Biochimica et Biophysica Acta 937 (1988) 145–152
Elsevier

BBA 73839

The localization of (Ca^{2+} or Mg^{2+})-ATPase in plasma membranes of renal proximal tubular cells

Monique Van Erum, Lut Martens, Luc Vanduffel and Henri Teuchy

Laboratorium voor Biochemie, Departement SBM, Limburgs Universitair Centrum, Diepenbeek (Belgium)

(Received 23 September 1987)

Key words: ATPase, (Ca^{2+} or Mg^{2+})-; Membrane fractionation; Brush-border membrane; Basolateral membrane; Electrophoresis; Free flow electrophoresis; (Pig kidney cortex)

Basolateral and brush-border vesicles from pig kidney cortex were prepared by differential centrifugation followed by free-flow electrophoresis. A low-affinity (Ca^{2+} or Mg^{2+})-ATPase which co-migrated with alkaline phosphatase was demonstrated. A considerable enrichment (by a factor of 10) of this ATPase activity was only observed in the brush-border and not in the basolateral membrane fractions. Maximal stimulation of this brush-border enzyme by Ca^{2+} was achieved when the ratio of Ca^{2+} to ATP reached a value between 1 and 2. The enzyme was not inhibited by excess Ca^{2+} or Mg^{2+}. A kinetic analysis of the azide-insensitive (Ca^{2+} or Mg^{2+})-ATPase gave a K_m of 0.43 mM for Ca-ATP and of 0.14 mM for Mg-ATP.

Introduction

A Mg^{2+}- or Ca^{2+}-stimulated ATPase activity has been described in plasma membranes, mitochondria, lysosomes and endoplasmic reticulum from a variety of tissues (skeletal, cardiac and smooth muscle, liver, lung, kidney, placenta, brain, pancreas) [1–9].

The Mg^{2+}- or Ca^{2+}-ATPases of these tissues are similar in that they are maximally stimulated at millimolar concentrations of either magnesium or calcium ions. In the rabbit kidney, the presence of such a low-affinity (Ca^{2+} or Mg^{2+})-ATPase has been demonstrated along the entire nephron by Katz and Doucet [10]. The exact localization of the enzyme in the plasma membranes of the proximal tubular cell is still unclear. For the rat kidney cortex, Kinne-Saffran and Kinne [11] localized a low-affinity Ca^{2+}-ATPase in the basolateral plasma membranes only. In contrast, Mörtl et al. [12] described a (Ca^{2+} or Mg^{2+})-dependent ATPase in rabbit kidney brush-border membranes. Finally, an azide-insensitive low-affinity ATPase stimulated by Ca^{2+} or Mg^{2+} was claimed to occur in both basolateral and brush-border membranes of pig kidney cortex by Ilsbroux et al. [13].

In order to evaluate these different findings we have purified basolateral and brush-border membranes with the free-flow electrophoresis method and have compared the distribution pattern of the azide-insensitive (Ca^{2+} or Mg^{2+})-ATPase with that of typical membrane marker enzymes. By the purification procedure reported in this paper we could demonstrate the presence of a low-affinity (Ca^{2+} or Mg^{2+})-ATPase activity in the brush-border membranes of pig renal cortex only.

Correspondence: M. Van Erum, Limburgs Universitair Centrum, Department SBM, B-3610 Diepenbeek, Belgium.

Materials and Methods

The sodium salts of ATP, $NADP^+$ and NADPH, the lyophilized enzymes glucose-6-phosphate dehydrogenase and hexokinase were obtained from Boehringer (Mannheim, F.R.G.). Ouabain was purchased from Calbiochem (La Jolla, CA, U.S.A.) and *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide from Serva Feinbiochemica (Heidelberg, F.R.G.). The Bio-Rad Protein Assay kit was obtained from Bio-Rad Laboratories (München, F.R.G.) and all other chemicals were from E. Merck (Darmstadt, F.R.G.) and were of analytical grade.

