

## Presence of Bicarbonate Stimulated ATPase in the Brush Border Microvillus Membranes of the Proximal Tubule (38186)

EVAMARIA KINNE-SAFFRAN AND ROLF KINNE  
(Introduced by I. L. Schwartz)

*Max-Planck-Institut für Biophysik, 6 Frankfurt 70, West Germany*

The proximal tubule of the kidney possesses a high transport capacity for bicarbonate; about 90% of the amount filtered by the glomerulus is reabsorbed in this part of the nephron (1). In terms of irreversible thermodynamics this transport can be defined as an active process (2). In other organs like stomach, pancreas and glandula submandibularis, which transport  $H^+/HCO_3^-$  actively, an ATP-phosphohydrolase stimulated by oxyanions such as bicarbonate and sulfite and inhibited by isothiocyanate and cyanate has been described (3-5). According to its behavior during cell fractionation procedures this enzyme is thought to be a constituent of the plasma membrane of the cell (3-5).

In the proximal tubule evidence for the presence of a plasma membrane bound  $HCO_3^-$ -ATPase is lacking. The main difficulty in studies on the distribution of this enzyme in the tubular cell is, as also pointed out by Katz and Epstein (6), the high  $HCO_3^-$  stimulated-ATPase activity in the mitochondria. In the present investigation free flow electrophoresis was utilized to separate plasma membranes from mitochondria and furthermore to distinguish between the plasma membranes derived from the apical region of the tubular cell (brush border microvilli) and the basal-lateral plasma membranes (7).

By comparison of the marker enzymes for the different membranes and the mitochondria it could be shown that the brush border microvilli, but not the basal-lateral plasma membranes, contain a non-mitochondrial oxybase-stimulated ATPase which in analogy to other buffer transporting or-

gans might be involved in the active proton transport in the proximal tubule (8, 9, 10).

*Methods.* Preparation of membrane fractions from rat kidney cortex: Plasma membranes of rat kidney cortex were obtained by differential centrifugation from the kidneys of 20 male Wistar rats (180-200 g body weight) in isotonic sucrose medium (250 mM sucrose, 10 mM triethanolamine-HCl, pH 7.6 at 20°) after homogenization with Teflon-glass homogenizers as described previously (11). This plasma membrane fraction was further subdivided into brush border microvilli and lateral-basal plasma membranes by preparative free flow electrophoresis. The electrophoresis was carried out in a FF4 electrophoresis apparatus (DESAGA, Heidelberg) according to Heidrich *et al.* (7).

*Determination of enzyme activities and protein.* Commonly used tests were applied to measure protein and enzymes. They have been described previously (7, 12).

The  $HCO_3^-$ -ATPase activity was measured using 75 mM Tris (hydroxymethylaminomethan)-HCl-buffer pH 7.6 in the presence of 6 mM  $MgSO_4$  and 2 mM ouabain with and without the addition of bicarbonate (sodium salt) to a final concentration of 25 mM. Three mM  $Na_2ATP$  was used as substrate. After 30 min of incubation at 37° the samples were heated for 1.5 min in boiling water, chilled and centrifuged. The amount of  $P_i$  liberated was determined in aliquots of the supernatants using the methods of Bartlett and Fiske-Subbarow (13, 14). The increase of ATPase activity in the presence of bicarbonate was considered as  $HCO_3^-$ -ATPase and

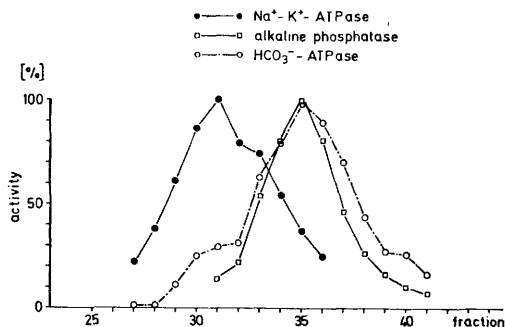


FIG. 1. Separation of plasma membrane fractions from rat kidney cortex by free flow electrophoresis. The activity in mU/ml of alkaline phosphatase, HCO<sub>3</sub><sup>-</sup>-ATPase and Na<sup>+</sup>-K<sup>+</sup>-ATPase in the fractions collected is expressed as percent of the maximal activity. The mean values of 4 experiments are given.

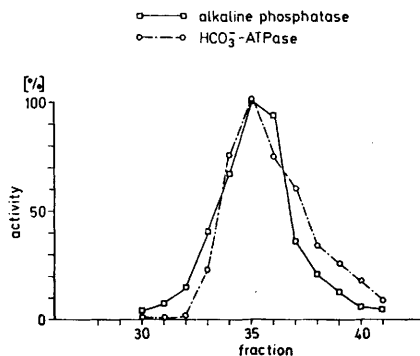


FIG. 2. Reelectrophoresis of the fractions 35–40 pooled from the separation shown in Fig. 1. The activity in mU/ml of alkaline phosphatase and HCO<sub>3</sub><sup>-</sup>-ATPase in the fractions collected is expressed as percent of the maximal activity. The mean values of 3 experiments are given.

amounted to about 20%–40% of the activity found in the presence of Mg<sup>2+</sup> alone.

