Biochimica et Biophysica Acta 905 (1987) 287–294
Elsevier

BBA 73801

Ion-dependent generation of the electrochemical proton gradient $\Delta\tilde{\mu}_{H^+}$ in reconstituted plasma membrane vesicles from the yeast *Metschnikowia reukaufii*

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(Received 23 June 1987)

Key words: Electrochemical proton gradient; Cation stimulation; Plasma membrane; Membrane vesicle; ATPase; (Yeast)

Plasma membrane vesicles were reconstituted by freezing and thawing of purified plasma membrane fraction from the yeast *Metschnikowia reukaufii* and phosphatidylcholine (type II-S from Sigma). The reconstituted plasma membrane vesicles generated a proton gradient (acidic inside) upon addition of ATP in presence of alkali cations. ΔpH generation was most efficient when K^+ was present both outside and inside the plasma membrane vesicles. Both ATPase activity and proton translocation in plasma membrane vesicles were inhibited by orthovanadate (50% inhibition at 100 μM). Plasma membrane vesicles reconstituted without added phosphatidylcholine generated in addition to ΔpH, also an electrical potential difference $\Delta\psi$ (inside positive). $\Delta\psi$ generation exhibited no K^+ specificity. 50 μM dicyclohexylcarbodiimide inhibited completely $\Delta\psi$ generation whereas the K^+-channel blocker quinine (5 μM) caused an 8-fold increase of $\Delta\psi$. The proton gradient was much less affected by the agents. Taking into account the K^+-dependent stimulation of the plasma membrane ATPase of *M. reukaufii*, these results further support the conclusion that the ATPase operates as a partially electrogenic H^+/K^+ exchanger, as was also suggested for other yeast plasma membrane ATPases.

Introduction

Plasma membrane of eukaryotic microorganism and plant cells are equipped with H^+-translocating ATPase generating electrochemical proton gradient across the membrane which is capable of driving nutrient uptake into the cells by symport with H^+ [1,2]. Proton translocating plasma membrane ATPases were found in yeast [3,4], fungi [5,6] and higher plants [7–9]. In two yeasts, *Rhodotorula glutinis* [10,11] and *Metschnikowia reukaufii* (unpublished), the proton extrusion was accompanied with a charge compensating accumulation of potassium in the cells. An unsolved problem remains whether the plasma membrane ATPases operate as pure electrogenic H^+-pump or as partially electrogenic H^+/K^+ exchanger similar to the $(Na^+ + K^+)$-ATPase of animal epithelial cells [12]. The latter mode of operation has been favoured by Villalobo [13] for *Schizosac-*

Abbreviations: ATPase, adneosine triphosphate; ACMA, 9-amino-6-chloro-2-methoxyacridine; DCCD, N,N-dicyclohexylcarbodiimide; CCCP, carbonyl cyanide m-chlorophenylhydrazone; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid.

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charomyces plasma membrane ATPase and by Sze [14] for that of plant cells.

It has been shown that the plasma membrane ATPase activity of *M. reukaufii* was stimulated almost four times in the presence of 80 mM K^+ [15]. In the present communication, the generation of the electrochemical proton gradient $\Delta\tilde{\mu}_{H^+}$ (both (ΔpH and $\Delta\psi$) driven by the plasma membrane ATPase was characterized in reconstituted plasma membrane vesicles from *M. reukaufii*. The effect of the ATPase-inhibitors orthovanadate and dicyclohexylcarbodiimide (DCCD) and of the K^+-channel blocker quinine thereon was studied. The results support the view that the plasma membrane ATPase is a partially electrogenic proton/potassium exchanger.

Materials and Methods

Isolation of plasma membranes

The facultative anaerobic yeast *Metschnikowia reukaufii* (CBS 5834) was grown for 14–17 h at 24 °C and harvested as described by Aldermann and Höfer [16]. Protoplasts were produced by enzymatic digestion of cell walls essentially as described by Aldermann and Höfer [17] except for substituting the snail gut extract (helicase) by a mixture of cytohelicase (1.7% (w/v)), novozym (0.025% (w/v)), bovine serum albumin (0.3% (w/v)). The following steps, the coating of the protoplast membrane by silica microbeads, the lysis of coated protoplasts and the purification of plasma membranes by differential centrifugation were described by Gläser and Höfer [15]. The purified plasma membrane fraction (about 2 mg protein/ml) was stored at −70 °C, its ATPase activity was maintained for a period of at least two months.

