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Indications for a K^+/H^+ cotransport system in plasma membranes from two acidophilic microorganisms

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The unicellular green alga *Dunaliella acidophila* grows optimally at pH 1.0 while maintaining a neutral cytoplasmic pH and a positive membrane potential. To study the mechanism of K^+ uptake in this organism and in the acidophilic yeast *Metschnikowia reukaufii*, ^{86}Rb was trapped in plasma membrane vesicles of these organisms, by freezing and thawing with phospholipid vesicles. Both preparations possess K^+-stimulated, vanadate-sensitive H^+-ATPases. ATP stimulates ^{86}Rb efflux from the reconstituted vesicles, and the stimulation is abolished by vanadate. The protonophore SF-6847 also inhibits the ATP-dependent ^{86}Rb efflux. In contrast, the lipophilic anion SCN^-, which abolishes the ATP-induced membrane potential, does not inhibit ^{86}Rb efflux. An artificial pH difference, acid inside, stimulates ^{86}Rb efflux in reconstituted *D. acidophila* plasma membranes and the stimulation is not affected by vanadate but is inhibited by SF-6847. These results suggest the existence of a K^+/H^+ cotransport system acting in parallel to the H^+-ATPase in the plasma membranes of these organisms. It can explain how acidophiles accumulate K^+ against a large electrochemical potential gradient.

Introduction

Plants and algae take up K^+ from the medium and sustain concentration gradients of 10^2–10^4-fold similar to animal cells in spite of the fact that they do not possess an ouabain-sensitive Na^+/K^+-ATPase. Potassium uptake in plants and algae is dependent on metabolic energy but its mechanism is not fully understood. It is generally assumed that potential-dependent K^+ channels in plant plasma membranes are the major mechanism of K^+ transport, and that the driving force for K^+ uptake is the membrane potential, generated by the vanadate-sensitive H^+-ATPase, although other mechanisms have also been considered [1]. The fact that the H^+-pump is stimulated by K^+, along with several measurements of large K^+ gradients which cannot be accounted for by the membrane potential, led to suggestions that the vanadate-sensitive H^+-ATPase may act as an H^+/K^+ exchange pump (reviewed in Ref. 2). The only report of direct measurements of K^+ transport catalyzed by a plasma membrane H^+-ATPase comes from a yeast-reconstituted enzyme, in which ATP or an imposed membrane potential was shown to stimulate K^+ efflux, and which was inhibited by vanadate [3]. A different K^+/H^+ antiport system which is activated by cytoplasmic pH perturbations has been described in *Chlorella*. However, the function of this system seems to be mainly in pH homeostasis and not in K^+ uptake [4]. The group of Blatt and Slayman has produced strong kinetic and thermodynamic evidence for a H^+/K^+ cotransport system in *Neurospora* [5,6] which may resemble the low-affinity K^+ transport system (Trk) in *E. coli* and similar bacteria [7,8], whereas in the alga *Chara* recent evidence suggests the existence of a Na^+/K^+ cotransport mechanism [9].

Potassium uptake in extreme acidophiles is particularly problematic since extreme acidophiles possess a positive-inside membrane potential and a pH gradient which do not allow K^+ uptake via a channel or by K^+/H^+ antiport [8,10]. In this study we have investi-

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Abbreviations: SF-6847, 3,5-di(*tert*-butyl)-4-hydroxybenzylidenemalonitrile; ACMA, 9-amino-6-chloromethoxyacridine; Mes, 4-morpholinoethanesulphonic acid; oxonol VI, bis-(3-propyl-5-oxoisoxazol-4-yl)pentamethine oxonol.

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gated the involvement of vanadate-sensitive H^+-ATPases from plasma membranes of two acidophiles in K^+ transport. The organisms that were studied are *Dunaliella acidophila,* an extremely acidophilic green alga, which grows optimally at pH 1, accumulates K^+ at least 100-fold with respect to the medium and sustains neutral cytoplasmic pH and a positive-inside membrane potential [11,12] and the yeast *Metschnikowia reukaufii.* Plasma membranes from both organisms possess vanadate-sensitive electrogenic H^+-ATPases, which are stimulated 3–4-fold by K^+ (Refs. 13, 14; Sekler et al., unpublished observations).