Membrane preparation

Basolateral plasma membranes were isolated from slices of pig kidney cortex by differential centrifugation followed by free-flow electrophoresis. 25 g kidney cortical slices were homogenized (1 : 6, w/v) in buffer I (250 mM sucrose in 4 mM Tris titrated to pH 7.6 with HCl) using a Sorvall Omni-mixer for 75 s. Complete homogenization was performed with a teflon/glass homogenizer (0.15 mm clearance, 10 strokes at 600 rev./min). The homogenate was centrifuged for 15 min at $2800 \times g$ in a JA-20 rotor (Beckmann J-21B refrigerated centrifuge). The resultant supernatant was centrifuged for 20 min at $24000 \times g$ [14]. Each fluffy layer, was washed with 20 ml of buffer I and again centrifuged at $24000 \times g$ for 20 min. Following this last centrifugation the fluffy layers were resuspended in buffer I in a total volume of 15 ml (protein concentration ± 15 mg/ml) and injected into a Desaga FF 48 free-flow electrophoresis apparatus through the port above fraction 39. The electrophoresis buffer (buffer II) contained 250 mM sucrose and 8 mM Tris-HCl (pH 7.6, conductivity 500 μS/cm). In the electrode vessels 80 mM Tris-HCl (pH 7.6) was used as a buffer. The conditions of the run were the following: 700 V, 70 mA, temperature 4°C, electrophoresis buffer flow-rate 110 ml/h, injection rate ± 0.5 ml/h. After separation, all fractions were assayed for enzyme activities and protein concentration.

For re-electrophoresis the selected fractions were pooled, centrifuged at $24000 \times g$ for 30 min. The precipitate was resuspended in buffer II to a final protein concentration of about 10 mg/ml and subjected to re-electrophoresis under the same conditions as above.

Brush borders of pig kidney cortex were prepared by differential centrifugation in isotonic sucrose medium (250 mM sucrose, 4 mM Tris-HCl (pH 7.6)) as described by Ilsbroux et al. [13]. The microsomal pellet was resuspended in 2.5 ml of buffer II (protein concentration ± 50 mg/ml) and injected in the free-flow electrophoresis apparatus at a rate of about 0.5 ml/h. The other conditions of the run were the same as above.

For re-electrophoresis, the selected fractions were pooled, centrifuged at $105000 \times g$ for 1 h. The precipitate was resuspended in a minimal volume of buffer II (protein concentration ± 7 mg/ml) and injected at a rate of about 1.5 ml/h.

Enzyme and protein determination

For the determination of alkaline phosphatase (EC 3.1.3.1) the method of Cathala et al. [15] was used. $(Na^+ + K^+)$-ATPase (EC 3.6.1.3) was determined according to Kinne et al. [16] with minor modifications. A higher buffer concentration (125 mM Tris) and $MgCl_2$ instead of $MgSO_4$ was used. Trehalase (EC 3.2.1.28) was measured as described by Ilsbroux et al. [13]. Succinate dehydrogenase (EC 1.3.99.1) activity was measured as described by Earl and Korner [17]. Glucose-6-phosphatase (EC 3.1.3.9) was assayed by the method of Baginski et al. [18]. β-*N*-Acetylglucosaminidase (EC 3.2.1.30) was measured by the method of Stirling [19]. The azide-insensitive (Ca^{2+} or Mg^{2+})-ATPase activity was assayed as follows: the assay medium contained 320 mM Tris-HCl (pH 8.5), 7 mM $CaCl_2$ or 5 mM $MgCl_2$, 5 mM NaN_3 as an inhibitor of mitochondrial ATPase and 5 mM Na_2ATP in a final volume of 1 ml. Variations of this composition will be presented in the figure legends. Incubation at 37°C was started by the addition of 100 μl membrane suspension to 900 μl concentrated assay mixture. Care was taken that less than 10% of the substrate present was hydrolyzed during the incubation. Inorganic phosphate was determined by an adaptation of the method of Lebel et al. [20] as described by Ilsbroux et al. [13]. Protein was measured by the method of Bradford, using the Bio-Rad Protein Assay Kit, with bovine γ-globulin as a standard.

Electron microscopy

Pooled fractions obtained after re-electrophoresis were spun down at 24000 × *g* for 20 min for the basolateral membranes, and at 105000 × *g* for 1 h for brush borders. The pellets were fixed with glutaraldehyde (2.5%) in 0.1 M sodium cacodylate buffer (pH 7.2) and postfixed in 1% OsO_4 in the same buffer at 4° C. Specimens were dehydrated and embedded in SPURR medium. Ultrathin sections were cut with a Diatome diamond knife on a Reichert OMU4 Ultramicrotome and were subsequently stained with saturated uranyl acetate in 50% ethanol and 0.4% lead citrate. The specimens were viewed with a Philips E.M. 301G.

For negative staining of the brush borders, samples of the free-flow electrophoresis fractions were directly stained with 2% phosphotungstic acid pH 7.6 (droplet method) and examined with a Philips E.M. 400.