Reconstitution experiment

Crude soybean phospholipids (L-α-phosphatidylcholine type II-S) were partially purified as described for asolectine by Kagawa and Racker [18], dissolved in 10 mM Mes (pH 6.5) (35 mg/ml) and sonicated until the solution was clear. Purified plasma membranes (20–40 μg protein) were added to a solution containing 1.8–3.5 mg of phospholipid and the mixture was frozen in liquid nitrogen. By thawing of the frozen material plasma membrane vesicles reconstituted. To enclose ions into plasma membrane vesicles the corresponding salts were added to the membrane-phospholipid mixture prior to freezing. After thawing the mixture was centrifuged for 2 min at 12000 rpm in an Ecco centrifuge to remove excess phospholipids and salts (if added). By this step the ionic composition of the outside medium could be manipulated. Since plasma membrane vesicles still contained heavy silica microbeads the short period of centrifugation was sufficient to spin them down.

Substituting for the type II-S with purified phosphatidylcholine (Phospholipon 100) resulted in 60% reduced H^+-translocating activity of reconstituted plasma membrane vesicles. An alternative method of reconstitution, sonification of the phospholipid plasma membrane mixture [19] instead of freezing and thawing led to a strong inhibition of the plasma membrane ATPase activity.

In experiments in which the electrical potential difference was estimated by oxonol (see below) plasma membrane vesicles were reconstituted without added phospholipids following the same procedure. In these plasma membrane vesicles both $\Delta\psi$ and ΔpH were generated upon the addition of ATP. However, attempts to enclose salts into the vesicles failed.

Determination of Mg^{2+}-dependent ATPase activity

The ATPase activity was measured photometrically by a modified method of Serrano [20]. The assay contained 20–40 μg plasma membrane protein, 10–50 mM Mes (pH 6.5), and 2 mM $MgSO_4$ or $MgCl_2$. The reaction was started by the addition of 2–3.3 mM ATP at 30 °C and stopped after 3–5 min.

Determination of ΔpH and $\Delta\psi$ by means of fluorescent probes

Mg^{2+}-ATPase dependent translocation of protons into plasma membrane vesicles was estimated by measuring the quench of fluorescence of 9-amino-6-chloro-2-methoxyacridine (ACMA, 1.3 μM [3]). Plasma membrane vesicles (20–40 μg protein) were suspended in 10 mM Mes or 20 mM Hepes buffer, (pH 6.5), containing 2 mM $MgSO_4$ and 20–40 mM alkali salts. The reaction was started at 22 or 30 °C by addition of 2 mM ATP. The fluorescence of ACMA was measured at

412/480 nm excitation/emission wavelength in a Hitachi fluorescence spectrophotometer type 650-10M.

Generation of $\Delta\psi$ was observed only in plasma membrane vesicles reconstituted without added phospholipids. It was followed by increasing quench of oxonol V [6]. The assay contained 20–40 μg plasma membrane protein, 20 mM Hepes (pH 6.5), 2 mM $MgSO_4$, 0–40 mM alkali salts, and 2.4 μM oxonol V. After the start by 2 mM ATP at 22° C the relative fluorescence quenching of oxonol V was measured at 580/640 nm excitation emission-wavelengths in the same Hitachi fluorescence spectrophotometer.

In all experiments concerning cation specificity of the measured activity the pH of used buffer was adjusted by titration to the desired value with LiOH.

To prove that the fluorescence quenching in suspension of plasma membrane vesicles was caused by operation of the Mg^{2+}-dependent plasma membrane ATPase control experiments were carried out. Boiling of plasma membrane vesicles, omission of Mg^{2+} or substitution of ATP with either ITP, CTP or glucose 6-phosphate (2 mM each) prevented completely any fluorescence quench of both ACMA and oxonol V.

