Biochimica et Biophysica Acta, 471 (1977) 78—91

BBA 77841

IS THERE A PLASMA-MEMBRANE-LOCATED ANION-SENSITIVE ATPase?

II. FURTHER STUDIES ON RABBIT KIDNEY

J.M.M. VAN AMELSVOORT [a], J.J.H.H.M. DE PONT [a], A.L.H. STOLS [b] and S.L. BONTING [a]

Departments of [a] Biochemistry and [b] Submicroscopic Morphology, University of Nijmegen, Nijmegen (The Netherlands)

(Received March 23rd, 1977)

Summary

A study has been made to determine whether renal plasma membranes contain an HCO_3^- stimulated, ouabain insensitive Mg ATPase. Purified mitochondrial, microsomal and brush border membrane fractions have been isolated from rabbit kidney.

The microsomal anion-sensitive ATPase activity appears to be entirely of mitochondrial origin on the basis of the effects of inhibitors of mitochondrial Mg ATPase.

The brush border membrane fraction is contaminated with mitochondrial fragments and contains an Mg ATPase activity with low anion-sensitivity. Further purification of this fraction causes parallel decreases in anion-sensitivity of the Mg ATPase activity and in cytochrome *c* oxidase activity.

These results indicate that conclusions previously reached by other investigators for a role of anion-sensitive Mg ATPase in the bicarbonate reabsorption of the proximal tubule may no longer be tenable.

Introduction

Of the various ATPases thought to be involved in ion transport across cell membranes, the anion-sensitive ATPase is still the most dubious one. Although a role of anion-sensitive ATPase in anion transport and indirectly in H^+ transport has been suggested [1—3], no direct proof for such a role has been supplied so far.

In an earlier study [4] we have investigated in three tissues with active anion transport whether any anion-sensitive ATPase activity is located in the plasma membrane. Subcellular fractions have been prepared from rainbow trout gill, rabbit gastric mucosa and rabbit kidney by means of differential and density gradient centrifugation and subjected to enzyme studies. In the case of trout

gill and gastric mucosa an exclusively mitochondrial localization of the enzyme has been found. Possible explanations for earlier erroneous conclusions of other investigators have been offered. Other reports for gastric mucosa and other tissues support a mitochondrial origin of the enzyme [5—8].

For rabbit kidney we were unable to reach a definite conclusion, because the mitochondrial and plasma membrane marker enzymes greatly overlapped in the subfractions obtained by density gradient centrifugation of a light mitochondrial (20 min 20000 × *g*) and a microsomal (60 min 100000 × *g*) fraction of rabbit kidney. A mitochondrial anion-sensitive ATPase activity appears to be present in this tissue, but the possibility of the existence of such activity in the plasma membrane could not be excluded.

Previously, Katz and Epstein [9] have found a parallel distribution of HCO_3^- stimulated ATPase activity and of the mitochondrial marker enzyme succinic dehydrogenase in various subcellular fractions of rat kidney. Kinne-Saffran and Kinne [10] find this enzyme to be present in the brush border fraction of rat kidney, and Liang and Sacktor [11] have further characterized the enzyme from the same structure in rabbit kidney cortex. Since these authors [10,11] suggest that this enzyme would be involved in bicarbonate reabsorption (or proton secretion) in the proximal tubule, we have re-examined the characteristics of the anion-sensitive ATPase activity in whole rabbit kidney and especially of that in the brush border fraction of the kidney cortex.

Methods and Materials

Preparation of mitochondrial and microsomal fraction

Kidneys of New Zealand white rabbits are obtained immediately after killing the animals by a blow on the neck, followed by carotic exsanguination. They are cooled on ice, rinsed and decapsulated. The tissue is then minced with scissors and homogenized in 9 volumes 0.25 M sucrose, 25 mM Tris/maleate (pH 7.6) in a teflon/glass homogenizer (Potter/Elvehjem) with 4—5 strokes at 850 rev./min. All operations are performed at 0—4°C. Mitochondrial (10 min 10000 × *g*) and microsomal fractions (60 min 100000 × *g*) are prepared as described previously [4]. Both fractions are further fractionated by resuspending in the homogenization buffer and layering them on top of a linear gradient of 30 to 55% (w/v) sucrose in 25 mM Tris/maleate (pH 7.6). Gradients are routinely centrifuged for 16—17 h at 24000 rev./min (64000 × g_{av}) in the SB 110 rotor of the IEC type B 60 ultracentrifuge. Approximately 25 fractions are collected by means of a density gradient removing apparatus (Auto-Densiflow IIc, Buchler Instruments, Searle Analytic Inc., Fort Lee N.J., U.S.A.) connected to a fraction collector (LKB, Bromma, Sweden). Sucrose concentrations are measured in an Abbe refractometer at 20°C.

Appropriate fractions, selected on the basis of the cytochrome *c* oxidase distribution pattern (mitochondrial fraction; fraction numbers 4—8 in Fig. 1) or of the (Na^+ + K^+)-ATPase distribution pattern (microsomal fraction; between 34.5 and 41% (w/v) sucrose, fraction numbers 13—18 in Fig. 2), are pooled, diluted with an equal volume 25 mM Tris/maleate (pH 7.6), centrifuged for 60 min at 100000 × *g* and resuspended in homogenization buffer for further studies.

Preparation of brush border fraction

The method of Aronson and Sacktor [12] is used. Sometimes this fraction is resuspended in 0.25 M sucrose, 25 mM Tris/maleate (pH 7.6) and further purified by layering it on a linear gradient of 38 to 49% (w/v) sucrose in 25 mM Tris/maleate (pH 7.6) with a cushion of 64.5% (w/v) sucrose in the same buffer and centrifuging and processing it as described above.

Protein determination

Protein concentrations are estimated by the method of Lowry et al. [13] with bovine serum albumin, dissolved in homogenization buffer, serving as standard.

