SHORT COMMUNICATIONS

BBA 73046

The presence of K^+-dependent phosphatase in intestinal epithelial cell brush borders isolated by a new method

A K^+-activated phosphatase has been reported in preparations of membranes from red blood cells[1], brain[2–5], kidney[5,6], liver[5,7] and the electric organ of Electrophorus[8]. We here describe in preparations of brush borders isolated from intestinal epithelial cells the presence of an enzyme which hydrolyses a synthetic phosphatase–substrate (*p*-nitrophenyl phosphate) and which is activated by K^+ at neutral pH.

MILLER AND CRANE[9] and EICHHOLZ AND CRANE[10] have described procedures for preparing sheets of microvilli from hamster intestine and which are free of nuclei or other subcellular components, but other attempts have failed to separate the brush borders from the nuclei[11]. By our procedure the epithelial cell brush borders are harvested uncontaminated by the presence of other subcellular structures. The brush borders are separated from sucrose–EDTA solutions of increasing dilution. The mucosa is removed from the small intestine of a single animal (hamster, rat, chicken or pigeon) and homogenized in 0.3 M sucrose–5 mM EDTA (adjusted to pH 7.0 with Tris) (1 g wet wt. in 50 ml). Minimum clearance between homogenizer pestle and tube, 0.01 cm. The tissue is homogenized with 30 strokes (1 cycle up-down = 1 stroke) and filtered through nylon mesh. The homogenate is centrifuged (15000 × *g*·min, 0–2°), the supernatant discarded and the precipitate resuspended by homogenizing (6 strokes) in 50 ml of 300 mM sucrose–5 mM EDTA. The procedure is repeated, but the precipitate is taken up in 50 ml 225 mM sucrose–5 mM EDTA, and subsequently in 50 ml of 150 mM, 75 mM and then 37.5 mM sucrose solutions, all with 5 mM EDTA. The final precipitate is taken up in an appropriate buffer. Throughout the whole fractionation procedure it is essential to monitor the appearances of the successive precipitates with a high quality phase contrast microscope. In electronmicrographs (Fig. 1) the microvilli of brush borders thus prepared seem to overlie a thin felt-like network of microfibrillae (the terminal web), but to be free of inclusions such as nuclei; a variable amount of lateral cell membrane remains. The individual microvilli appear to have maintained their structural integrity extremely well; thus a glycocalyx is sometimes to be seen outside the triple membrane.

The *p*-nitrophenylphosphatase activity was assayed by measuring the rate of release of either *p*-nitrophenol by a modification of the method of FUJITA *et al.*[4], or of inorganic phosphate, by the method of FISKE AND SUBBAROW[12]. During a 30-min incubation the activity was linearly related to the amount of preparation used. All cations were added as their chlorides. Protein was determined by the method of LOWRY *et al.*[13]. *p*-Nitrophenylphosphatase activity was detected in the brush borders of the pigeon, hamster and rat. With the pigeon the specific activity of the phos-

phatase that required the presence of K^+ was over 3 times greater in the brush border preparation than in the original homogenate.

In the absence of a divalent cation, the activity of pigeon brush border *p*-nitrophenylphosphatase was low. The addition of Mg^{2+}, Zn^{2+}, Mn^{2+} or Ca^{2+} stimulated