Chemicals

Valinomycin, Hepes, nucleoside triphosphates and glucose 6-phosphate were from Boehringer. MES and L-α-phosphatidylcholine type II-S were from Sigma and Phospholipon 100 from Nattermann. Bovine serum albumine was from Serva and orthovanadate from ICN Pharmaceuticals. Cytohelicase was from Réactif Biologique Française and novozym from Novo Industri AS. FCCP was from Fluka and oxonol V from Molecular Probes Inc. A suspension of silica microbeads was kindly supplied by Dr. B. Jacobson, University of Massachusetts, Amherst, U.S.A., and ACMA was from own synthesis as described by Prelog et al. [21]. All other chemicals were from Merck. Solution were made from quartz double-distilled water.

Results

The plasma membrane ATPase of *M. reukaufii* exhibits a distinct specificity for ATP [15]. When ATP was added to plasma membrane vesicles suspensions, ATP dependent generation of a proton gradient (ΔpH) and of a membrane potential $\Delta\psi$ was induced. There was no H^+ translocation induced when ITP, CTP, or glucose 6-phosphate were added instead of ATP. The proton translocation was strictly dependent on the presence of Mg^{2+}. Since ATP cannot cross the vesicle membrane $\Delta\tilde{\mu}_{H^+}$ generation could be only measured with inside out vesicles, i.e. with those having the ATP-splitting part of ATPase oriented towards the outside of vesicles. However, the percentage of inside out vesicles in the individual reconstituted preparations was not determined.

Specificity of stimulation by cations and anions of ATPase dependent H^+ translocation into plasma membrane vesicles

In order to test ion specificity of salt-stimulated generation of ΔpH in plasma membrane vesicles, given concentrations of salts were enclosed in plasma membrane vesicles during reconstitution from a mixture of plasma membranes and phospholipids. Reconstituted plasma membrane vesicles were resuspended in the same salt concentration. Table I summarizes the obtained results. Whereas anions displayed no significant specificity, K^+ proved to be the most effective stimulant of ΔpH generation in plasma membrane vesicles. In the presence of Li^+ ΔpH generation was very slow, there was no net H^+ translocation in the absence of added salt (except buffer and $MgSO_4$). This pattern of effects corresponds fully to that of salts effect on ATP-splitting activity of plasma membrane vesicles (not shown) as well as of isolated plasma membranes [15]. Table II presents the concentration dependence of ΔpH generation on K^+ enclosed inside plasma membrane vesicles and/or added to the outside of plasma membrane vesicles. The highest stimulation of H^+ translocation was reached with K^+ present on both sides of plasma membrane vesicles.

Proton gradients already built up were dissipated following additions of either an uncoupler (5 μM FCCP) or of the inhibitor of plasma membrane ATPase (0.5 mM orthovanadate,). ΔpH dissipation induced by vanadate was considerably slower as compared with the effect of FCCP (Fig. 1A). However, the effect of vanadate demonstrates