Materials and Methods

Plasma membrane preparations

D. acidophila (Masyuk strain No SAG 1985 in algal collection of the Institute of plant physiology, Göttingen) was a generous gift from Dr. Schlösser, Göttingen. The cells were cultured as previously described in a growth medium adjusted to pH 1 [15]. The preparation of *D. acidophila* plasma membranes was done as follows. 2–15 litres of algae, 10^7 cells/ml, were collected by centrifugation ($500 \times g$, 10 min) and washed once in 1 litre of glycerol buffer containing 0.7 M glycerol, 30 mM Tris-Mes (pH 6.5) and 2 mM $MgCl_2$. From this stage all purification steps were performed at 0–4°C. The cells were resuspended in 30–40 ml of glycerol buffer and osmotically ruptured by dilution to a final volume of 270–350 ml in bursting buffer, containing 5 mM EDTA, 5 mM β-mercaptoethanol, 20 mM Tris-Mes (pH 6.5), 1% poly(vinylpyrrolidone) (PVP-40), 5 mM γ-aminocaproic acid and 1 mM benzamidine. Following 10 min incubation, the cells were passed twice through a Yeda press apparatus (pressure of 1000 atmosphere, flow rate of approx. 100 ml/min). 200 mM ethanolamine chloride, 100 mM choline chloride and 50 mM KCl were added to the ruptured cells. Unbroken cells and cell debris were removed by a brief centrifugation ($5000 \times g$ 10 min) and the resulting supernatant was supplemented with 10% glycerol. Fractionation of plasma membranes was obtained by layering 30 ml of this mixture onto 30/43% sucrose, 18 ml each, containing also 2 mM EDTA, 20 mM Tris-Mes, 0.5% PVP-40, 50 mM KCl and 200 mM ethanolamine chloride. The gradients were centrifuged at $250000 \times g$ for 120 min and the interphase between the 30 and 43% sucrose was carefully collected with a syringe, diluted to 300 ml with suspension buffer containing 1 mM EDTA, 10 mM Tris-Mes (pH 6), 10 mM KCl, 250 mM sucrose and 1 mM β mercaptoethanol and centrifuged again. The pellet was resuspended in 4–10 ml suspension buffer, homogenized with a Potter homogenizer, centrifuged at $500 \times g$ for 10 min to remove residual aggregates and kept at −196°C. Preparation of plasma membranes from *M. reukaufii* was done as described by Gläser and Höfer [14].

Reconstitution of plasma membrane vesicles

Soybean phosphatidylcholine 40 mg/ml was dissolved in 10 mM Tris-Mes (pH 6), sonicated to clarity, dialyzed 4 h at 22°C against the same buffer and dialyzed again for another 12 h at 4°C. Plasma membranes, stored in liquid nitrogen, were dialyzed for 2 h at 4°C before the reconstitution against 0.5 litre of 10 mM Tris-Mes (pH 6), 0.25 M sucrose, 2 mM mercaptoethanol and 0.5% (w/v) poly(vinylpyrrolidone).

Sonicated phospholipids (8 mg) were mixed on vortex with plasma membranes (200–300 μg protein) and ^{86}Rb (20–30 μM) in 800 μl buffer containing 10 mM Tris-Mes (pH 6) and alkali salt and frozen in liquid nitrogen. After thawing at room temperature the samples were either used directly for ^{86}Rb efflux measurements or passed first through Sephadex-G-50 columns, equilibrated with the same buffer by the centrifugation procedure to remove untrapped ^{86}Rb [16].

^{86}Rb efflux measurements

ATP-dependent ^{86}Rb efflux. After reconstitution 2 mM $MgSO_4$, 1 mg/ml bovine serum albumin and in part of the experiments an ATP-regenerating system containing 4 mM phospho*enol*pyruvate and 5 U/ml pyruvate kinase was also added to the vesicle suspension. Following a preincubation of 5 min at 24°C the reaction was started by addition of 0.7–2 mM ATP. Trapped ^{86}Rb content was determined by separation of external ^{86}Rb on Dowex-50 columns. Samples of 50 μl were applied to 3 ml Dowex-50/Tris columns preequilibrated with 0.2 M sucrose and 10 mM Tris-Mes (pH 6) and washed with 2.5 ml of the same buffer as previously described [17].