Results

Localization of the azide-insensitive (Ca^{2+} or Mg^{2+})-ATPase

In the fluffy layer obtained by differential centrifugation the specific activity of ($Na^+ + K^+$)-ATPase (marker enzyme for basolateral plasma membranes) is higher than in the microsomal pellet. ($Na^+ + K^+$)-ATPase is enriched by a factor of 4.9 in the fluffy layer and in the microsomal pellet by a factor of 1.2 as compared to the homogenate. The total amount of ($Na^+ + K^+$)-ATPase activity is also higher in the fluffy later (26% of the total activity in the homogenate) than in the microsomal pellet (9.2%). Moreover, the contamination by brush-border membranes is lower in the fluffy layer (12.6% of the total activity in the homogenate) than in the microsomal pellet (26%). However, there is no considerable difference between the enrichment of alkaline phosphatase (marker enzyme for brush-border membranes) in the fluffy layer (3.1-fold) and in the microsomal pellet (3.9-fold). For these reasons the fluffy layer was used to purify basolateral membranes whereas the microsomal pellet was used as the starting material for the preparation of the brush-border membranes.

In Fig. 1A the distribution of alkaline phosphatase, ($Na^+ + K^+$)-ATPase and azide-insensitive (Ca^{2+} or Mg^{2+})-ATPase after free-flow electrophoresis of a fluffy layer is shown. The distribution of alkaline phosphatase is similar to that of trehalase, another brush-border marker (results not shown). The fluffy layer is subdivided by free-flow electrophoresis into brush-border microvilli and basolateral plasma membranes. But partial cross-contamination of the basolateral membrane fractions by brush-border membranes does

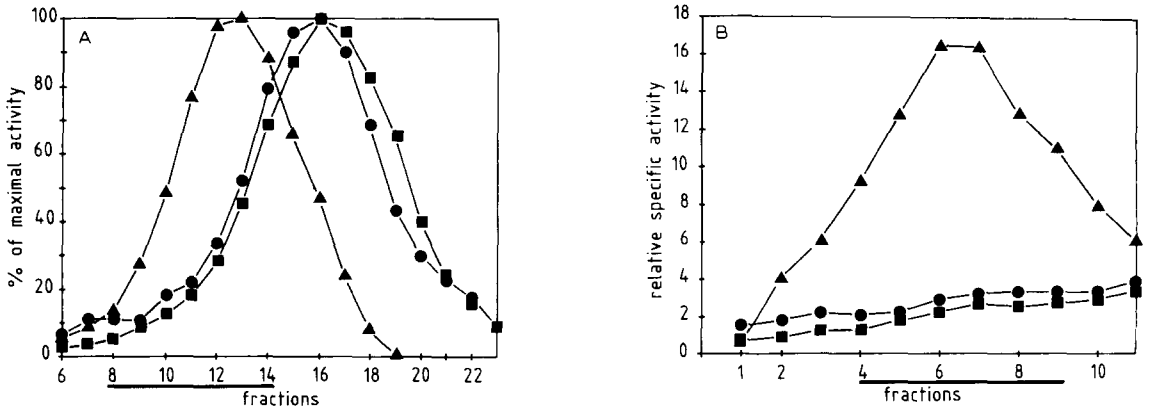

Fig. 1. (A) Distribution of ($Na^+ + K^+$)-ATPase (▲), alkaline phosphatase (■) and azide-insensitive Ca^{2+}-ATPase (●) after free-flow electrophoresis of a fluffy layer of pig kidney cortex. The activity of each enzyme is expressed as a percentage of the maximal activity. (B) Distribution of ($Na^+ + K^+$)-ATPase (▲), alkaline phosphatase (■) and azide-insensitive Ca^{2+}-ATPase (●) after re-electrophoresis of the basolateral membrane fractions. After the first free-flow electrophoresis (Fig. 1A), the basolateral membrane fractions (8–14) were pooled, concentrated and re-electrophorized under the same conditions. For every enzyme the relative specific activity (compared to the homogenate) in each fraction is given.

occur as indicated by the overlapping enzyme activities. The azide-insensitive (Ca^{2+} or Mg^{2+})-ATPase follows the distribution of alkaline phosphatase. To prove that no appreciable (Ca^{2+} or Mg^{2+})-ATPase activity is localized in the basolateral membranes, fractions 8–14 were pooled and re-electrophorized. The results are shown in Fig. 1B. The relative specific activity for ($Na^+ + K^+$)-ATPase in both peak fractions has a value of 16. This indicates that these fractions consist primarily of basolateral membranes. The relative specific activities of the other subcellular marker enzymes are only slightly increased, indicating that contamination by other cellular components is low (Table I). Since the relative specific activity for (Ca^{2+} or Mg^{2+})-ATPase did not increase during the re-electrophoresis (Fig. 1B) in contrast with the relative specific activity for ($Na^+ + K^+$)-ATPase, the (Ca^{2+} or Mg^{2+})-ATPase activity does not represent a basolateral membrane bound enzyme.