In gradient fractions protein concentrations are determined by measuring 280 nm absorbance (A_{280}) after dilution with 1.5 volumes of 2% (w/v) sodium dodecylsulphate to eliminate scattering in the cuvet. Corrections are made for the absorbance of sucrose.

Enzyme assays

Cytochrome c oxidase is estimated after Cooperstein and Lazarow [14] at room temperature (22°C) in the presence of 0.6% Tween-80 (w/v) and expressed as the rate of the initial decrease in 550 nm absorbance with an initial absorbance of 0.5 (equivalent to a cytochrome *c* concentration of approx. 17 μM). A unit of enzyme activity is defined as a decrease of one absorbance unit per minute.

5'Nucleotidase is measured according to Heppel and Hilmoe [15].

γ-Glutamyltranspeptidase is measured after Orlowski and Meister [16].

(Na^+ + K^+)-ATPase is measured as described by Bonting (ref. 17, p. 261) in the modification of Schuurmans Stekhoven et al. [18].

Alkaline phosphatase is estimated by adding a 20 μl aliquot to 200 μl incubation mixture containing (final concentrations): 4.5 mM $MgCl_2$, 4.5 mM *p*-nitrophenylphosphate, 0.45 M 2-amino-2-methyl-propanol-1-HCl (pH 9.5). After a suitable period of incubation at 37°C, the reaction is stopped by addition of 3 ml 0.1 N NaOH. The liberated *p*-nitrophenol is determined by reading the absorbance at 410 nm against suitable blanks and standards.

Anion-sensitive Mg ATPase is measured at pH 8.4 in the presence of 10^{-4} M ouabain in media of constant ionic strength, containing predominantly either HCO_3^- or Cl^- [4].

When inhibitors in ethanolic solution are applied, controls are run containing the same ethanol concentration (<2% v/v). Preincubation for 15 min at 0°C (dicyclohexylcarbodiimide: 60 min 0°C) and 5 min at 37°C in the absence of ATP precedes the incubation in this case.

Electron microscopy

The brush border fraction, after resuspension in 0.25 M sucrose, 25 mM Tris/maleate (pH 7.6) is fixed by addition of 2 volumes 3% glutaraldehyde in 0.25 M sucrose, 25 mM Tris/NaOH (pH 7.6), followed by centrifugation for 10 min at 27000 × *g*. The pellet is left with part of the supernatant at 4°C overnight. The next day the pellet is postfixed with 2% OsO_4 and then dehydrated

and embedded in Epon. Thin sections, stained with uranyl acetate and lead citrate, are examined in a Philips EM 300 electron microscope.

Materials

Cytochrome *c* (horse heart), Na_2ATP, Na_2AMP and L-α-glutamyl-*p*-nitranilide are obtained from Boehringer (Mannheim, G.F.R.), Tween-80 from Atlas Goldschmidt GmbH (G.F.R.), sodium dodecyl sulphate (SDS) from Sigma (St. Louis, Missouri, U.S.A.). Oligomycin (Sigma, St. Louis, Missouri, U.S.A.) consists of 15% oligomycin A and 85% oligomycin B (average molecular weight 400). Dicyclohexylcarbodiimide, from Nutritional Biochemical Corp., is vacuum-distilled before use. Aurovertin D (isolated by Dr. R. Bertina and donated by the B.C.P. Jansen Institute, University of Amsterdam) is dissolved in absolute ethanol and the concentration is determined spectrophotometrically at 367.5 nm using an absorbance coefficient of 42.7 $mM^{-1} \cdot cm^{-1}$ [19]. All other reagents are from E. Merck (Darmstadt, G.F.R.) and are of analytical grade.

Results

Mitochondrial and microsomal fractions

Anion-sensitive ATPase activities of various subcellular fractions of rabbit kidney, viz. nuclear (10 min $1000 \times g$), heavy mitochondrial (10 min $10000 \times g$), light mitochondrial (20 min $20000 \times g$), microsomal (60 min $100000 \times g$) and supernatant, have been represented in an earlier paper [4].

Figs. 1 and 2 show the distribution patterns of the enzyme activities in the mitochondrial (10 min $10000 \times g$) and microsomal (60 min $100000 \times g$) fractions, subjected to sucrose density gradient centrifugation. In the mitochondrial fraction (Fig. 1) the anion-sensitive ATPase activity (measured in the stimulatory HCO_3^--rich medium and in the intermediate Cl^- medium) and the mitochondrial marker enzyme cytochrome *c* oxidase coincide completely. $(Na^+ + K^+)$-ATPase activity is extremely low or negligible.

In the microsomal fraction (Fig. 2) there is a rather poor separation between cytochrome *c* oxidase activity, which probably results from mitochondrial fragments as postulated previously for gastric mucosa [4], and $(Na^+ + K^+)$-ATPase activity. Hence, no conclusions can be drawn about the nature of these subfractions. Anion-sensitive ATPase activity is distributed over a large range of the gradient, while an anion-insensitive ATPase activity is seen at the top of the gradient only. An anion-sensitive ATPase activity, not coinciding with peak activities of cytochrome *c* oxidase, appears to be present in subfractions 13—18 (Fig. 2).