Acid-base treatment. Reconstituted proteoliposomes supplemented with $MgSO_4$ and bovine serum albumin as above were incubated with 40 mM sodium succinate at pH 4.85 for 4 min. Unbuffered Tris (60 mM) was added to bring the pH to 7.9 and samples of 50 μl were taken to Dowex-50 columns as described above for determination of trapped ^{86}Rb.

Base-acid treatment. Proteoliposomes were reconstituted in buffer containing 100 mM Tris-HCl (pH 8.5) by freezing and thawing, transferred through Sephadex G-50 columns preequilibrated with 100 mM Tris-HCl, 50 mM Na_2SO_4 (pH 8.5) and supplemented with $MgSO_4$ and bovine serum albumin as above. ^{86}Rb uptake was initiated by addition of unbuffered Mes (50 mM, final pH 5.2) and ^{86}Rb. Samples of 50 μl were taken to Dowex-50 columns as described above.

Measurements of ΔpH and $\Delta\psi$ in reconstituted vesicles

Internal acidification of proteoliposomes was measured by fluorescence quenching of 9-amino-6-chloro-

methoxyacridine (ACMA). Proteoliposomes (20–30 μg protein) were suspended in 10 mM Tris-Mes (pH 6), 5 mM $MgSO_4$, 2 μM ACMA and 10–20 mM alkali salt. The reaction was started by addition of 0.5 mM ATP at 25 °C. Fluorescence changes were followed in a Perkin-Elmer MPF-44A spectrofluorimeter at excitation and emission wavelengths 412 and 480 nm, respectively. Electrical potential differences were measured by following absorbance changes of oxonol VI at 603 and 590 nm in an Aminco DW 2a spectrophotometer. The reaction mixture was identical to that described above, except that 1 μM oxonol VI replaced ACMA.

Analytical methods

ATPase activity was measured by the release of inorganic phosphate according to Ames [18]. In brief, 50 μl reconstituted vesicles containing 10–15 μg protein were suspended in 200 μl reaction mixture containing 10 mM Tris-Mes (pH 6), 2 mM $MgSO_4$, 1 mM ATP and 10 mM alkali salt at 26 °C. The reaction was stopped with 0.8 ml containing 5 mM ammonium molybdate, 0.5 M suphuric acid, 1% (w/v) sodium dodecyl sulphate and 1.7% (w/v) ascorbate. Protein concentration was measured according to Markwell et al. [19].

Results

In order to check whether there is an ATP-dependent K^+ transport activity in plasma membranes of acidophiles, ^{86}Rb had to be introduced into the vesicles. Attempts to load native plasma membrane vesicles with ^{86}Rb by prolonged incubations resulted in trapping of very small amounts of the isotope, either due to a small

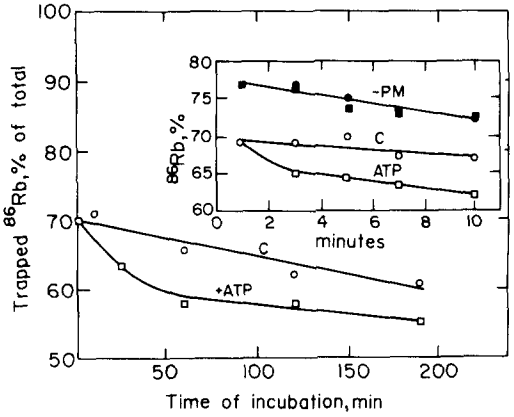

Fig. 1. Time-course of ATP-stimulated ^{86}Rb efflux. *D. acidophila* plasma membrane vesicles were loaded by reconstitution with ^{86}Rb and 10 mM K_2SO_4 and the untrapped ^{86}Rb was removed by centrifugation through Sephadex G-50 columns as described under Materials and Methods. The reconstituted proteoliposomes were incubated at 22 °C in a K_2SO_4 buffer and the reaction was started by addition of 2 mM ATP. Results are expressed as percent of the total trapped ^{86}Rb in the proteoliposomes. C, control proteoliposomes without ATP. −PM, control phospholipid vesicles reconstituted without plasma membranes.