Examination of these basolateral membrane fractions by electron microscopy revealed the presence of closed membranous vesicles of various sizes, open sheets of membranes and some amorphous material. Other recognizable cell particles such as lysosomes and mitochondria were not detected (Fig. 2).

TABLE I

ENRICHMENT OF MARKER ENZYMES IN THE BASOLATERAL AND BRUSH-BORDER MEMBRANE PREPARATION

The activities of the marker enzymes were determined in pooled (4–9) basolateral membrane fractions (Fig. 1B) and in pooled (18–23) brush-border membranes (Fig. 3B), obtained after re-electrophoresis. For every enzyme the relative specific activity (compared to the homogenate) is given.

Enzyme	Relative specific activity	
	basolateral membranes	brush-border membranes
Alkaline phosphatase	2.0	8.8
Trehalase	1.5	11.0
(Ca^{2+} or Mg^{2+})-ATPase	2.8	10.0
($Na^+ + K^+$)-ATPase	11.2	0.1
β-*N*-Acetylglucosaminidase	1.1	1.0
Succinate dehydrogenase	0.5	0.3
Glucose-6-phosphatase	2.0	3.4

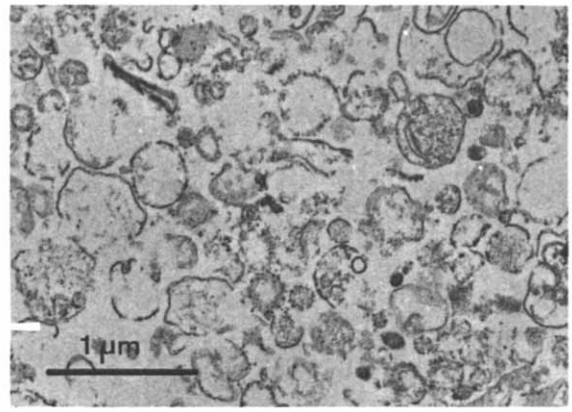

Fig. 2. Electron micrograph of pig renal basolateral membranes in re-electrophoresis fractions (4–9). Thin sectioned preparation.

In the experiment presented in Fig. 3A, a resuspended microsomal pellet was used for free-flow electrophoresis. The distribution pattern is similar to the one obtained with the fluffy layer (Fig. 1A). However, the absolute activity of ($Na^+ + K^+$)-ATPase in the alkaline phosphatase peak fraction is only 20% of the activity in the corresponding fraction of the first experiment (Fig. 1A). This means that the brush-border peak fraction is much less contaminated with basolateral membranes after free-flow electrophoresis of a microsomal pellet as compared to a fluffy layer. To obtain a higher purification of the brush-border membranes, fractions 15–22 were pooled and re-electrophorized. As shown in Fig. 3B the distribution of (Ca^{2+} or Mg^{2+})-ATPase correlates very closely with the distribution of alkaline phosphatase and nearly the same enrichment is achieved for both enzymes. Almost no ($Na^+ + K^+$)-ATPase activity can be detected in the brush-border membrane fractions. In the pooled fractions (18–23) the specific activities of alkaline phosphatase, trehalase and (Ca^{2+} or Mg^{2+})-ATPase increased by a factor of respectively 8.8, 11.0 and 10.0 relative to the homogenate (Table I). Glucose-6-phosphatase is enriched 3.4-fold, indicating a small contamination of the preparation by endoplasmic reticulum. In contrast, the contamination by basolateral membranes, mitochrondria and lysosomes is minimal (Table I). These results indicate that the (Ca^{2+} or Mg^{2+})-ATPase is localized in the brush-border membranes of the renal tubular cells.