In order to characterize the anion-sensitive ATPase activity, coinciding with $(Na^+ + K^+)$-ATPase in the microsomal fraction, we have pooled subfractions 13—18 (Fig. 2) and compared this material with the mitochondrial fraction (subfractions 4—8, Fig. 1) for inhibition by oligomycin, aurovertin D and dicyclohexylcarbodiimide. The effects of oligomycin on both fractions is shown in Fig. 3a. The anion-sensitive ATPase activity in the microsomal fraction appears to be even more sensitive to oligomycin (pI_{50} 7.8) than that in the mitochrondrial fraction. This may be due to the shape of the inhibition curve, which pos-

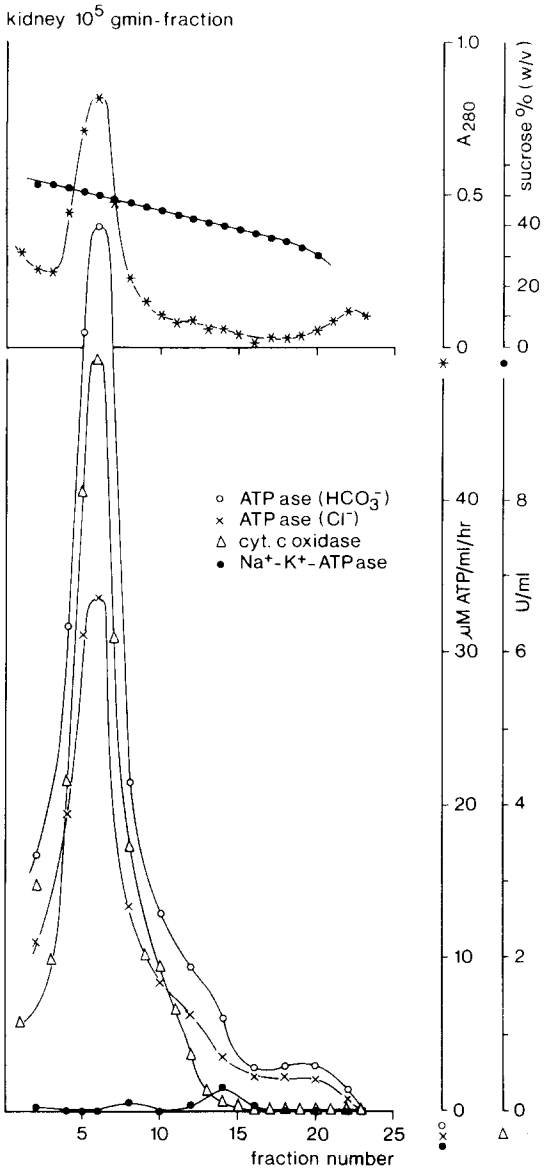


Fig. 1. Enzyme distribution patterns after density gradient centrifugation (16 h 64000 × g_{av}) of a mitochondrial fraction (10 min 10000 × g) of rabbit kidney. Typical experiment representative for two experiments. Symbols, upper section: ●———●, distribution of sucrose; ★———★, 280 nm absorbance, after dilution and correction; lower section: ○———○, ATPase activity in HCO_3^- medium; ×———×, ATPase activity in Cl^- medium; ●———●, (Na^+ + K^+)-ATPase activity; △———△, cytochrome *c* oxidase activity.

sibly reflects the presence of a mixture of Mg ATPase activities with different sensitivities towards oligomycin. This idea is further supported by the different rest activities remaining at maximal inhibition (mitochondrial fraction 10%, microsomal fraction 26%, see Table I). Fig. 3b shows the effects of aurovertin D, which inhibits the stimulation by HCO_3^- of the mitochondrial Mg ATPase