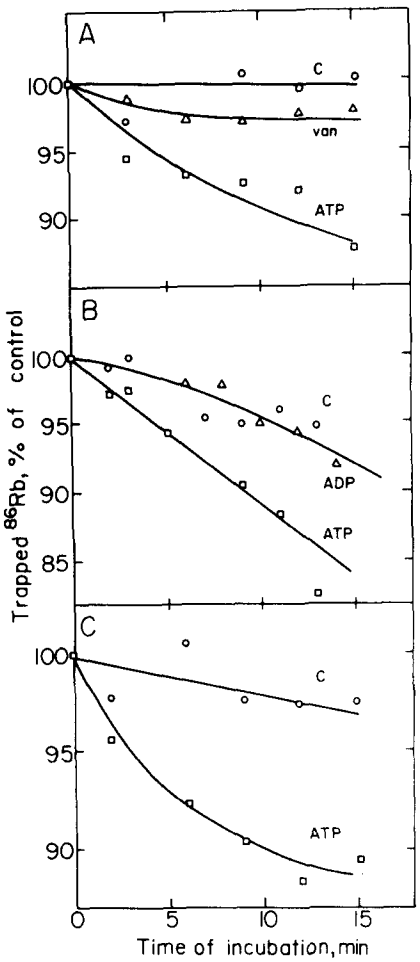

Fig. 2. Effects of vanadate and ADP on ^{86}Rb efflux. Proteoliposomes of *D. acidophila* (A, B) or *M. reukaufii* (C) were reconstituted in Na_2SO_4 buffer and were used either immediately (A, C) or centrifuged through Sephadex G-50 columns (B) as in Fig. 1. Additions in (A) are 0.7 mM ATP with or without 0.5 mM orthovanadate. In (B) 2 mM ATP or 2 mM ADP, and in (C) 1 mM ATP with or without 0.5 mM orthovanadate. Other details are described under Materials and Methods.

trapping capacity or because of leakiness of the plasma membranes. Therefore, ^{86}Rb was trapped in plasma membranes by freezing and thawing with sonicated phospholipids. This procedure yields tightly-sealed reconstituted proteoliposomes with a large trapping capacity [16]. The preparations that were chosen are plasma membranes of the green alga *Dunaliella acidophila*, which grows optimally at pH 1, and from the acidophilic yeast *Metschnikowia reukaufii*. The ^{86}Rb-trapping procedure was performed by rapid freezing of plasma membranes with sonicated phospholipid vesicles (at a ratio of 1 : 40 w/w). Untrapped ^{86}Rb was removed in part of the experiments (Fig. 1) by a Sephadex Penefsky column, and the trapped ^{86}Rb content was measured by transferring the reconstituted proteoliposomes through a cation-exchange minicolumn [17]. Fig. 1 demonstrates that ATP stimulates ^{86}Rb efflux from the proteoliposomes, but not from pure-lipid

vesicles (inset). The relative stimulation is quite variable (4–20-fold), due to the large variability in the passive permeability of soybean phospholipids to K^+ which seems to be due to the presence of K^+ channels which can be partly inactivated by dialysis at elevated temperature [20]. It may be noted that the ATP stimulated ^{86}Rb release is transient, leading to a decrease of about 15% of the trapped ^{86}Rb in the preparation. This is probably the fraction of vesicles which contain plasma membrane components in the reconstituted preparation. A similar ATP-stimulated ^{86}Rb efflux is obtained in reconstituted plasma membrane preparations from *D. acidophila* (Fig. 2A and B) and *M. raukaufii* (Fig. 2C). The stimulation is inhibited by the ATPase inhibitor orthovanadate (Fig. 2A) and is not observed when ADP replaces ATP (Fig. 2B), suggesting that ^{86}Rb efflux is induced by the activity of the vanadate-sensitive H^+-ATPase.