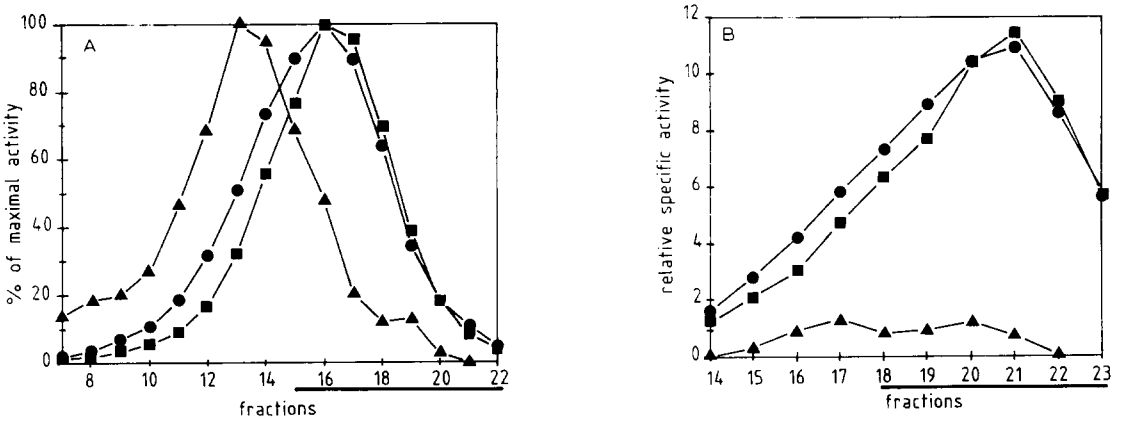

Fig. 3. (A) Distribution of $(Na^+ + K^+)$-ATPase (▲), alkaline phosphatase (■) and azide-insensitive Ca^{2+}-ATPase (●) after free-flow electrophoresis of a microsomal pellet of pig kidney cortex. The activity of each enzyme is expressed as a percentage of the maximal activity. (B) Distribution of $(Na^+ + K^+)$-ATPase (▲), alkaline phosphatase (■) and azide-insensitive Ca^{2+}-ATPase (●) after re-electrophoresis of the brush-border membranes. After the first free-flow electrophoresis (Fig. 3A), the brush-border membrane fractions (15–22) were pooled, concentrated and re-electrophorized under the same conditions as the first run. The relative specific activity (compared to the homogenate) for every enzyme in each fraction is given.

Ultrathin sections were obtained from re-electrophorized material (Fig. 4A). Spherical vesicles of different sizes and a few elongated structures with an electron-opaque content can be observed. The former probably represent vesiculated brush-border membranes, the latter the original microvilli. The vesicles in the luminal membrane preparation seem to be more homogeneous in size and usually smaller than in the antiluminal membrane preparation. As is already evident from the enzymatic characterization, contamination with other cellular membranes is very low; the only organelles found are occasional lysosomes, as indicated by the presence of some small electron-dense vesicles. In negatively stained specimens, many elongated structures were found typical for the presence of microvilli (Fig. 4B).

Kinetic properties of the (Ca^{2+} or Mg^{2+})-ATPase

(Ca^{2+} or Mg^{2+})-ATPase activities were determined at various concentrations of calcium ions and ATP. Fig. 5A shows that maximal activity is

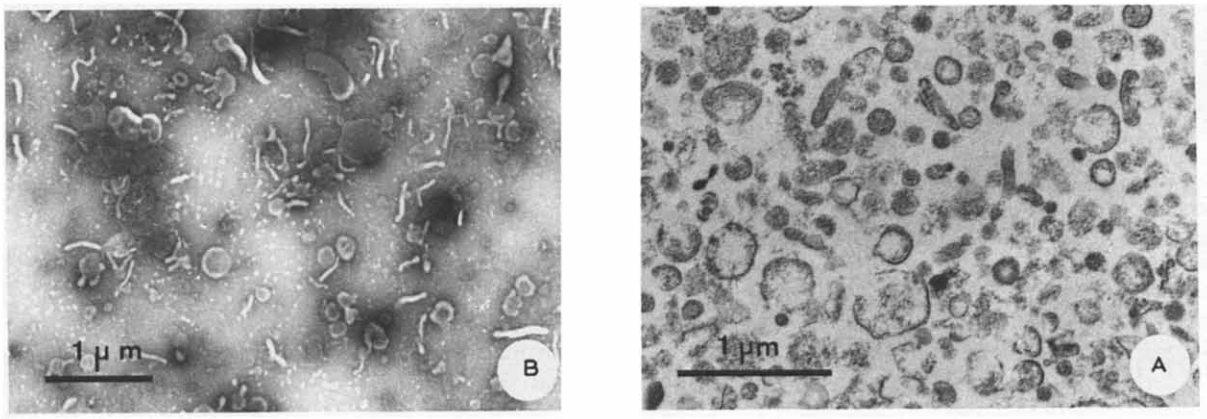

Fig. 4. Electron micrograph of pig renal brush-border membranes in re-electrophoresis fractions (18–23). (A) Thin sectioned preparation. (B) Negative stained preparation.

