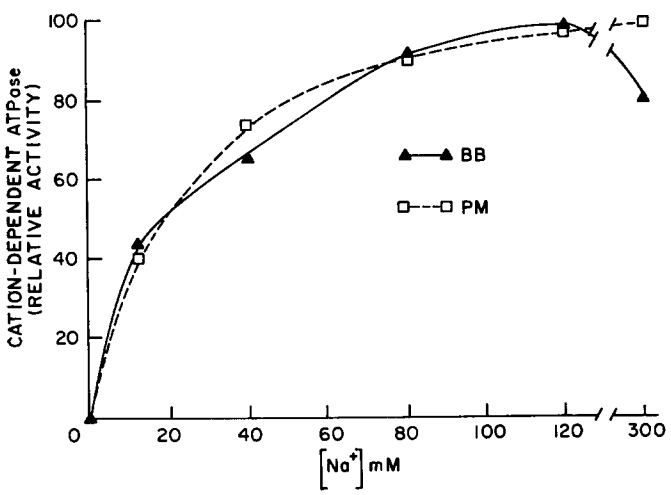

Fig. 2. Effect of Na^+ concentration on ATPase activity at constant K^+ (20 mM) concentration. The assay conditions and the specific activities at optimum conditions are given in the legend for Fig. 1. The buffer used was imidizole (30 mM), titrated to pH 7.0 with Tris. Cations were added as the chlorides. ▲—▲, BB (brush borders) (20 mM K^+); □-----□, PM (plasma membrane) (20 mM K^+).

substrate, ATP. Fig. 4 illustrates this difference in the form of a plot of activity versus ATP concentration, as well as a double-reciprocal plot from which the K_m for each enzyme system was calculated. It is important to point out that the values obtained at all substrate concentrations were rigorously determined. The enzyme at each substrate concentration was assayed in triplicate and the results in this experiment

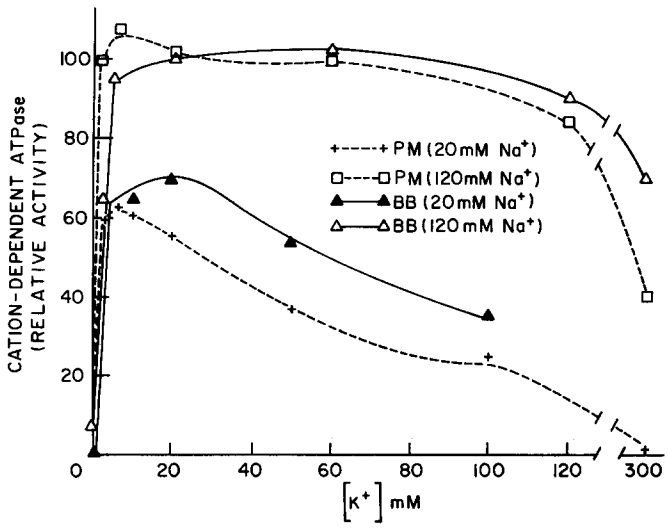

Fig. 3. Effect of K^+ concentration on ATPase activity at two separate Na^+ concentrations (20 mM and 120 mM). Conditions are the same as those described in Figs. 1 and 2. +-----+, PM (plasma membrane) (20 mM Na^+); □-----□, PM (120 mM Na^+); ▲—▲, BB (brush borders) (20 mM Na^+); and △—△, BB (120 mM Na^+).