The succinic dehydrogenase was measured according to Gibbs and Reimer after pretreatment of the membranes with 0.1% DOC (15).

**Results.** During the isolation of a plasma membrane fraction from rat kidney cortex the HCO<sub>3</sub><sup>-</sup>-ATPase is enriched about 2 times ( $2.3 \pm 0.3$ ,  $n = 4$ ) compared to the homogenate, whereas the activity of succinic dehydrogenase (SDH) as a marker enzyme for mitochondria is reduced from 24  $\mu$ U/mg protein to 4  $\mu$ U/mg protein. If this membrane fraction is further subdivided by free flow electrophoresis into brush border microvilli (marker enzyme: alkaline phosphatase) and lateral-basal plasma membranes (marker enzyme: Na<sup>+</sup>-K<sup>+</sup>-ATPase), the HCO<sub>3</sub><sup>-</sup>-ATPase follows

the alkaline phosphatase (Figs. 1 and 2).

The increase of alkaline phosphatase activity in the purified brush border microvilli fraction is accompanied by an increase in HCO<sub>3</sub><sup>-</sup>-ATPase activity (Table I). Since the SDH activity is further reduced in this membrane fraction the HCO<sub>3</sub><sup>-</sup>-ATPase activity measured cannot be attributed to mitochondrial contamination but represents a brush border membrane bound enzyme.

The brush border membrane HCO<sub>3</sub><sup>-</sup>-ATPase is maximally stimulated by oxybases when ATP is used as substrate. If ATP is replaced by CTP, GTP, ITP or UTP only up to 20% of the activity observed in the presence of ATP can be obtained.

The naturally occurring oxybase bicarbonate activates the enzyme optimally in the range from 25 to 50 mM, half maximal

TABLE I. The Enzyme Activities Measured in the Different Membrane Fractions are Expressed in mU/mg Protein, for Succinic Dehydrogenase in  $\mu$ U/mg Protein. The Mean Values  $\pm$  SE of 4 Experiments are given.

Membrane fraction	Alkaline phosphatase	HCO <sub>3</sub> <sup>-</sup> -ATPase	Na-K-ATPase	Succinic dehydrogenase
Plasma membrane fraction	$1.1 \pm 0.2$	$0.13 \pm 0.02$	$0.16 \pm 0.02$	$4.0 \pm 0.2$
Brush border membranes	$1.8 \pm 0.2$	$0.18 \pm 0.04$	$0.05 \pm 0.02$	$2.1 \pm 0.1$
Lateral-basal plasma membranes	$0.3 \pm 0.1$	$0.06 \pm 0.01$	$0.42 \pm 0.02$	$3.6 \pm 0.2$

stimulation is found at a bicarbonate concentration of 16 mM. Of the other oxyanions tested, sulfite (25–50 mM) is slightly more effective than bicarbonate, whereas thiocyanate (25 mM) and cyanate (25 mM) inhibit the HCO<sub>3</sub><sup>-</sup>-ATPase almost completely.

*Discussion.* On the basis of studies in several H<sup>+</sup>/HCO<sub>3</sub><sup>-</sup>-transporting organs it has been postulated that a membrane bound HCO<sub>3</sub><sup>-</sup>-ATPase is involved in the active buffer transport (3, 4). The presence of a bicarbonate stimulated ATPase in the brush border microvilli can lead to analogous considerations for the proximal tubule.

Bicarbonate reabsorption in the proximal tubule has been attributed to an active transfer of protons through the membrane of the epithelial cell (8, 9, 10). It is not clear however, whether this transport is linked directly to the cellular metabolism via ATP or whether it is coupled to another energy dependent transport performed by the proximal tubule such as sodium transport. Our results clearly favor the former view, and so do recently performed electrophysiological studies (16).

The existence of 2 different ATP-phosphohydrolases: a bicarbonate stimulated ATPase in the brush border and a Na<sup>+</sup>-K<sup>+</sup>-activated ATPase in the basal-lateral membranes might explain the only partial inhibition of sodium transport in the proximal tubule by ouabain (18), but complete blockade by metabolic inhibitors like cyanide (17). Ouabain probably only blocks that part of the sodium transfer which is linked to the action of Na<sup>+</sup>-K<sup>+</sup>-ATPase directly, whereas the remaining transport is maintained by other factors such as the bicarbonate ATPase-mediated proton secretion, which is not affected by ouabain (16). If however the activity of both ATPases is reduced by lack of ATP-production in the presence of metabolic inhibitors a complete inhibition of the sodium reabsorption should be expected.

*Summary.* A non-mitochondrial bicarbonate-stimulated adenosine triphosphatase has been localized in the brush border microvilli of rat proximal renal tubular cells. This finding is in accord with the putative role of this enzyme in the active transport of protons by the proximal tubule.

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