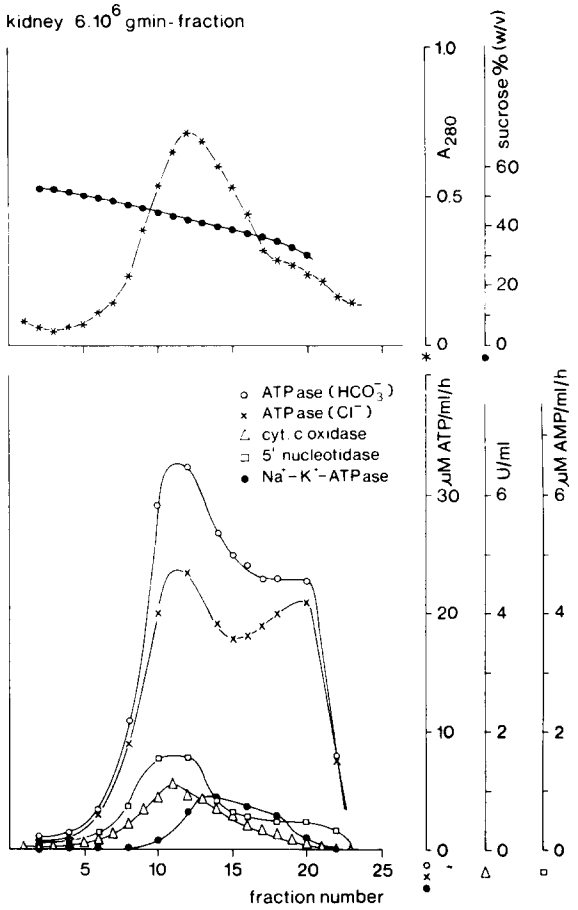


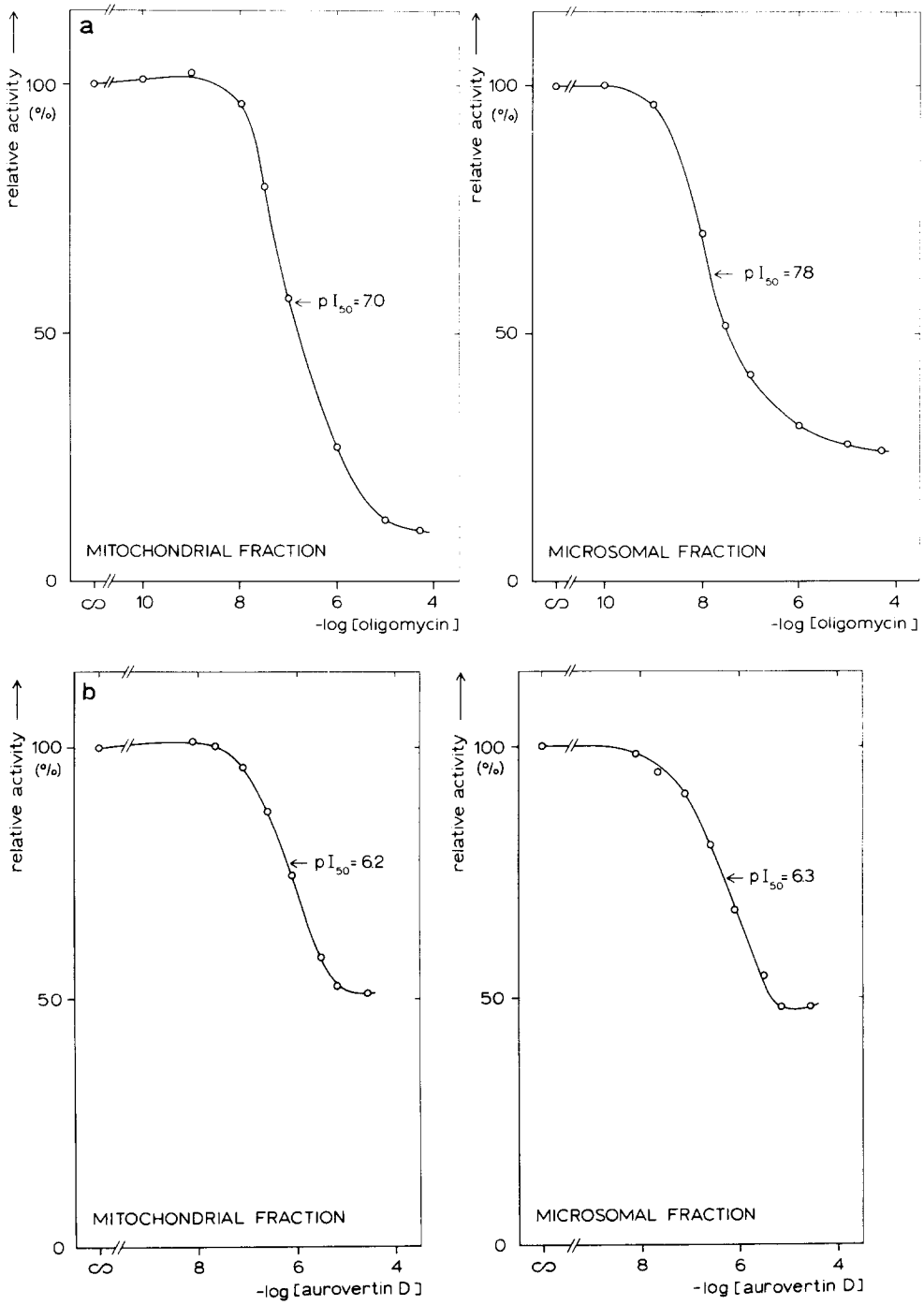
Fig. 2. Enzyme distribition patterns after density gradient centrifugation (16 h 64000 X g_{av}) of a microsomal fraction (60 min 100000 X g_{av}) of rabbit kidney. Typical experiment representative for five experiments. Symbols as in Fig. 1. Nucleotidase activity.

activity [20]. The mitochondrial Mg ATPase activity appears to be inhibited by only 49% in HCO_3^--containing medium at maximally inhibitory concentrations of aurovertin D. The microsomal enzyme is inhibited to the same extent and shows the same half-inhibition concentration (Table I). The effects of dicyclohexylcarbodiimide on the anion-sensitive ATPase activities of both fractions is shown in Fig. 3c. Similar results as for oligomycin are obtained. These results, summarized in Table I, clearly indicate that the major part of the anion-sensitive Mg ATPase activity in this pooled microsomal subfraction, even though coinciding with a relatively high (Na^+ + K^+)-ATPase activity, must be of mitochondrial origin.

Brush border fraction

In the course of this work, Liang and Sacktor [11] reported the presence of an anion-sensitive ATPase activity in a purified brush border fraction of rabbit kidney cortex. They suggested a possible interrelationship between this enzyme

and bicarbonate reabsorption in the renal tubule. Since the degree of mitochondrial contamination in their preparation may have been underestimated, we have repeated and extended their studies.



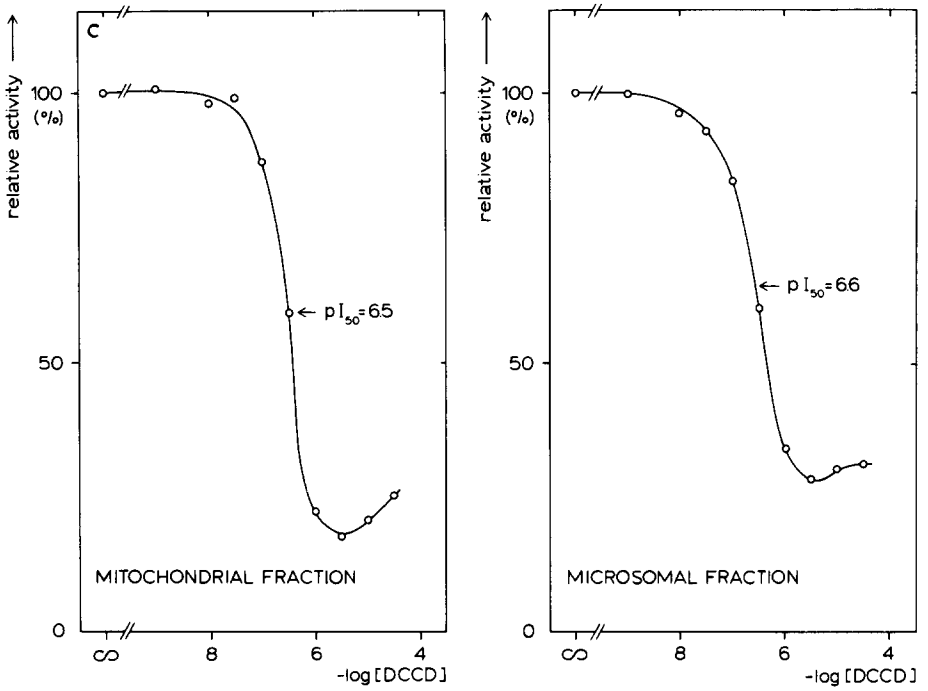


Fig. 3. Relative ATPase activity in HCO_3^- medium, as a function of the negative logarithm of oligomycin (a), aurovertin D (b) or dicyclohexylcarbodiimide (DCCD) (c) concentration in purified mitochondrial and microsomal fractions. Mean ratios over the activity without added inhibitor (≡100%) are shown for two experiments in each case.