Several different mechanisms may lead to an ATP-dependent ^{86}Rb efflux in the reconstituted proteoliposomes. One possibility is that the ATPase is a K^+/H^+ antiporter, catalyzing K^+ (^{86}Rb) efflux in exchange with H^+ uptake. Another is that ^{86}Rb efflux is driven by the transmembrane potential generated by the electrogenic H^+-pump via K^+ channels. A third mechanism is that the pH gradient generated by the H^+ pump induces ^{86}Rb efflux via a K^+/H^+ cotransporter.

In order to try to estimate the contribution of the membrane potential ($\Delta\psi$) and pH gradient to ^{86}Rb efflux we have compared the formation of ATP dependent $\Delta\psi$ and ΔpH in proteoliposomes reconstituted in sulphate and chloride media. The direction of the membrane potential (positive inside), estimated from oxonol VI absorbance changes, was verified by comparison to an inwardly directed K^+ concentration difference in the

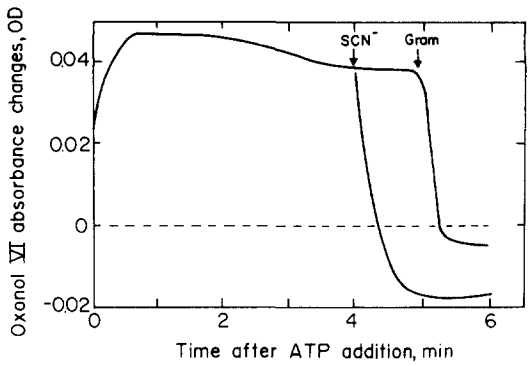

Fig. 4. Effect of isothiocyanate on ATP-induced $\Delta\psi$. *D. acidophila* plasma membranes were reconstituted in Na_2SO_4 buffer and the oxonol VI absorbance changes were measured as in Fig. 3. 20 mM NaSCN or 1 μM gramicidin D were added where indicated. The broken line represents a control sample containing gramicidin D before addition of ATP.

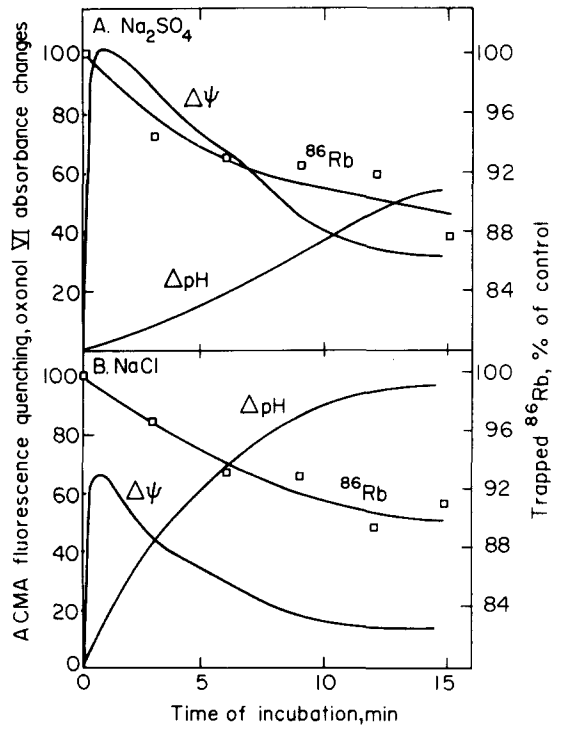

Fig. 3. Comparison of ATP-induced ^{86}Rb efflux, ΔpH and $\Delta\psi$ generation in chloride and sulphate media. *D. acidophila* plasma membranes were reconstituted either in 10 mM Na_2SO_4 (A) or in 20 mM NaCl (B) and analyzed in the same medium for ^{86}Rb efflux, ACMA fluorescence quenching (ΔpH) or oxonol VI absorbance changes ($\Delta\psi$) following addition of ATP as described under Materials and Methods. ΔpH: 100 units correspond to 10% of total ACMA fluorescence. $\Delta\psi$: 100 units correspond to 0.0025 *A* units. ^{86}Rb: 100% correspond to trapped ^{86}Rb content at zero time.