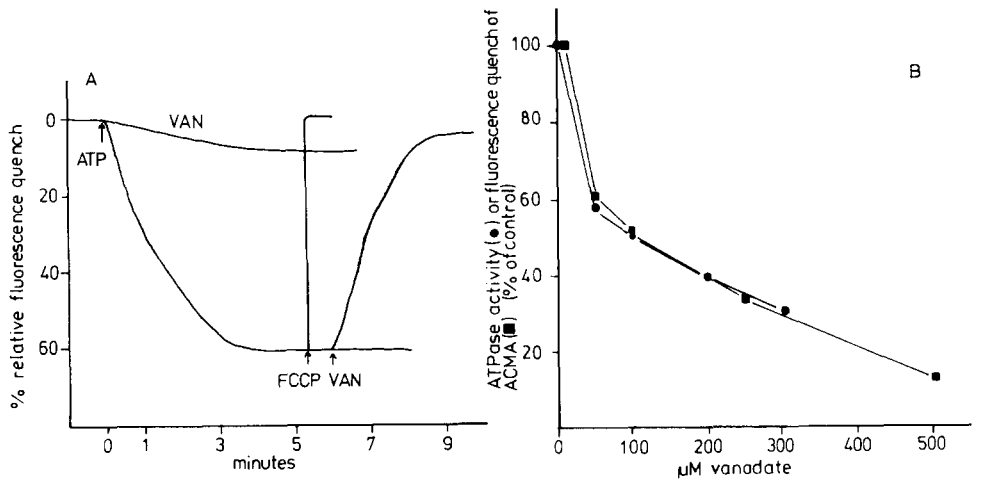

Fig. 1. Generation of ΔpH gradient in plasma membrane vesicles and effect of uncoupler and of vanadate thereupon. Part A: ΔpH generation in plasma membrane vesicles and its dissipation by 5 μM FCCP or 500 μM orthovanadate. Plasma membrane vesicles (20 μg protein) were suspended in 10 mM Mes buffer (pH 6.5) containing 2 mM $MgSO_4$, 40 mM KCl and 1.3 μM ACMA (when ΔpH was measured). The reaction was started by addition of 2 mM ATP, temperature 30 °C. The respective agent was added as indicated by arrows. Part B: Inhibition by orthovanadate of ATPase activity (●) and of ΔpH generation (■). ATPase activity was measured as indicated in Materials and Methods, ΔpH generation as in part A. Data are means of three experiments and expressed in % of control without inhibitor.

TABLE I

EFFECT OF DIFFERENT IONS ON THE GENERATION OF ΔpH GRADIENT (ACMA FLUORESCENCE QUENCH)

The assay samples contained 10 mM Mes (pH 6.5), 2 mM $MgSO_4$, 20 or 40 mM alkali salt, 1.3 μM ACMA, 10–15 μg plasma membrane protein in a total volume 1.5 ml. The same concentrations of alkali salt were also enclosed into plasma membrane vesicles during reconstitution. Carbonate and phosphate salt solution were adjusted to pH 6.5 with concentrated acetic acid. The reaction was started at 30 °C by addition of 2 mM ATP. The data represent arithmetic means ± S.D. (number of measurements). The values are related to control experiments in the presence of 40 mM KCl (100%). Under these conditions the relative ACMA fluorescence quench was 53%.

Added salt	ACMA fluorescence quench (ΔpH generation) (% of control)
40 mM KCl (control)	100 (37)
40 mM KNO_3	78 ± 19 (9)
20 mM K_2CO_3	82 ± 7 (6)
20 mM K_2HPO_4	100 (2)
20 mM K_2SO_4	105 ± 19 (8)
40 mM LiCl	19 ± 5 (21)
40 mM NaCl	37 ± 15 (15)
40 mM RbCl	61 ± 14 (5)
40 mM CsCl	46 ± 9 (6)
No added salt	0 (13)

that plasma membrane vesicles reconstituted into phospholipids are significantly permeable to protons and also the compensating K^+. Increasing the plasma membrane vesicles permeability to K^+ by

TABLE II

EFFECT OF K^+ ENCLOSURE INSIDE AND/OR K^+ ADDITION TO THE OUTSIDE OF RECONSTITUTED PLASMA MEMBRANE VESICLES ON ΔpH GENERATION

The assay samples containing 10 mM Mes (pH 6.5), 2 mM $MgSO_4$, 1.3 μM ACMA and KCl as indicated in a total volume of 1.5 ml. The reaction was started at 30 °C by addition of 2 mM ATP. Plasma membrane vesicles were reconstituted either in the absence of in the presence of increased KCl concentrations. The data given are representative of two experiments and are expressed in % of relative ACMA fluorescence quench relative to that obtained in the presence of 40 mM KCl both inside and outside. Under these conditions the ACMA fluorescence quench amounted to about 50%.

mm KCl (outside)	ΔpH generation, mm KCl (inside)			
	0	5	20	40
0	0	8	20	36
5	20	24	37	57
20	24	50	61	80
40	24	45	65	100

application of valinomycin (0.3 to 1 μM) did not enhance the rate and extent of proton translocation into plasma membrane vesicles. The inhibition by vanadate of ΔpH generation in plasma membrane vesicles goes parallel with that of the ATP-splitting activity of plasma membrane vesicles (Fig. 1B). FCCP, on the other hand, stimulated the ATPase activity of plasma membrane vesicles, in the presence of 40 mM KCl by about 20%. The specific ATPase activity of plasma membrane fraction in the absence of K^+ was stimulated by 5 μM CCCP from 0.13 to 0.17 U/mg protein, whereas 80 mM K^+ enhanced the ATPase activity of the same fraction to 0.70 U/mg.