The brush border fraction is isolated by the procedure of Aronson and Sacktor [12] and is examined by electron microscopy. The preparation is characterized by coinciding peaks of alkaline phosphatase and γ-glutamyltranspeptidase activities in density gradient centrifugation, both of which are thought to be marker enzymes for the brush border membrane [10,11]. The specific activity of anion-sensitivite ATPase of this fraction and the effects of oligomycin

TABLE I

EFFECTS OF VARIOUS INHIBITORS ON ANION-SENSITIVE Mg ATPase ACTIVITIES IN SUBFRACTIONS FROM RABBIT KIDNEY

pI_{50} is the negative logarithm of the molar inhibitor concentration at half-maximal inhibition. pI_{99} is the negative logarithm of the molar inhibitor concentration giving 99% of the maximal inhibition. Rest activity is percent ATPase activity remaining at maximal inhibition. The HCO_3^- medium was used in all experiments. DCCD, dicyclohexylcarbodiimide. For the brush border fraction means of 3 experiments with standard errors and for the mitochondrial and microsomal fractions means of 2 experiments are presented.

Fraction	Oligomycin			Aurovertin D			DCCD			*n*
	pI_{50}	pI_{99}	Rest activity (%)	pI_{50}	pI_{99}	Rest activity (%)	pI_{50}	pI_{99}	Rest activity (%)	
Mitochondrial	7.0	4.7	10	6.2	5.0	51	6.5	5.7	18	2
Microsomal	7.8	4.8	26	6.3	5.1	48	6.6	5.6	29	2
Brush border	7.6 ± 0.2	4.9 ± 0.1	68 ± 2	—	—	83 ± 1	—	—	68 ± 3	3

are comparable to those of the preparation of Liang and Sacktor [11].

Fig. 4 shows the inhibition of anion-sensitive ATPase in the brush border fraction by oligomycin in a HCO_3^- medium and a Cl^- medium. The ATPase activity in the HCO_3^- medium appears to be more sensitive to oligomycin than the activity in the Cl^- medium with inhibitions of 32% (S.E. 2, $n = 3$) and 23% (S.E. 1, $n = 3$), respectively. These figures closely agree with those of Liang and Sacktor [11], who reported 36 and 23%, respectively. This indicates that our brush border fraction is very similar to theirs.

In Table II the effects of various inhibitory agents on the specific activity of anion-sensitive ATPase in the brush border membrane fraction are shown. Concentrations are chosen which are maximally inhibitory for the mitochondrial ATPase (see Fig. 3a—c) of rabbit kidney. The HCO_3^--stimulated ATPase activity in the brush border fraction, expressed as the difference between the activities in the HCO_3^- and the Cl^- medium, is strongly inhibited (62—65%) by these agents. This is especially the case for aurovertin D, where bicarbonate stimulates the activity by only 20% instead of by 51% in the absence of inhibitor. The activity in the Cl^- medium is inhibited much less (4—24%).

Since we also find cytochrome *c* oxidase activity in this fraction, we have further purified it. Care was taken to remove the small brown mitochondrial pellet, which is below the brush border pellet after the final centrifugations in the isolation procedure of Aronson and Sacktor [12]. The final preparation was subjected to density gradient centrifugation by layering it on a very shal-

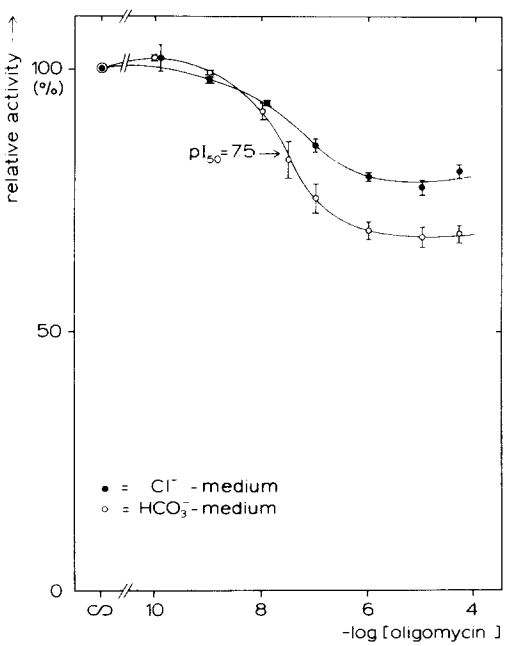


Fig. 4. Relative ATPase activity in HCO_3^- medium (○) and Cl^- medium (●) as a function of the negative logarithm of oligomycin concentration in the brush border fraction of rabbit kidney cortex. Mean ratios over the activity without added oligomycin (≡100%) with the standard errors are shown for three experiments.

TABLE II

EFFECTS OF VARIOUS INHIBITORS ON ANION-SENSITIVE Mg ATPase ACTIVITIES IN BRUSH BORDER FRACTION FROM RABBIT KIDNEY CORTEX

Inhibitors are applied in concentrations which are maximally inhibitory for mitochondrial Mg ATPase. Specific activity is expressed in $\mu mol \cdot h^{-1} \cdot mg^{-1}$ protein with the standard error for 4 experiments. DCCD, dicyclohexylcarbodiimide.