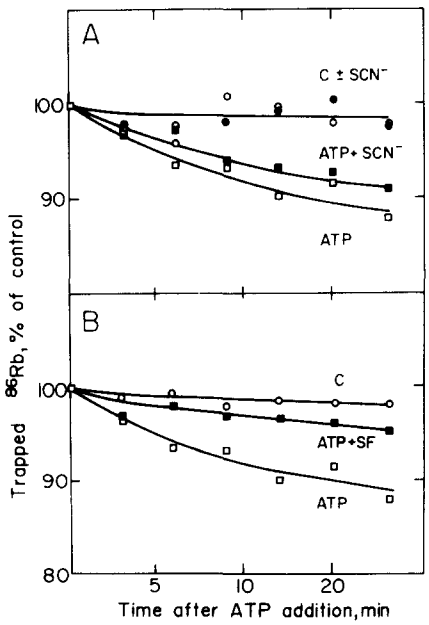

Fig. 5. Effects of isothiocyanate and a protonophore on ^{86}Rb efflux. *D. acidophila* plasma membranes were reconstituted in 20 mM NaCl buffer. In (A) the reaction was started by addition of 0.7 mM ATP in the presence (○, □) or absence (○, ■) of 20 mM NaSCN. Controls (C) contained 0.5 mM orthovanadate in addition to ATP. In (B) 10^{-7} M SF-6847 was added where indicated.

TABLE I

Effects of the protonophore SF-6847 and isothiocyanate on ATP hydrolysis, ATP-induced ^{86}Rb efflux and $\Delta\psi$ formation

Plasma membranes were reconstituted in the presence of either 10 mM Na_2SO_4 or K_2SO_4 or 20 mM NaCl or KCl. SF-6847 (10^{-7} M) or NaSCN (20 mM) were added before ATP. ATPase activity is corrected for non-specific ATP hydrolysis in the presence of 0.5 mM vanadate (less than 20%). Initial rates of ^{86}Rb efflux are expressed as % decrease of total trapped isotope per min, and $\Delta\psi$ is expressed as the maximal change in absorbance of oxonol VI. The number of experiments is shown in brackets.

Salt	^{86}Rb efflux (% decrease /min)	$\Delta\psi$ (*A* units $\times 10^3$)	ATPase (μmol/mg protein per min)
A. *D. acidophila*			
Na_2SO_4	0.54 ±0.12 (4)	2.20	0.21
Na_2SO_4 + SF	0.08	0.70	0.24
Na_2SO_4 + NaSCN	0.49	0.00	0.19
NaCl	0.57 ±0.11 (3)	0.95	0.24
NaCl + SF	0.22 ±0.01 (2)	0.15	0.23
NaCl + NaSCN	0.47 ±0.01 (2)	0.00	0.24
K_2SO_4	0.11	0.60	0.32
KCl	0.15	0.25	0.24
B. *M. reukaufii*			
Na_2SO_4	0.57 ±0.07 (2)	1.70	0.28
Na_2SO_4 + SF	0.015 ±0.01 (2)	0.45	0.34
Na_2SO_4 + NaSCN	0.45 ±0.05 (2)	0.40	0.31
C. Phospholipid vesicles			
Na_2SO_4	0	0	–

presence of valinomycin. As is demonstrated in Fig. 3, in sulphate medium the formation of $\Delta\psi$ is larger, whereas ΔpH, estimated from ACMA fluorescence quenching is smaller than in chloride medium. Nevertheless, the rate of ^{86}Rb efflux is not significantly different in the two preparations. In order to obtain a more conclusive answer about the effect of $\Delta\psi$ and ΔpH on ^{86}Rb efflux we looked for a way to abolish them completely. Fig. 4 demonstrates that the lipophilic anion isothiocyanate (SCN^-) completely inhibits the ATP induced $\Delta\psi$. Yet, isothiocyanate only slightly inhibits ^{86}Rb efflux from proteoliposomes under similar conditions (Fig. 5A, Table I), indicating that $\Delta\psi$ is not the major driving force for ^{86}Rb efflux. In contrast, the protonophore SF-6847 almost completely inhibits ^{86}Rb efflux (Fig. 5B, Table I). SF-6847, completely inhibits ΔpH (not shown) and does not inhibit but rather slightly stimulates ATP hydrolysis under these conditions. These results indicate that ΔpH is the driving force for ^{86}Rb efflux.