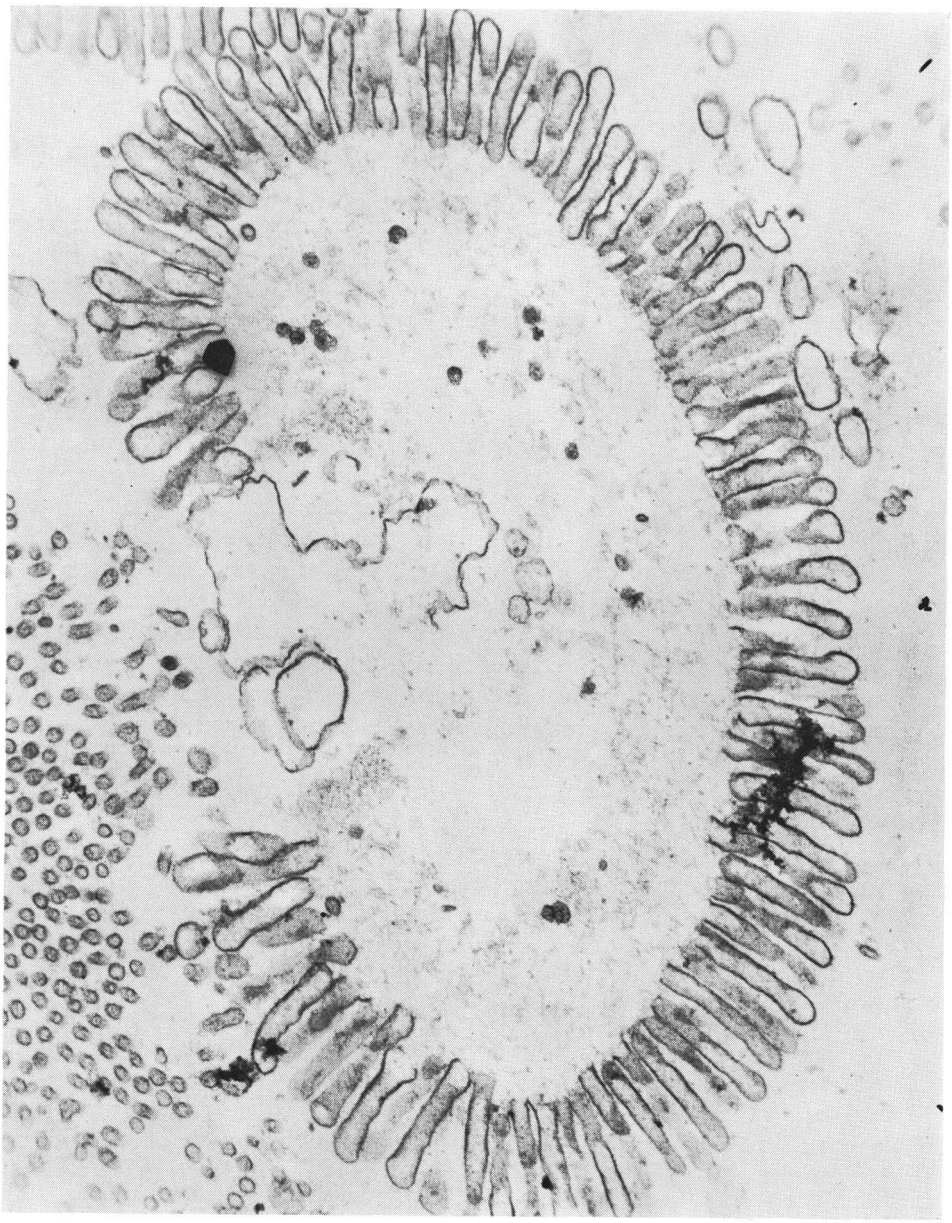

Fig. 1. Electron micrograph of single brush border from final precipitate of a preparation of pigeon intestinal epithelial cell brush borders isolated as described. Fixed with glutaraldehyde and post-osmicated. × 22 500.

the activity in decreasing order of effectiveness; the concentration of Mg^{2+} leading to half maximal increase in activity was 1.20 ± 0.19 (4) mM. In the presence of an optimal concentration of Mg^{2+} (5 mM), the addition of Ca^{2+}, Mn^{2+} or Zn^{2+} was inhibitory.

TABLE I

THE EFFECT OF Mg^{2+}, K^+ AND Na^+ ON *p*-NITROPHENYLPHOSPHATASE ACTIVITY, EXPRESSED AS PERCENTAGE MAXIMAL ACTIVITY, OF BRUSH BORDERS

30 min incubation at 37.5°. Incubation volume 1.1 ml containing 0.1 ml brush border preparation, 1.0 ml substrate (5 mM) + histidine imidazole buffer (pH 6.8; 50 mM) + ions indicated (added as their chlorides). Reaction stopped with 0.2 ml 30% trichloroacetic acid and *p*-nitrophenol absorbance determined at 410 mμ after adding 4 ml 0.15 M NaOH. Specific activities for pigeon 100 = 5.9, rat 100 = 5.0, hamster 100 = 5.3 μM *p*-nitrophenyl phosphate·mg protein^{-1}·h^{-1}.