Agent	Specific activity		Difference $HCO_3^- - Cl^-$	Ratio $HCO_3^- : Cl^-$
	HCO_3^- medium	Cl^- medium		
Control	27.1 ± 4.2	17.7 ± 2.1	9.4 ± 2.1	1.51 ± 0.06
Oligomycin, $5 \cdot 10^{-5}$ M	16.0 ± 1.3	13.0 ± 0.8	3.0 ± 0.7	1.23 ± 0.04
Aurovertin D, $2.2 \cdot 10^{-5}$ M	20.1 ± 2.0	16.8 ± 1.5	3.3 ± 0.7	1.20 ± 0.04
DCCD, $3.2 \cdot 10^{-6}$ M	16.2 ± 1.3	12.8 ± 0.8	3.4 ± 0.7	1.26 ± 0.05

low gradient of 38 to 49% (w/v) sucrose (see Methods). The enzyme distribution pattern thus obtained (Fig. 5) shows that the alkaline phosphatase and γ-glutamyltranspeptidase activities, which are thought to be localized in renal brush border membranes [10,11], sediment in a single peak between 42 and 45% (w/v) sucrose. The $(Na^+ + K^+)$-ATPase and cytochrome *c* oxidase activities (the latter being rather low) are spread over a larger range of the gradient. The

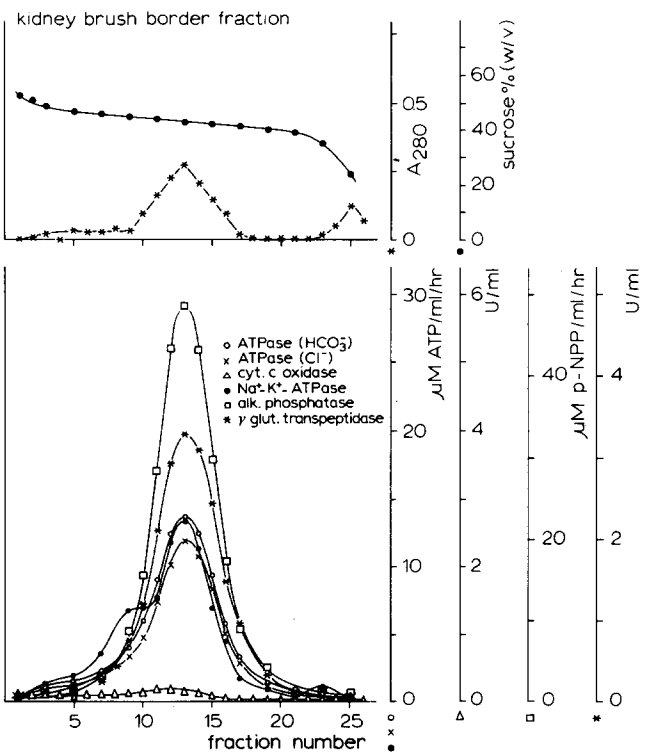
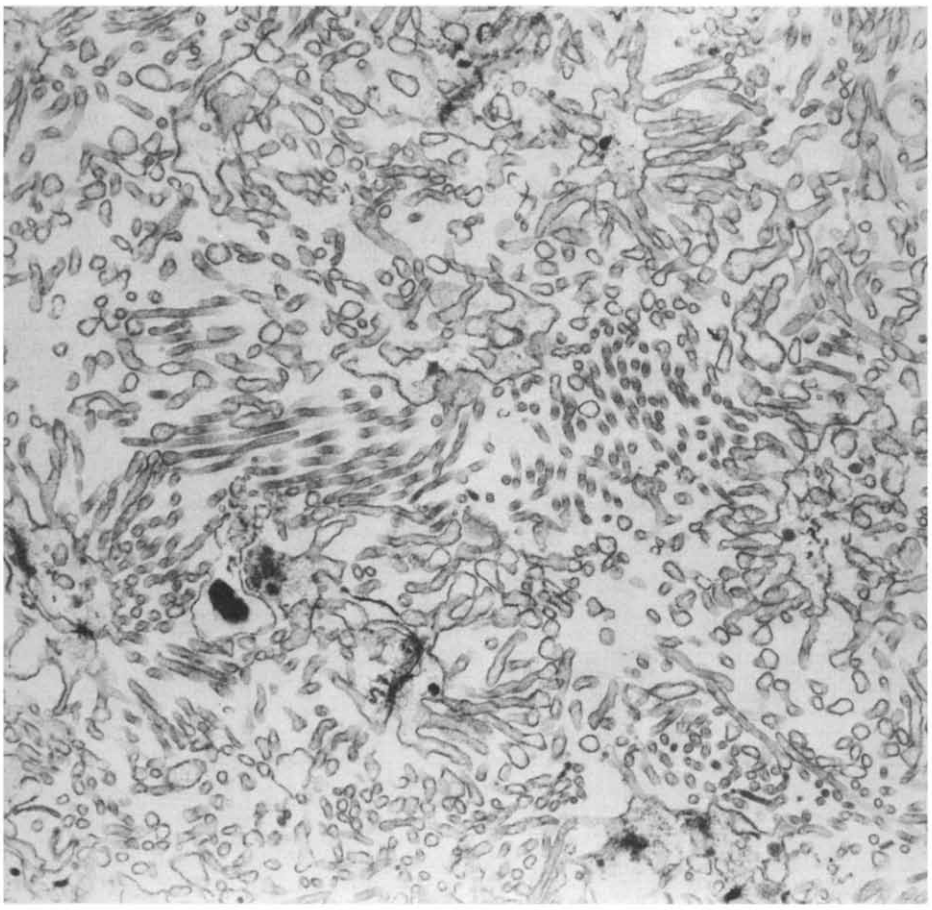


Fig. 5. Enzyme distribution patterns after density gradient centrifugation (16 h 64000 × g_{av}) of brush border membrane fraction of rabbit kidney cortex. Typical experiment representative for three experiments. Symbols: □———□, alkaline phosphatase; ★———★, γ-glutamyltranspeptidase; other symbols as in Fig. 1.