The minor inhibition of ^{86}Rb efflux by the collapse of $\Delta\psi$, indicated by the small effect of SCN^-, does not appear to be consistent with a K^+/H^+ cotransport, which is expected to be driven by both ΔpH and $\Delta\psi$. This result may be a consequence of a compensatory increase in ΔpH generated by the H^+-ATPase due to

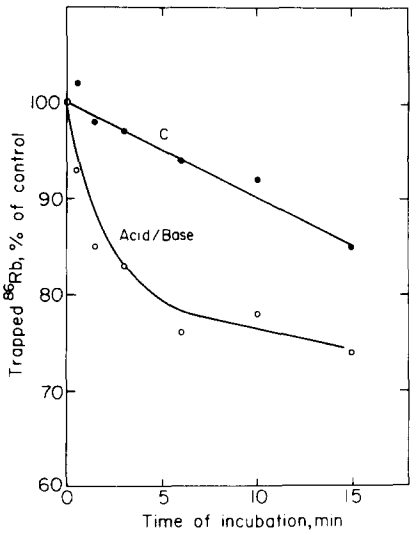

Fig. 6. ^{86}Rb efflux induced by an artificial pH gradient. Reconstituted *D. acidophila* proteoliposomes were incubated with 40 mM sodium succinate at pH 4.85 and transferred to pH 7.9 (zero time) to induce the artificial ΔpH as described under Materials and Methods. Control vesicles were incubated for the same time in the final acid base mixture.

$\Delta\psi$ dissipation. Indeed we have observed a small enhancement of ATP-induced ACMA fluorescence quenching by SCN^- which is consistent with this interpretation (data not shown). Further indirect support for a K^+/H^+ cotransport is the much smaller oxonol signal measured with trapped K^+ salts (Table I), which is consistent with a large efflux of K^+ and protons through the cotransporter, that reduces the ATP-generated $\Delta\psi$.

In order to check more directly the effect of a pH gradient on ^{86}Rb efflux, artificial pH gradients were imposed in proteoliposomes and their effects on ^{86}Rb transport was studied. Fig. 6 demonstrates that an

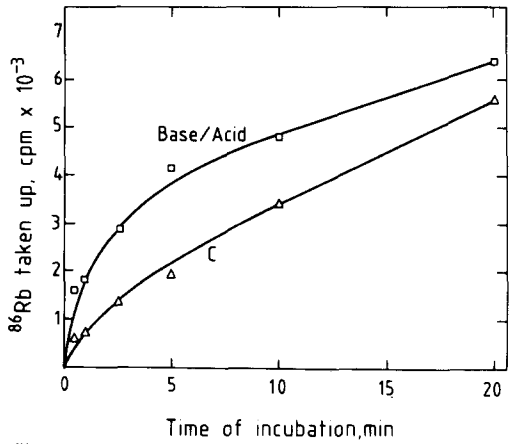

Fig. 7. ^{86}Rb uptake induced by an artificial base-acid treatment. Proteoliposomes of *D. acidophila* were reconstituted in Tris-HCl (pH 8.5). At zero time a mixture of ^{86}Rb and unbuffered Mes (final pH 5.2), or Tris-HCl (final pH 8.5, control) were added and samples were taken for analysis of ^{86}Rb content. Other details are described under Materials and Methods.

TABLE II

Properties of acid-base induced ^{86}Rb efflux

Plasma membranes of *D. acidophila* were reconstituted and treated by acid-succinate (pH 4.95) and basic Tris (pH 8.05) as described in Fig. 6. Vanadate (0.5 mM), SF-6847 ($2 \cdot 10^{-7}$ M), isothiocyanate (20 mM) or ATP (0.5 mM) were added during the acid-succinate treatment. A/B, acid-base treated; C, control without treatment. Averages of two experiments are presented.