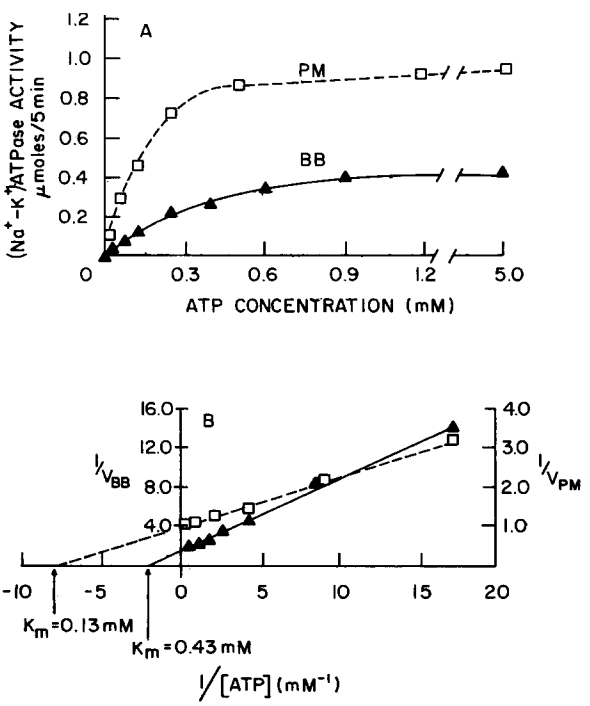

Fig. 4. Effect of ATP concentration on (Na^+–K^+)-ATPase activity. Mg^+/ATP ratio was kept at 2.0 at all ATP concentrations. Pyruvate kinase (20 μg) and phosphoenolpyruvate (2.0 mM) were added to the assay systems described in Figs. 1 and 2. □----□, PM (plasma membrane) (Na^+–K^+)-ATPase; ▲—▲, BB (brush borders) (Na^+–K^+)-ATPase.

were similar to those obtained in four separate experiments. Two techniques for the determination of phosphate were used and both short (30 sec) and long (10 min) incubation times were employed. An ATP generating system was used, for which the optimal concentrations of phosphoenolpyruvate and pyruvate kinase were determined in separate experiments. The optimum Mg^{2+}/ATP ratio, also determined separately, was held constant. Concentrations of substrate as low as $3 \cdot 10^{-5}$ M ATP were used, a value which was well below the calculated K_m. The final results demonstrated approximately a 3–4-fold difference in the K_m's measured for the two enzyme systems.

The (Na^+–K^+)-ATPase from the brush border fraction and the plasma mem-

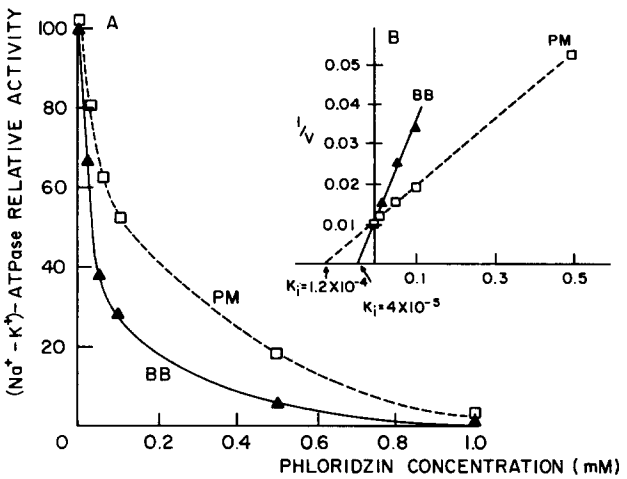

Fig. 5. Phloridzin inhibition of (Na^+–K^+)-ATPase activity. Assay conditions, specific activities and enzyme preparations were the same as described in Figs. 1 and 2. □-----□, PM (plasma membrane) (Na^+–K^+)-ATPase; ▲—▲, BB (brush border) (Na^+–K^+)-ATPase.

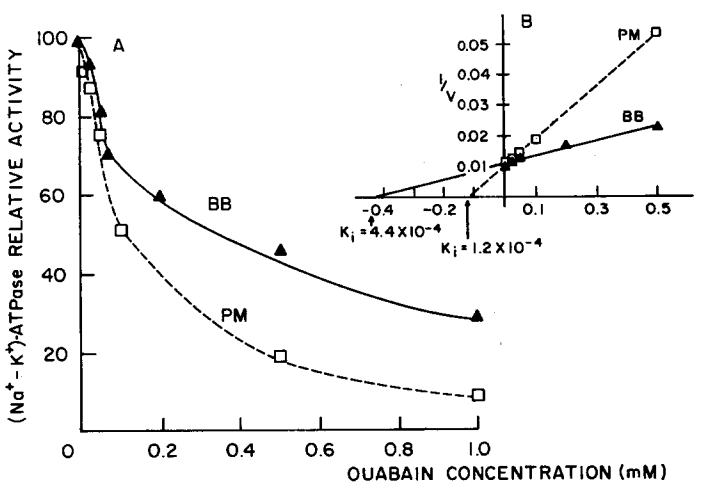

Fig. 6. Ouabain inhibition of (Na^+–K^+)-ATPase activity. Assay conditions, specific activities and membrane preparations are the same as described in Figs. 1 and 2. 1.0 mM ouabain had no effect on the ATPase activity assayed in the absence of Na^+ and K^+ (Mg^{2+}-ATPase). □-----□, PM (plasma membrane) (Na^+–K^+)-ATPase; ▲—▲, BB (brush border) (Na^+–K^+)-ATPase.