achieved when the ratio of Ca^{2+} to ATP reaches a value above 1. This suggests that the brush-border (Ca^{2+} or Mg^{2+})-ATPase attacks a Ca – ATP complex as its true substrate. The enzyme is not inhibited when the concentration of calcium ions exceeds the ATP concentration. On the other hand, there is an inhibitory effect when the ATP level exceeds the calcium level. Fig. 5B indicates that both Ca-ATP and Mg-ATP are effective substrates. But Ca-ATP is much more effective (50%) than Mg-ATP in promoting the ATPase activity. The optimal concentration of both substrates is 5 mM. With Ca-ATP as substrate, a K_m value of 0.43 mM could be calculated from Lineweaver-Burk plots. The K_m for Mg-ATP was 0.14 mM (Fig. 5C).

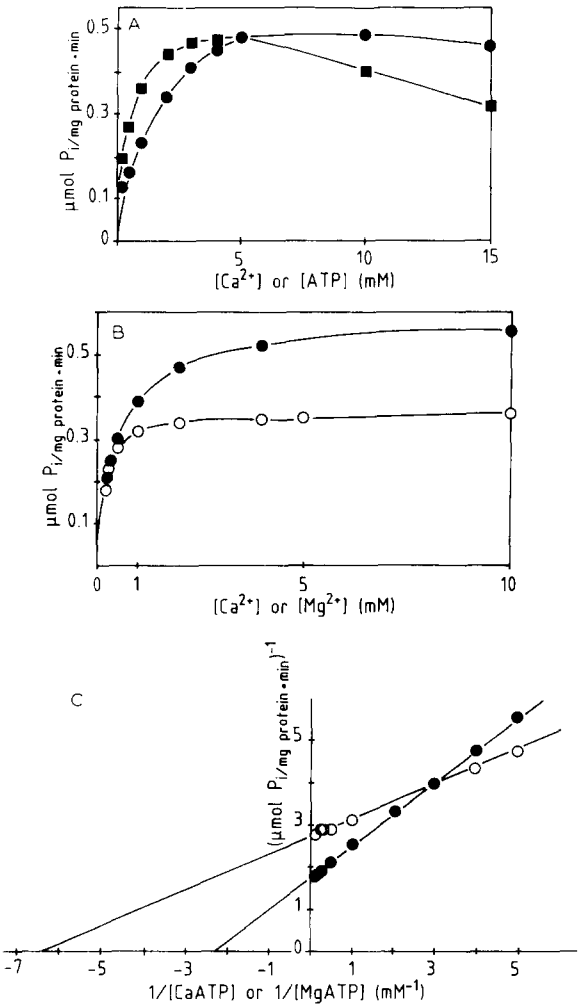

Fig. 5. (A) Effect of ATP and Ca^{2+} concentration on ATPase activity of brush-border membranes. Brush-border membranes were obtained as described in Fig. 3C. ●, The ATPase activity was tested at a constant ATP concentration (5 mM) and varying concentrations of Ca^{2+} (from 0.25 to 15 mM). ■, The ATPase activity was tested at a constant Ca^{2+} concentration (5 mM) and varying concentrations of ATP (from 0.25 to 15 mM). (B) Effect of Ca^{2+} and Mg^{2+} concentration on ATPase activity of brush-border membranes. Brush-border membranes were obtained as described in Fig. 3C. Assays were in the presence of equimolar concentrations of Na_2ATP and either $CaCl_2$ (●) or $MgCl_2$ (○). (C) Lineweaver-Burk plots of the Ca^{2+}-ATPase and Mg^{2+}-ATPase activities in brush-border membranes. The ATPase activities were tested in the presence of equimolar concentrations of Na_2ATP and either $CaCl_2$ (●) or $MgCl_2$ (○).

Discussion

Several investigators have described the existence of a membrane bound non-mitochondrial (Ca^{2+} or Mg^{2+})-ATPase activity in renal plasma membranes [13], but the localization of the enzyme is still doubtfull. In this study we have tried to elucidate this problem by purification of basolateral and brush-border membranes.

Depending on the membranes we wished to obtain, homogenates of renal cortex tissue were subjected to two different centrifugation procedures. For the purification of brush-border membranes, a microsomal pellet was fractionated by free-flow electrophoresis as described by Ilsbroux et al. [13]. We used such a pellet because it contains a high concentration of brush-border membranes, and because contamination by basolateral membranes is low.