Relation between ATP-dependent generation of chemical gradient of H^+ (ΔpH) and of electrical potential difference (Δψ) in plasma membrane vesicles

The demonstration of Δψ generation was based on quenching of oxonol V fluorescence. Such a quench was observed only in suspensions of plasma membrane vesicles reconstituted without added phospholipids. These plasma membrane vesicles did not generate ΔpH in the absence of added salt. However, the addition of ATP induced a transient increase of Δψ (Fig. 2). The generation

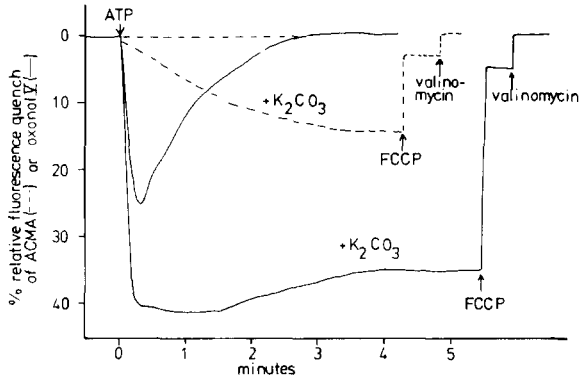

Fig. 2. Generation of ΔpH and of Δψ in plasma membrane vesicles with and without added K_2CO_3 (20 mM). For measurements of two translocations plasma membrane vesicles (40 μg protein) were suspended in 20 mM Hepes buffer (pH 6.5), 2 mM $MgSO_4$ and 1.3 μM ACMA (ΔpH generation, broken line) or 2.4 μM oxonol V (Δψ generation, full line) in a total volume of 1.5 ml. The reaction was started by addition of 2 mM ATP, temperature 22° C. The protonophore FCCP (5 μM) and the ionophore valinomycin (0.3 μM) were added as indicated by arrows.

of Δψ was significantly increased and the resulted Δψ stabilized in the presence of 20 mM K_2CO_3. Concomitantly, ΔpH was also built up, so that under these conditions the overall $\Delta\tilde{\mu}_{H^+}$ across the plasma membrane of vesicles consisted of both of its components. They were both dissipated almost completely following application of the pro-

TABLE III

COMPARISON OF SALT EFFECT ON THE GENERATION OF ΔpH (ACMA FLUORESCENCE QUENCH) AND Δψ (OXONOL V FLUORESCENCE QUENCH)

The assay sample contained 20 mM Hepes (adjusted to pH 6.5 by LiOH), 2 mM $MgSO_4$, 20 or 40 mM alkali salt, 2.4 μM oxonol V or 1.3 μM ACMA and 20–40 μg plasma membrane protein in a total volume of 1.5 ml. Plasma membrane vesicles reconstituted without added phospholipids. The reaction was started at 22° C by 2 mM ATP. Carbonate and phosphate salt solutions were adjusted to pH 6.5 with concentrated acetic acid. The data represent arithmetic means ± S.D. (number of measurements).

(A) Varying anions in potassium salts. The values are related to control experiments in the presence of 20 mM K_2CO_3 (100%), corresponding to 60% relative fluorescence quench of oxonol (Δψ) or to 17% of ACMA (ΔpH).

Added salt	%	
	Oxonol V fluorescence quench (Δψ generation)	ACMA fluorescence quench (ΔpH generation)
20 mM K_2CO_3 (control)	100 (21)	100 (3)
40 mM KCl	31 ± 7 (7)	459 ± 17 (3)
40 mM KNO_3	0 ± 0 (5)	435 (2)
20 mM K_2HPO_4	79 ± 11 (11)	115 ± 6 (3)
20 mM K_2SO_4	54 ± 2 (7)	130 (2)
No added salt	77 ± 9 (19)	0 ± 0 (5)

(B) Varying cations in carbonate and phosphate salts. The values are related to the respective fluorescence quench in the presence of corresponding K^+ salt (100%) (see above).

Added salt	%	
	Oxonol V fluorescence quench (Δψ generation)	ACMA fluorescence quench (ΔpH generation)
20 mM Na_2CO_3	105 ± 5 (3)	43 (1)
20 mM Li_2CO_3	89 ± 7 (3)	0 (2)
20 mM Na_2HPO_4	106 ± 5 (3)	not measured

TABLE IV

EFFECT OF DIFFERENT DCCD CONCENTRATIONS ON ATPase ACTIVITY OF PLASMA MEMBRANE VESICLES AND ON THEIR CAPACITY TO GENERATE ΔpH AND $\Delta\psi$

Experimental conditions as in Figs. 1 and 2. Data are means of two experiments. Numbers in brackets give % of control without DCCD in the presence of 40 mM KCl for ATPase activity or of 20 mM K_2CO_3 for fluorescence measurements. n.m., not measured.

