

DISTRIBUTION OF BICARBONATE-STIMULATED ATPase IN RAT INTESTINAL EPITHELIUM

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Since the report of a HCO_3^- -sensitive ATPase in microsomal fractions from frog gastric mucosa (4) there has been speculation about a possible role of this enzyme in H^+ or HCO_3^- transport across epithelial cells. In organs such as stomach (17, 9), pancreas (12), salivary gland (11), renal proximal tubules (7), and choroid plexus (6), which transport $\text{H}^+/\text{HCO}_3^-$, an ATPase activity has been described, which is increased by various oxyanions and decreased by thiocyanate. In most of these tissues the HCO_3^- -sensitive ATPase was present in a microsomal fraction of tissue homogenates. Studies on the distribution of this enzyme are complicated by the fact that ATPase activity in mitochondria is stimulated by the same oxyanions as the presumed transport ATPase (2). In fact, recent reports on rat kidney (5), rat fundus mucosa (13), and dog salivary gland (3) provide evidence that the HCO_3^- -ATPase in these microsomal fractions is of mitochondrial rather than plasmalemmal origin.

There has been no report so far on HCO_3^- -ATPase in intestinal epithelium although this epithelium is known to transport HCO_3^- in large amounts (10). Mircheff and Wright (8) have recently described an analytical isolation procedure which separates both the brush-border and the basolateral membranes from each other and from the intracellular membranes, with good resolution and high recoveries. This powerful analytical procedure was used to determine the subcellular distribution of HCO_3^- -ATPase and thus to evaluate the possible role of this enzyme in intestinal HCO_3^- -transport.

MATERIALS AND METHODS

The small intestine was removed from 200-mg Sprague-Dawley male rats, and epithelial cells were harvested from the mucosa by gentle scraping. Isolation and purifi-

cation of plasma membranes was carried out according to Mircheff and Wright (8). Briefly, an initial centrifugation of the homogenate at 45,000 g for 20 min separated the soluble protein and light microsomes (S_0) from heavier components (P_0). Then, basolateral fragments together with mitochondria were separated from brush-border fragments by repeated differential centrifugation at 450 g for 10 min. Crude basolateral membranes (BLM) (P_4') and crude brush-border membranes (BBM) (P_9) were purified further by centrifugation on linear density gradients of 25% to 65% sorbitol for 24 h at 20,000 rpm in a Beckman SW 25.1 rotor (Beckman Instruments Inc., Spinco Div., Palo Alto, Calif.). Fourteen fractions and the pellet were collected from the density gradient and washed free of sorbitol by two centrifugations at 100,000 g for 20 min. The initial fractions, S_0 and P_0 , the crude BLM and BBM fractions, P_4' and P_9 , and the density gradient fractions were analyzed for their content of protein, Na-K-ATPase (a BLM marker), sucrase (a BBM marker), and succinic dehydrogenase (SDH) (a mitochondrial marker). The assays have been described previously (8). In addition, all the fractions were analyzed for their content of HCO_3^- -ATPase. HCO_3^- -ATPase activity was estimated as the difference in the rate of phosphate release from ATP between two media, one containing 100 mM NaHCO_3 and the other containing 100 mM NaSCN . In addition, both media contained 5 mM MgCl_2 , 50 mM TrisHCl , 3 mM NaATP , and 3 mM NaEDTA . De Pont et al. (1) have shown that the pH optimum for an anion-sensitive ATPase from gastric mucosa is between 8.4 and 9.0 for all the anions tested. For this reason, we set the pH of our assay mixture at pH 8.4 (i.e., the spontaneous pH of the HCO_3^- -containing solution). As a control, we determined the rate of phosphate release from ATP in Cl^- -containing medium. In homogenates of intestinal epithelium, the ATPase activity in Cl^- medium was about 40% lower than in HCO_3^- medium.

RESULTS

Table I shows the distributions of protein, Na-K-ATPase, sucrase, SDH, and HCO_3^- -ATPase

TABLE I
Distribution of Enzyme Activities among Fractions of Rat Small Intestine Obtained by Differential Centrifugation

	S ₀	P ₀	P ₄ '	P ₉
Na-K-ATPase	11.0	89.0	60.6	3.3
HCO ₃ ⁻ -ATPase	9.3	90.7	68.5	4.0
SDH	4.2	95.8	74.3	3.6
Sucrase	2.7	97.3	16.3	63.7
Protein	30.6	69.4	18.5	4.2

The results are mean values obtained from three preparations. S₀ and P₀ represent the initial centrifugation at 45,000 g for 20 min. P₄' and P₉ represent crude basolateral and crude brush-border fractions, respectively. Values presented are percentages of the total initial amounts. Since the first homogenate was difficult to sample correctly, initial quantities were taken as the sum of the amounts present in S₀ and P₀. The difference between the yield of enzyme markers (P₄' + P₉) and the starting material is accounted for by the differential fractions arising during the preparation of the crude plasma membranes (see reference 8, Table I).

among the initial centrifugation and crude brush-border and basolateral fractions. The overall recovery of all enzyme markers during differential centrifugation was 80%. This table shows clearly that most of the HCO₃⁻-ATPase activity is associated with either basolateral or mitochondrial membranes. Although the crude brush-border fraction contains a small amount of this enzyme, comparable amounts of Na-K-ATPase and SDH are present. Fig. 1 shows the results of purification of crude BLM on a density gradient. HCO₃⁻-ATPase activity follows closely the mitochondrial marker. Evidently, there is no HCO₃⁻-ATPase associated with purified basolateral plasma membranes. Sucrase (not shown in Fig. 1, for reasons of clarity) was recovered from fractions 7 through 11, and protein was distributed rather equally among all fractions. The recovery of markers from all density gradients was at least 78%. Since the recovery was comparable for all markers, protein included, no inactivation took place during purification. Recoveries reported here were identical to those previously reported (8).