Mg ATPase activity, coinciding with the peak activities of alkaline phosphatase and γ-glutamyltranspeptidase, is very insensitive towards anions, resulting in an activity ratio of 1.12 (S.E. 0.01, $n = 3$) in HCO_3^- versus Cl^- medium, compared to a ratio of 1.51 (S.E. 0.06, $n = 4$) in the original preparation (Table II). Hence, the decrease in cytochrome *c* oxidase activity seems to parallel the decrease in the stimulatory effect of HCO_3^-.

The small residual stimulation by HCO_3^- is inhibited only slightly by oligomycin and aurovertin D. This may be due to enclosure of mitochondria by brush border membranes, making these mitochondria inaccessible to these inhibitors. Our electronmicrographs provide evidence for such a phenomenon. While brush border membranes, the major part of which consist of open structures with finger-like projections and whose appearance is very similar to that of preparations previously shown by Berger and Sacktor [21], dominate the picture (Fig. 6a), mitochondria surrounded by membranes are frequently observed (Fig. 6b). When this fraction, after purification by density gradient centrifugation, is frozen and thawed, the ratio of ATPase activity in HCO_3^- versus Cl^-

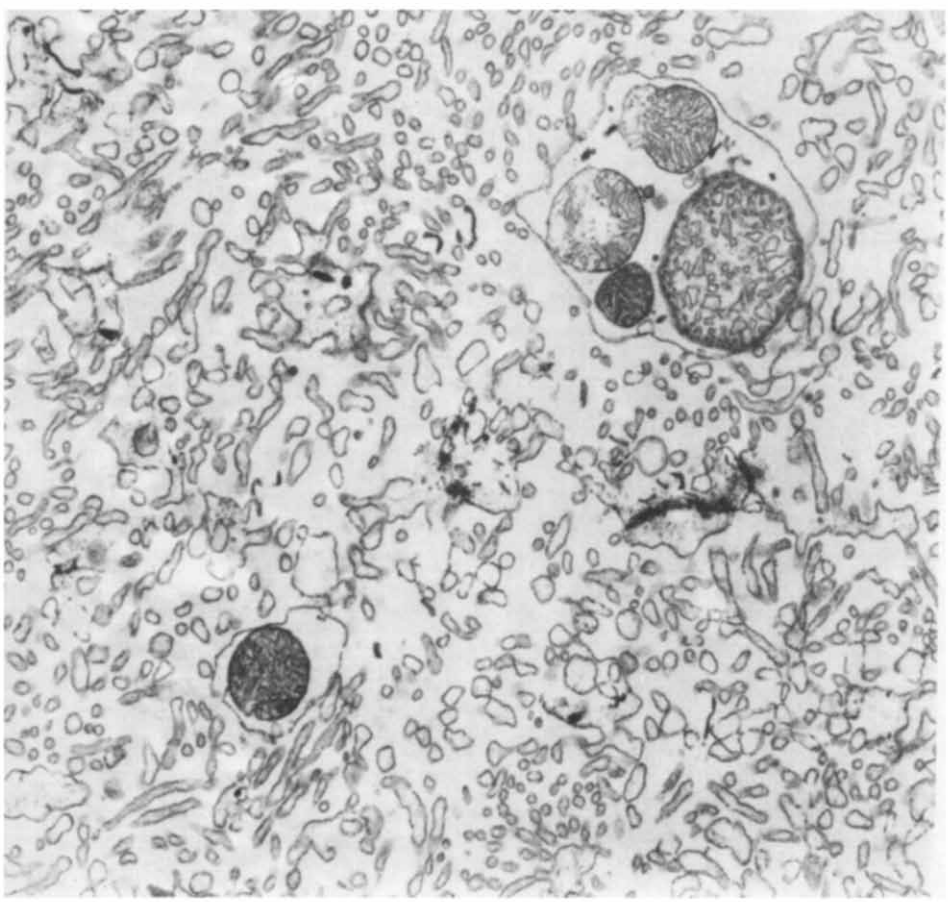


a

medium increases from 1.12 (S.E. 0.01, $n = 3$) to 1.28 (S.E. 0.03, $n = 3$). The electronmicrographs suggest that there may be sufficient mitochondrial material present to explain the small residual HCO_3^--stimulated ATPase in this fraction. All results together indicate that the brush border fraction contains a mixture of two Mg ATPase activities. The major activity is an inherent property of the brush border membrane, is rather anion-insensitive and shows little sensitivity to the inhibitors used. The minor activity is a Mg ATPase of mitochondrial origin, which is sensitive towards anions and towards the various inhibitors. This impurity is also reflected by the minor cytochrome *c* oxidase activity in this fraction.

Discussion

Requirement for accepting a role of anion-sensitive ATPase in anion or proton transport across the cell membrane is the presence of the enzyme in the



b

Fig. 6. Electron micrographs of rabbit kidney brush border fraction. (a), brush border fraction (14000 ×); (b), mitochondria entrapped in brush border membranes (14000 ×).

plasma membrane. This is more difficult than in the case of (Na^+ K^+)-ATPase, since anion-sensitive ATPase is invariably present in mitochondria and (Na^+ K^+)-ATPase is not. Hence, in order to determine the presence of anion-sensitive ATPase in plasma membranes, contamination with mitochondrial fragments must be excluded.

In our previous paper [4] we have established for rabbit gastric mucosa and trout gill that the anion-sensitive ATPase can be entirely attributed to mitochondrial contamination. For rabbit kidney the results were not completely conclusive. Hence, we have further investigated this tissue in the present paper.

Recent reports about the occurrence of anion-sensitive ATPase activity in renal brush border membranes [10,11] suffer from the fact that such contamination has not been adequately excluded. Kinne-Saffran and Kinne [10] have used free-flow electrophoresis for the isolation; they find in their final distribution pattern coincidence between HCO_3^--stimulated ATPase and alkaline phosphatase, but not with (Na^+ + K^+)-ATPase. However, they do not show the distribution pattern of a mitochondrial marker enzyme after separation of the brush border membranes from the lateral-basal membranes, but assume that a lowering of the succinic dehydrogenase to HCO_3^- ATPase ratio during isolation is sufficient proof. Liang and Sacktor [11] estimate the mitochondrial contamination in their brush border fraction to be only 10% on the basis of the succinic dehydrogenase activity, and conclude that less than 15% of the anion-sensitive ATPase activity in this fraction could be of mitochondrial origin. However, in both cases an increase in the anion-sensitive ATPase/succinic dehydrogenase ratio may reflect activation through loss of an ATPase inhibitor protein or an increase in substrate accessibility of the ATPase during fractionation [4,22].