Treatment	^{86}Rb efflux (% decrease/min)
A. *D. acidophila* proteoliposomes	
A/B	6.3
A/B + vanadate	5.8
A/B + SF	4.4
A/B + NaSCN	5.4
A/B + ATP	6.5
C	0.6
B. Phospholipid vesicles	
A/B	0.9
C	0.7

acid/base treatment induced by incubating proteoliposomes at pH 5, in the presence of succinic acid which serves as an effective internal buffer, followed by rapid alkalinization of the medium stimulates a rapid efflux of about 20% of the trapped ^{86}Rb from the proteoliposomes. The ^{86}Rb efflux is not observed in pure lipid vesicles and is not inhibited by vanadate or by isothiocyanate, but is partially inhibited by SF-6847 (Table II). Conversely, a base/acid pH gradient, induced by trapping alkaline Tris buffer in proteoliposomes followed by rapid acidification of the external medium stimulates ^{86}Rb uptake into proteoliposomes (Fig. 7). These results are consistent with the idea that ΔpH is the driving force for ^{86}Rb efflux, probably via a K^+/H^+ cotransporter.

Discussion

The presence of a K^+/H^+ cotransport system in parallel with a H^+-ATPase has several advantages for an acidophile. First, it provides a system for K^+ uptake which is driven by the existing pH gradient across the plasma membrane that is generated and maintained by the H^+-pump. By assuming a K^+/H^+ stoichiometry of 1 : 1, and by ignoring the transmembrane electrical potential the existing pH gradient is large enough to concentrate K^+ up to 10^6-fold in *D. acidophila* or 10^4-fold in *M. reukaufii*. The advantage in this combination over a primary K^+/H^+ pump is that there is no obligatory connection between H^+ extrusion and K^+ accumulation, which is a dangerous option in case of pH stress in the absence of external K^+. To date there are only a few reports about K^+/H^+ cotransport activities, and none of them from direct studies in membrane vesicles. The most prominent work, by Blatt et al., comes from electrophysiological studies in *Neurospora*, which is a mild acidophile [5,6]. The Trk K^+ transport system in *E. coli* and related bacteria was suggested to be a K^+/H^+ cotransport, although a direct demonstration for such a mechanism has not yet been obtained [7]. It may be noted that a similar K^+ transport mechanism exists in acidophilic bacteria [8], which may well be the evolutionary origin of this transport system. The only report of an ATP driven K^+ efflux from membrane vesicles is the work of Vilalobo [3] with the reconstituted H^+-ATPase from the yeast *Schizosaccharomyces pombe*. However, contrary to our results, this ATP-stimulated K^+ efflux is driven by the primary pump itself or by the membrane potential generated by the pump, and not by the pH gradient [3].

A second possible role of a K^+/H^+ cotransport system in acidophilic organisms is in creating a positive-inside $\Delta\psi$. Such a reversed membrane potential has been observed in acidophilic bacteria at very acidic pH [8] and in *D. acidophila* [12]. A positive $\Delta\psi$ is probably crucial for acidophiles in decreasing the protonmotive force across the plasma membrane in order to reduce the proton leak into the cells and to decrease the back-pressure for H^+-extrusion via respiratory and H^+-ATPase systems. A K^+/H^+ cotransport acting in parallel with a H^+ pump can explain how a positive-inside $\Delta\psi$ is generated and sustained in spite of the opposite polarity of the H^+-ATPase. Such a situation is possible if in the steady-state the overall flux of charge through the cotransporter ($H^+ + K^+$) is larger, while the net H^+ influx is smaller than the rate of H^+ efflux through the H^+-extruding ATPase. Such a mechanism has been proposed before for acidophilic bacteria [10].

One of the interesting questions for which we have no information at present, is the control mechanism of the H^+ pump and K^+/H^+ cotransporter in acidophiles. It may be surmised that the two systems have to function in concert in order to sustain the intracellular pH, intracellular K^+ and a positive $\Delta\psi$. In preliminary experiments we observed that K^+ uptake in *D. acidophila* cells in the light is 3–5-fold faster than in the dark (Gläser, unpublished observations). It has also been reported recently that $\Delta\psi$ becomes less positive inside upon illumination, indicating enhanced activity of the H^+-ATPase [12]. These observations seem to indicate that in the light there is an increased recycling of protons across the plasma membrane brought about by activation of the H^+-ATPase and the cotransporter. How the two transport systems are activated remains to be elucidated in the future.

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