In Fig. 2 the purification of crude brush-borders is shown. Again, the small percentage of the HCO₃⁻-ATPase activity in the sucrase peak coincides with an equal recovery of SDH activity. All the HCO₃⁻-ATPase measured in the epithelium is associated with mitochondria.

DISCUSSION

Transport of H⁺ and HCO₃⁻ across epithelial tissues is common to most epithelia studied so far (14). Absorption of Na⁺ is frequently accompanied by oppositely directed H⁺ secretion, and in most of these tissues HCO₃⁻ ions have a stimulating effect on Na⁺ transport (14). SCN⁻ is well known as an inhibitor of acid secretion in stomach, and recently it has been shown to inhibit salt and water transport in gallbladder epithelium (15). Therefore, it is not surprising that demonstration of a Mg-ATPase which could be stimulated by HCO₃⁻ and inhibited by SCN⁻ inspired models in which this ATPase is linked directly with H⁺/HCO₃⁻ transport. Inherent in such a model is the requirement that a transport-related ATPase activity be associated with plasma membranes. HCO₃⁻-ATPase activity has been demonstrated in preparations of renal brush-border membranes (7) and in microsomes from several epithelia (17, 12, 11, 16). It should be noted that the subcellular origins of microsomal fractions (meaning any small particles, even mitochondrial fragments) are

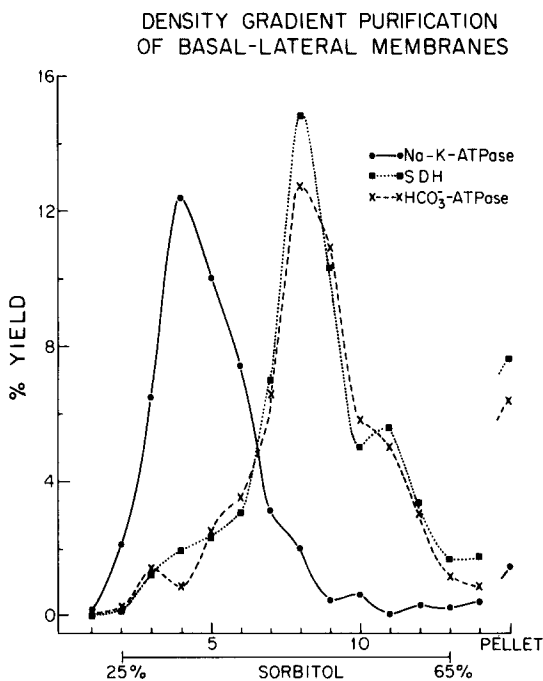


FIGURE 1 Distribution of markers following equilibrium density gradient centrifugation of crude baso-lateral membranes, P₄'. Yields are expressed as percentages of the total initial marker activities. Identical results were obtained in three different preparations.

DENSITY GRADIENT PURIFICATION
OF BRUSH-BORDER MEMBRANES

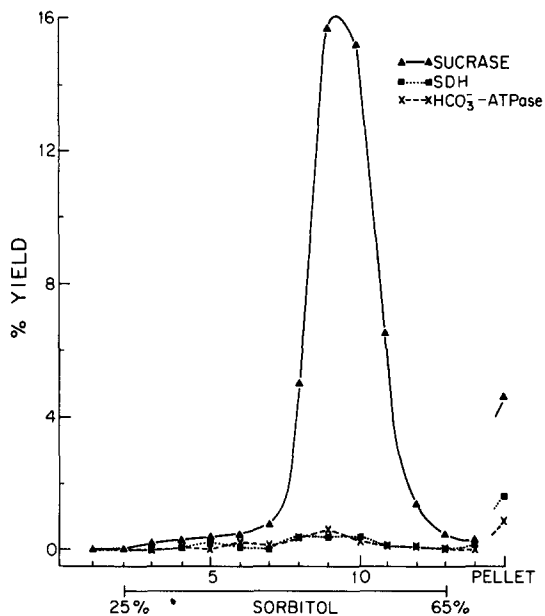


FIGURE 2 Distribution of markers following equilibrium density gradient centrifugation of crude brush-border membranes, P_3 . Yields are expressed as percentages of the total initial marker activities. Identical results were obtained in three different preparations.

not well defined. The present work shows unambiguously that all the HCO_3^- -ATPase activity measured in the intestinal epithelium is associated with mitochondrial membranes. By implication, there is no ground for speculation that plasma membrane HCO_3^- -ATPase plays a role in rat intestinal $\text{H}^+/\text{HCO}_3^-$ transport.

SUMMARY

This study reports on the distribution of bicarbonate-stimulated ATPase in rat intestinal epithelial cells. Brush-border membranes and basolateral membranes were separated from each other and from mitochondrial and other intracellular membranes by differential and density gradient centrifugation. Bicarbonate-sensitive ATPase activity followed the mitochondrial marker succinic dehydrogenase closely throughout all the centrifugation steps. The low HCO_3^- -ATPase activity in purified brush-border and basolateral plasma membranes could be accounted for quantitatively by the small mitochondrial contamination. Consequently, there are no grounds for postulating that

this enzyme has a direct role in H^+ or HCO_3^- transport across the rat small intestine.

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