	ATPase activity (U/mg protein)		% relative fluorescence quench of	
	without K^+	40 mM KCl	ACMA (ΔpH)	oxonol V ($\Delta\psi$)
No inhibitor	0.24 (24)	1.02 (100)	27 (100)	70 (100)
+10 μM DCCD	n.m.	n.m.	22.7 (84)	18 (26)
+50 μM DCCD	0.30 (29)	0.67 (66)	19.4 (72)	0 (0)
+300 μM DCCD	0.21 (21)	0.42 (41)	0.4 (1)	n.m.

tonophore FCCP (5 μM). The small rest gradients were sensitive to 0.3 μM valinomycin (Fig. 2).

Depending upon the ionic composition of added salts, either ΔpH- or $\Delta\psi$-generation was favoured (Table III). Permeant anions of potassium salts facilitated the building up of ΔpH whereas less-permeant anions enhanced $\Delta\psi$ generation. The relation of the two component of $\Delta\tilde{\mu}_{H^+}$ was always reverse proportional. Earlier studies with purified plasma membranes demonstrated an almost 4-fold stimulation of plasma membrane-ATPase by K^+ [15]. The results of Table III show that whereas added alkali cation (at best K^+) was necessary prerequisite for building up ΔpH, $\Delta\psi$ was generated transiently also in absence of added salt. Moreover, $\Delta\psi$ generation was almost independent of added cation, however, it was increasingly diminished in the presence of permeant anions.

Effect of various inhibitors on H^+ translocation into plasma membrane vesicles

The inhibitor of membrane-bound ATPases, DCCD (0.3 mM), inhibited the K^+-stimulated ATPase activity of plasma membrane vesicles. At the same time, electroneutral H^+ translocation (ΔpH generation) was also suspended (Table IV). On the other hand, the electrogenic H^+ translocation (building up $\Delta\psi$) was completely inhibited already by 50 μM DCCD, under which conditions plasma membrane vesicles were still capable of ΔpH generation by about 80% capacity.

The K^+-channel blocker quinine at concentrations below 50 μM did not affect the ATPase activity of plasma membrane vesicles. Its effect on ΔpH generation was dependent on the K^+ salt added. In the presence of low-permeant anion (CO_3^{2-}) the size of ΔpH built up was about one-fifth of that built up in the presence of a permeant anion (Cl^-). Quinine (20 μM) had a minor effect on ΔpH in carbonate containing suspensions whereas it inhibited the electroneutral H^+ translocation by 50% in the presence of chloride. Contrary to its effect on electroneutral H^+ translocation, quinine distinctly stimulated the generation of $\Delta\psi$. Already 5 μM quinine increased the fluorescence quench of oxonol V 8-fold. $\Delta\psi$ was generally lower (by a factor of three) in the presence of KCl as compared with K_2CO_3. $\Delta\psi$ built up under these conditions was stable for at least 5 min and was rapidly dissipated by 5 μM FCCP. FCCP-induced depolarization of plasma membrane vesicles was still accelerated by added 0.3 μM valinomycin. Valinomycin alone depolarized plasma membrane vesicles rather slowly, presumably due to the K^+ concentration gradient built up during $\Delta\tilde{\mu}_{H^+}$ generation which supports the positive membrane potential.

Discussion

Earlier work demonstrated that the ATPase activity of *M. reukaufii* plasma membrane was up to 4-fold stimulated by added K^+ [15]. The present results show that driven ΔpH generation by ATP in reconstituted plasma membrane vesicles was maximal in the presence of K^+. Other alkali cations supported ΔpH generation less effective ($K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$). There was no measurable H^+ translocation in the absence of

added salt (Table I). Changing of the anion of added K^+ salt did not have a significant effect on H^+ translocation. The stimulatory effect of cations on ΔpH generation was independent of whether plasma membrane vesicles were reconstituted with or without added phospholipids. The cations (K^+) were effective when added to either side of plasma membrane vesicles, however, they were more effective when presented on both sides.