Additions							
Mg^{2+}, $5 \cdot 10^{-3}$ M	—	+	—	+	+	+	+
K^+, $10 \cdot 10^{-3}$ M	—	—	+	—	+	+	+
Na^+, $50 \cdot 10^{-3}$ M	—	—	—	+	—	+	—
Ouabain, $0.1 \cdot 10^{-3}$ M	—	—	—	—	—	—	+
Brush borders from							
Pigeon	18	32	19	30	100	55	32
Rat	—	61	—	58	100	74	61
Hamster	—	74	—	69	100	79	74

In the presence of Mg^{2+}, the addition of K^+ increased the activity about 3-fold (Table I). Thus in 31 experiments on preparations from 16 pigeons the mean basal *p*-nitrophenylphosphatase activity without K^+ was 38.1 ± 7.6% of that in the presence of 10 mM K^+. The optimal concentration of K^+ was 10–15 mM; further addition of K^+ led to some decrease in activity. In the absence of other monovalent cations, the concentration of K^+ yielding half maximal increase in activity was 0.84 ± 0.20 (8) mM. This K^+-dependent activity had a specific activity of 4.20 ± 0.67 (5) μmoles *p*-nitrophenol liberated per mg protein per h and the substrate K_m was 1.1 mM. The K^+-dependent, but not the basal activity, was inhibited by ouabain (Strophantin G) (K_i $1.5 \cdot 10^{-5}$ M) and ATP (K_i $7 \cdot 10^{-4}$ M) but not by phloridzin. P_i ($K_i \approx 1 \cdot 10^{-3}$ M),

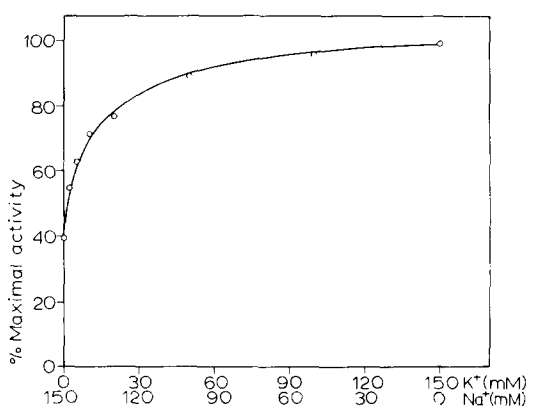

Fig. 2. Effect of K^+ and Na^+ concentration on pigeon brush border *p*-nitrophenylphosphatase activity. Conditions of assay as in Table I.

ADP ($K_i \approx 1.5 \cdot 10^{-3}$ M) and adenosine 3′-phosphate and adenosine 3′,5′-cyclic phosphate in decreasing order of effectiveness, inhibited both basal and K^+-sensitive activity. Rb^+, NH_4^+, Cs^+, in decreasing order of effectiveness, could substitute for K^+; Li^+ was scarcely effective, while both choline and Na^+, in the presence of Mg^{2+} only, caused a slight decrease in activity. However, Na^+ was unique in their ability to inhibit specifically the K^+-dependent activity; 50 % inhibition was caused by some 30 mM Na^+. This inhibition was kinetically complex; the addition of Na^+ not only decreased the overall rate of K^+-dependent *p*-nitrophenylphosphate hydrolysis at high K^+ concentrations, but also resulted in a decrease in the K^+ activation constant of the enzyme. As a result of this inhibition, the total phosphatase activity in a medium resembling an "intracellular" environment (high potassium, low sodium) is nearly twice that in one resembling the "extracellular" environment (high sodium, low potassium) (Fig. 2).

It would appear that the K^+-dependent ouabain-sensitive phosphatase of brush borders is similar to that reported in other membranes. The relation of this enzyme to the ($Na^+ + K^+$)-dependent ATPase first described by SKOU[14] is not clear. However, the effect of Na^+ on the K^+-dependent ouabain-sensitive phosphatase may indicate that the enzyme has a role in the membrane transport function of the microvilli.

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