To purify basolateral membranes, a fluffy layer prepared according to the procedure of De Smedt et al. [14] was subjected to free-flow electrophoresis. The content of basolateral membranes in this fraction is higher than in a microsomal pellet. Moreover, the ratio of basolateral over brush-border membranes is much more favourable.

From the data obtained with the free-flow electrophoresis technique, it is clear that the azide-insensitive (Ca^{2+} or Mg^{2+})-ATPase activity coincides with that of alkaline phosphatase. The co-purification of these enzymes strongly suggests that the brush-border plasma membranes of renal tubular cells contain a low-affinity (Ca^{2+} or Mg^{2+})-ATPase that is intensitive to azide. There is no indication for the presence of this enzyme in

the basolateral membranes, since ATPase is not enriched during purification of these membranes.

Furthermore the possibility of an inactivation of the enzyme can be excluded since recoveries of upto 90% were consistently obtained after free-flow electrophoresis.

Our results do not agree with the findings of Kinne-Saffran and Kinne [11] who demonstrated the presence of a low-affinity Ca^{2+}-ATPase in basolateral membranes of rat kidney cortex. However, these authors did not suppress the mitochondrial ATPase by addition of azide to the assay medium.

Furthermore we can not confirm the study of Ilsbroux et al. [13] who located an azide-insensitive (Ca^{2+} or Mg^{2+})-ATPase in both basolateral and brush-border membranes. For the preparation of basolateral membranes by free-flow electrophoresis, these authors used a microsomal pellet as starting material, which had a very unfavourable ratio of basolateral over brush-border membranes. It is our experience that a sufficiently high purification of basolateral membranes can not be reached from such a pellet by free-flow electrophoresis. Consequently no definite conclusion can be made about the exact localization of the (Ca^{2+} or Mg^{2+})-ATPase from their experiments.

On the other hand Mörtl et al. [12] identified a non-mitochondrial (Ca^{2+} or Mg^{2+})-ATPase in brush-border membranes of rabbit kidney cortex. This is in accordance with our findings.

It is highly improbable that the low-affinity (Ca^{2+} or Mg^{2+})-ATPase activity in our brush-border preparation is due to enzymes from other subcellular components. Mitochondrial ATPase in both solubilized and membrane bound form was completely inhibited by using azide [21]. A contribution by the lysosomal (Ca^{2+} or Mg^{2+})-ATPase [2] is unlikely since the enrichment of β-*N*-acetylglucosaminidase, a lysosomal marker, is negligible in contrast with the high enrichment factor of (Ca^{2+} or Mg^{2+})-ATPase in the brush-border preparation. The possibility that the measured enzyme activity is associated with the endoplasmic reticulum [3] can also be ruled out since both basolateral and brush-border membrane preparations show a similar, rather small enrichment for glucose-6-phosphatase whereas only the brush-border preparation is highly enriched in (Ca^{2+} or Mg^{2+})-ATPase activity.

Since alkaline phosphatase is not considered to be a very specific brush-border marker [22] we also measured the trehalase activity. The enrichment for this enzyme in the brush-border preparation was even higher than for alkaline phosphatase. From these results and from electron microscopic observations we conclude that we have prepared a highly enriched brush-border fraction and that the ATPase activity is due to a brush-border membrane bound enzyme.

The (Ca^{2+} or Mg^{2+})-ATPase activity found in our brush-border fractions is not specific for renal tubular cells. A (Ca^{2+} or Mg^{2+})-ATPase with very similar properties was also found in low-density vesicles isolated from brain, lung, heart, liver, spleen, skeletal muscle and adipose tissue [8]. By kinetic analysis of (Ca^{2+} or Mg^{2+})-ATPase from a purified brush-border fraction, a K_m of 0.14 mM for Mg-ATP was found in our study. This is exactly the same value as in the study of Busse et al. [6]. This value is also comparable with those reported for other cell types [8]. With Ca-ATP as the substrate, the K_m value was 0.43 mM. Similar K_m values have been published for the (Ca^{2+} or Mg^{2+})-ATPases from rabbit kidney brush-border and T-tubular membranes of skeletal muscle [12,23].

Optimal conditions for the azide-insensitive low-affinity (Ca^{2+} or Mg^{2+})-ATPase are obtained when the ratio of divalent cation to ATP exceeds a value higher than 1, indicating that the enzyme accepts the cation-ATP complex as its true substrate. This is also in agreement with the study of Busse et al. [6].