Contrary to ΔpH, the ATP-driven generation of $\Delta\psi$ (in plasma membrane vesicles without added phospholipids) was independent of the present cation, However it depended strongly on the accompanying anion. There was a transient $\Delta\psi$ built up even without added salt (Table III). We attribute this behaviour to the different membrane permeability for the respective anions, NO_3^- being the best and CO_3^{2-} the least permeant anion. Chloride proved to be a medium permeant anion, capable of supporting both the highest ΔpH and a measurable $\Delta\psi$. Generally, the ratio of ΔpH and $\Delta\psi$ built up in the presence of various salts behaved reciprocally. In the presence of a well-permeant anion ΔpH was high and $\Delta\psi$ low whereas a less-permeant anion favoured $\Delta\psi$ generation (low ΔpH). The cations determined the size of the overall $\Delta\tilde{\mu}_{H^+}$ (Table III), very likely in response to their stimulating effect on plasma membrane ATPase [15].

The evidence for plasma membrane ATPase being the membrane-bound protein moiety translocating H^+ into plasma membrane vesicles from *M. reukaufii* has been proved by (1) the parallel inhibition of H^+ translocation and of ATPase activity by orthovanadate, (2) the equal nucleotide specificity of the two activities, and (3) the requirement of either activity for Mg^{2+}.

The interpretation of results obtained with the K^+-channel blocker quinine [22,23] and the ATPase inhibitor DCCD [24] is more complicated due to the dual action of alkali cations of which K^+ was the most effective. On one side, they stimulate the ATPase activity [15] (see also Table IV), on the other side, they are the prerequisite for the electroneutral H^+ translocation leading to building up of ΔpH gradient. In this sense, quinine reducing the ATPase-independent plasma membrane vesicle permeability for K^+ inhibited the generation of ΔpH under concomitant enhancement of $\Delta\psi$. This means that quinine reduced charge-compensating K^+ fluxes by pathway(s) other than the ATPase, thus enhancing the electrogenic H^+ translocation by the ATPase.

It is important to recall that the highest pH gradient was generated when K^+ was present on both membrane sides of the vesicles. For $\Delta\psi$ measurements, plasma membrane vesicles were reconstituted without enclosed salts, hence, their inner K^+ concentration depended on passive salt permeation, which was higher with a permeant anion, such as chloride. On the other hand, the stimulatory effect of K^+ on the plasma membrane ATPase activity was due to a specific interaction between K^+ and the ATPase since added uncouplers exhibited no significant effect on the ATPase activity.

DCCD applied at 50 μM concentration completely prevented the generation of $\Delta\psi$. Under these conditions plasma membrane vesicles built up still about 80% of the ΔpH gradient, thus demonstrating that preferentially the electrogenic part of H^+ translocation by the ATPase was affected by the inhibitor. Higher DCCD concentration (300 μM) inhibited also the electroneutral H^+ translocation. Correspondingly, the K^+-stimulated ATPase activity also was inhibited by 300 μM DCCD.

The fact that the plasma membrane ATPase of *M. reukaufii* was stimulated 4-fold in the presence of K^+ [15] (see also Table IV) and the results presented in this paper are consistent with the plasma membrane-bound ATPase operating as electrogenic H^+/cation exchanger. This means that the great majority of protons translocated are compensated for by opposite movement of another alkali cation, preferentially K^+; however, a small portion of protons are tanslocated without electrical compensation, thus generating the measurable membrane potential. At least a part of the compensating K^+ flux is through quinine-sensitive channels since quinine reduced ΔpH generation (in particular in the presence of KCl). Concomitantly, quinine increased the electrogenicity of the H^+-ATPase. On the other hand, the electrogenic H^+ translocation was sensitive to DCCD.

Among the plasma membrane-bound ATPases of eukaryotic cells there is one well characterized H^+/K^+-exchanging ATPase of gastric mucosa.

However, the work of Sachs and co-workers [25] presented evidence that this ATPase was an electroneutral cation pump. The yeast plasma membrane ATPase evidently generates $\Delta\psi$ in plasma membrane vesicles. Whereas the gastric ATPase was specific for K^+ the H^+ translocation by the yeast ATPase could be compensated in addition to K^+ partially by Rb^+, Cs^+ and Na^+. The passive unmediated permeation of anions (and cations), being the slowest of all ion translocations, bears importance only for salt diffusion into plasma membrane vesicles reconstituted without added phospholipids, i.e. without enclosed salt ($\Delta\psi$ measurements).