The enzyme distribution patterns of the rabbit kidney mitochondrial fraction show that a very high HCO_3^- stimulated ATPase activity is present in the mitochondria (Fig. 1). The microsomal fraction (Fig. 2) is contaminated with mitochondrial fragments, despite centrifugations for 10 min at 10000 $\times g$ and 20 min at 20000 $\times g$ preceding the 60 min 100000 $\times g$ centrifugation which yields the microsomal fraction. The presence of the peak activities of cytochrome *c* oxidase at low densities in the density gradient subfractions of the microsomal fraction suggests that the contamination consists of fragments of mitochondria. This is supported by the relatively high cardiolipin content of this this low density cytochrome *c* oxidase activity in rabbit gastric mucosal microsomes [4].

In an attempt to further establish this mitochondrial contamination, we have pooled the (Na^+ + K^+)-ATPase containing subfractions 13—18 (Fig. 2) and compared the inhibition characteristics of the anion-sensitive ATPase activity in this pooled subfraction with those of a mitochondrial fraction. The results in Fig. 3a—c and Table I clearly suggest that this microsomal anion-sensitive ATPase activity originates from mitochondrial contamination.

In addition, we have studied a brush border membrane fraction from kidney cortex, isolated as described by Liang and Sacktor [11]. Our fraction is very similar to theirs with respect to oligomycin inhibition, electron microscopic appearance and specific anion-sensitive ATPase activity. From the effects of various inhibitors on the anion-sensitive ATPase activity in this fraction, sum-

marized in Table II, it can be concluded that this fraction, which shows 51% stimulation of ATPase activity by HCO_3^-, must be heavily contaminated with mitochondrial material.

When this fraction is further purified by density gradient centrifugation, the remaining ATPase activity is stimulated for only 12% by HCO_3^-. The latter stimulation is poorly inhibited by oligomycin or aurovertin D, which is possibly due to inaccessibility of mitochondria enveloped by brush border membranes as shown in Fig. 6b and as confirmed by the increase in HCO_3^- stimulation upon freeze-thawing.

These results indicate that the anion-sensitive ATPase activities in the microsomal fraction and the brush border fraction of rabbit kidney cortex are probably largely due to mitochondrial contamination, and that the brush border membranes contain an Mg ATPase activity with low anion-sensitivity.

Acknowledgements

The skilful technical assistance of Mrs. Carolien van Dreumel-Hermens is gratefully acknowledged. This work was supported in part by the Netherlands Organization for Basic Research (ZWO) through the Netherlands Foundation for Biophysics.

References

1 Durbin, R.P. and Kasbekar, D.K. (1965) Fed. Proc. 24, 1377—1381
2 Blum, A.L., Shah, G., Pierre, T.St., Helander, H.F., Sung, C.P., Wiebelhaus, V.D. and Sachs, G. (1971) Biochim. Biophys. Acta 249, 101—113
3 Simon, B., Kinne, R. and Sachs, G. (1972) Biochim. Biophys. Acta 282, 293—300
4 Van Amelsvoort, J.M.M., De Pont, J.J.H.H.M. and Bonting, S.L. (1977) Biochim. Biophys. Acta 466, 283—301
5 Forte, J.G., Ganser, A.L. and Tanisawa, A.S. (1974) Ann. N.Y. Acad. Sci. 242, 255—267
6 Izutsu, K.T. and Siegel, I.A. (1975) Biochim. Biophys. Acta 382, 193—203
7 Izutsu, K.T. and Siegel, I.A. (1972) Biochim. Biophys. Acta 284, 478—484
8 Soumarmon, A., Lewin, M., Cheret, A.M. and Bonfils, S. (1974) Biochim. Biophys. Acta 339, 403—414
9 Katz, A.J. and Epstein, F.H. (1971) Enzyme 12, 499—507
10 Kinne-Saffran, E. and Kinne, R. (1974) Proc. Soc. Exp. Biol. Med. 146, 751—753
11 Liang, C.T. and Sacktor, B. (1976) Arch. Biochem. Biophys. 176, 285—297
12 Aronson, P.S. and Sacktor, B. (1975) J. Biol. Chem. 250, 6032—6039
13 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
14 Cooperstein, S.J. and Lazarow, A. (1951) J. Biol. Chem. 189, 665—675
15 Heppel, L.A. and Hilmoe, R.J. (1955) in Methods in Enzymology (Colowick, S.P. and Kaplan, N.O., eds.), Vol. II, p. 546 Academic Press, N.Y.
16 Orlowski, M. and Meister, A. (1965) J. Biol. Chem. 240, 338—347
17 Bonting, S.L. (1970) in Membranes and Ion Transport (Bittar, E.E., ed.), Vol. I, pp. 257—363, Wiley-Interscience, London
18 Schuurmans Stekhoven, F.M.A.H., De Pont, J.J.H.H.M. and Bonting, S.L. (1976) Biochim. Biophys. Acta 419, 137—149
19 Baldwin, C.L., Weaver, L.C., Brooker, R.M., Jacobsen, T.N., Osborne, Jr., C.E. and Nash, H.A. (1964) Lloydia 27, 88—95
20 Ebel, R.E. and Lardy, H.A. (1975) J. Biol. Chem. 250, 4992—4995
21 Berger, S.J. and Sacktor, B. (1970) J. Cell Biol. 47, 637—645
22 Horstman, L.L. and Racker, E. (1970) J. Biol. Chem. 245, 1336—1344