Substrate inhibition of the ATPase is observed when the ratio of cation to ATP is lower than 1. On the other hand no significant inhibition is noted when the Ca^{2+} concentration exceeds the ATP concentration. Therefore the inhibition observed is not caused by the Ca-ATP complex but is due to free ATP. The same inhibition was observed by Hidalgo et al. [23] in their study on (Ca^{2+} or Mg^{2+})-ATPase of transverse tubule membranes.

The low specificity of the ATPase towards divalent cations and nucleoside triphosphates (results not shown) resembles the (Ca^{2+} or Mg^{2+})-ATPase identified in pancreatic cells [9] and skeletal muscle

[8] which are assumed to be ecto-enzymes [24,4]. Further study is needed to prove that the renal brush-border enzyme is also an ecto-ATPase.

The physiological role of the (Ca^{2+} or Mg^{2+})-ATPase in kidney cortex is not known yet. An involvement in the secretion of protons into the lumen of the proximal tubule, as recently proposed by Kinne-Saffran and Kinne [25], is an intriguing possibility. Proof that the low- affinity (Ca^{2+} or Mg^{2+})-ATPase is the molecular entity responsible for proton transport in the renal brush border will be obtained only after reimplantation of the isolated enzyme into liposomes.

Acknowledgement

We express our gratitude to Dr. Antony Brain, E.M.-unit, King's College, London, for performing the electron microscopy.

References

1 Anand, M.B., Chauhan, M.S. and Dhalla, N.S. (1977) J. Biochem. 82, 1731–1739.
2 Schneider, D.L. (1977) J. Membr. Biol. 34, 247–261.
3 Black, B.L., McDonald, J.M. and Jarett, L. (1980) Arch. Biochem. Biophys. 199, 92–102.
4 Beeler, T.J., Gable K.S. and Keffer, J.M. (1983) Biochim. Biophys. Acta 734, 221–234.
5 Gupta, R.P., Venkitasubramanian, T.A. and Murti, V.V.S. (1983) Indian J. Biochem. Biophys. 20, 381–385.
6 Busse, D., Pohl, B., Bartel, H. and Buschmann, F. (1980) Arch. Biochem. Biophys. 201, 147–159.
7 Miller, R.K. and Berndt, W.O. (1973) Proc. Soc. Exp. Biol. Med. 143, 118–122.
8 Beeler, T.J., Wang, Gable, K. and Lee, S. (1985) Arch. Biochem. Biophys. 243, 644–654.
9 Martin, S.S. and Senior, A.E. (1980) Biochim. Biophys. Acta 602, 401–418.
10 Katz, A.I. and Doucet, A. (1980) Int. J. Biochem. 12, 125–129.
11 Kinne-Saffran, E. and Kinne, R. (1974) J. Membr. Biol. 17, 263–274.
12 Mörtl, M., Busse, D., Bartel, H. and Pohl, B. (1984) Biochim. Biophys. Acta 776, 237–246.
13 Ilsbroux, I., Vanduffel, L., Teuchy, H. and De Cuyper, M. (1985) Eur. J. Biochem. 151, 123–129.
14 De Smedt, H., Parys, J.B., Wuytack, F. and Borghgraef, R. (1984) Biochim. Biophys. Acta 776, 122–132.
15 Cathala, G., Brunel, C., Chappelet-Tordo, D. and Lazdunsky, M. (1975) J. Biol. Chem. 250, 6046–6053.
16 Kinne, R., Schmitz, J.E. and Kinne-Saffran, E. (1971) Pflügers Arch. 329, 191–206.
17 Earl, D.C.N. and Korner, A. (1965) Biochem. J. 94, 721–734.
18 Baginski, E.S., Foa, P.F. and Zak, B. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.), 2nd Edn., pp. 876–880, Academic Press, New York.
19 Stirling, J.L. (1984) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.), 3rd Edn., Vol. 4, pp. 269–277, Academic Press, New York.
20 LeBel, D., Poirier, G.G. and Beaudoin, A.R. (1978) Anal. Biochem. 85, 86–89.
21 Bowman, B.J., Mainzer, S.E., Allen, K.E. and Slayman, C.W. (1978) Biochim. Biophys. Acta 512, 13–28.
22 Mamelok, R.D., Tse, S.S., Newcomb, K., Bildstein, C.L. and Liu, D. (1982) Biochim. Biophys. Acta 692, 115–125.
23 Hidalgo, C., Gonzalez, M.E. and Lagos, R. (1983) J. Biol. Chem. 258, 13937–13945.
24 Hamlyn, J.M. and Senior, A.E. (1983) Biochem. J. 214, 59–68.
25 Kinne-Saffran, E. and Kinne, R. (1986) Pflügers Arch. 407, S180–S185.