brane fraction exhibited different sensitivities to phloridzin and ouabain, classic inhibitors of the active transport of glucose and sodium-potassium, respectively. Fig. 5 demonstrates the effect of phloridzin on the two enzyme systems. It can be seen that the brush border (Na^+–K^+)-ATPase was more sensitive to phloridzin inhibition than was the plasma membrane ATPase system. On the other hand, the ATPases exhibited the opposite relative sensitivities to ouabain (Fig. 6). The plasma membrane (Na^+–K^+)-ATPase appeared to be more sensitive to inhibition by ouabain than was the brush border enzyme, although at very low concentrations of inhibitor a parallel loss of activity occurred.

A summary of the properties of each enzyme system is tabulated in Table I.

TABLE I

COMPARISON OF THE PROPERTIES OF THE (Na^+–K^+)-ATPase ACTIVITIES OF BRUSH BORDER AND PLASMA MEMBRANE PREPARATIONS

Property	*Brush borders*	*Plasma membrane*
Location on sucrose gradient	30–40% interface	20–30% interface
pH optimum	7.3	6.8
K_m ATP	$4.3 \cdot 10^{-4}$ M	$1.3 \cdot 10^{-4}$ M
Optimum Na^+ concentration	120 mM	120 mM
Optimum K^+ concentration	20 mM	10 mM
Optimum Mg^{2+} concentration	2 × ATP concn.	2 × ATP concn.
Q_{10}	1.8	1.8
K_i phloridzin	$4 \cdot 10^{-5}$ M	$12 \cdot 10^{-5}$ M
K_i ouabain	$4.4 \cdot 10^{-4}$ M	$1.2 \cdot 10^{-4}$ M
Relative specific activity *	1	4–10
Relative purification of membrane	10–15-fold **	25–30-fold ***

* Specific activity of brush border arbitrarily assigned value of 1.
** Based on specific activity of invertase compared with that of total homogenate.
*** Based on specific activity of (Na^+–K^+)-ATPase compared with that of total homogenate.

DISCUSSION

Comparison of the (Na^+–K^+)-stimulated ATPase activity catalyzed by brush borders and plasma membranes isolated from rat intestinal mucosal homogenates revealed small, but reproducible quantitative differences, as summarized in Table I. The measured differences are probably minimum differences since the brush border fraction is no doubt contaminated by residual lateral plasma membrane in the form of the lateral tags seen on electron microscopic examination[1].

Both preparations are membrane-bound and are only partially purified. The specific activity of the plasma membrane fraction is 4–10 times greater than that of the isolated brush border fraction. The small quantitative differences between the fractions could be due to these factors. On the other hand, the response of the two preparations to the inhibitors, ouabain and phloridzin, suggests that the (Na^+-K^+)-stimulated ATPase activities in the two preparations may in fact represent two different enzymes. A three to four fold difference in apparent K_i was found for each inhibitor between the two preparations. More significantly, phloridzin was found to be more inhibitory to the brush border activity, while ouabain was more inhibitory to the plasma membrane activity. These differences parallel the findings that phloridzin

inhibits transport of D-glucose at the brush border pole of the intestinal epithelial cell[8,9], while ouabain exerts its inhibition of the active transport of D-glucose by acting at the serosal margin[8].

Active transport of the glucose and galactose and certain amino acids into the intestinal mucosal cell has been shown to be coupled to the downhill movement of Na^+ into the cell at the mucosal pole[10,11]. The extracellular to intracellular gradient of Na^+ is presumably maintained by the operation of an energy-dependent Na^+ pump. The roles of the two (Na^+–K^+)-stimulated ATPases suggested by the present studies in the active transport of sugars and amino acids remain to be clarified.

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