There have been some other indications in the literature as to a functional relation among eukaryotic plasma membrane ATPases such as $(Na^+ + K^+)$-ATPase of animal epithelial cells [12,26], $(H^+ + K^+)$-ATPase of gastric mucosa [27] or H^+-ATPase of yeast cells [13,28] suggesting their common mode of operation. This view has recently got support also from genetic work [29,30].

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft (Grant No. Ho 555).

References

1 Mitchell, P. (1966) Biol. Rev. 41, 445–502
2 Eddy, A.A. (1978) Adv. Microbiol. Physiol. 23, 1–78
3 Dufour, J.P., Goffeau, A. and Tsong, T.Y. (1982) J. Biol. Chem. 257, 9365–9371
4 Ahlers, J. (1984) Can. J. Biochem. Cell Biol. 62, 998–1005
5 Scarborough, G.A. (1980) Biochemistry 19, 2925–2931
6 Perlin, D.S., Kasamo, K., Broocker, R.J. and Slayman, C.W. (1984) J. Biol. Chem. 259, 7884–7892
7 Rasi-Caldogno, F., Pugliarello, M.C. and De Michelis, M.I. (1985) Plant Physiol. 77, 200–205
8 De Michelis, M.I. and Spanswick, R.M. (1986) Plant Physiol. 81, 542–547
9 Anthon, G.E. and Spanswick, R.M. (1986) Plant Physiol. 81, 1080–1085
10 Hauer, R., Uhlemann, G., Neumann, J. and Höfer, M. (1981) Biochim. Biophys. Acta 649, 680–690
11 Künemund, A. and Höfer, M. (1983) Biochim. Biophys. Acta 735, 203–210
12 Robinson, J.D. and Flashner, M.S. (1979) Biochim. Biophys. Acta 549, 145–176
13 Villalobo, A. (1982) J. Biol. Chem. 257, 1824–1828
14 Sze, H. (1980) Proc. Natl. Acad. Sci. USA 77, 5904–5908
15 Gläser, H.-U. and Höfer, M. (1986) J. Gen. Microbiol. 132, 2615–2620
16 Aldermann, B. and Höfer, M. (1981) Exp. Mycol. 5, 120–132
17 Aldermann, B. and Höfer, M. (1984) J. Gen. Microbiol. 130, 711–723
18 Kagawa, Y. and Racker, E. (1971) J. Biol. Chem. 246, 5477–5487
19 Racker, E. (1973) Biochem. Biophys. Res. Commun. 55, 224–230
20 Serrano, R. (1978) Mol. Cell. Biochem. 22, 51–63
21 Prelog, V., Rajner, E. and Stern, P. (1943) Helv. Chim. Acta 26, 1172–1180
22 Findlay, I., Dunne, M.J., Ullrich, S., Wollheim, C.B. and Petersen, O.H. (1985) FEBS Lett. 185, 4–8
23 Diwan, J.J. (1986) Biochem. Biophys. Res. Commun. 135, 830–836
24 Solioz, M. (1984) Trends Biochem. Sci. 9, 309–312
25 Sachs, G., Chang, H.H., Rabon, E., Schackman, R., Lewin, M. and Saccomani, G. (1976) J. Biol. Chem. 251, 7690–7698
26 Keynes, R.D. (1969) Quart. Rev. Biophys. 2, 177–281
27 Faller, L., Jackson, R., Malinowska, D., Mukidjam, E., Rabon, E., Saccomani, G., Sachs, G. and Smolka, A. (1982) in Annals of the New York Academy of Sciences (Carafoli, E. and Scarpa, A., eds.), Vol. 402, pp. 146–163, The New York Academy of Sciences, New York
28 Malpartida, F. and Serrano, R. (1981) J. Biol. Chem. 256, 4175–4177
29 Serrano, R., Kielland-Brandt, M.C. and Fink, G.R. (1986) Nature 319, 689–693
30 Addison, R. (1986) J. Biol. Chem. 261, 14896–14901