Proton Gradient Across the Tonoplast of *Riccia fluitans* as a Result of the Joint Action of Two Electroenzymes[1]

Eva Johannes[2] and Hubert Felle*

*Botanisches Institut I, Justus-Liebig-Universität, Senckenbergstrasse 17-21,
D-6300 Giessen, Federal Republic of Germany*

ABSTRACT

Using pH-sensitive microelectrodes (*in vitro*) and acridine orange photometry (*in vivo*), the actions of the two tonoplast phosphatases, the tp-ATPase and the tp-PPase, were investigated with respect to how effectively they could generate a transtonoplast pH-gradient. Under standard conditions the vacuoles of the aquatic liverwort *Riccia fluitans* have an *in vivo* pH of 4.7 to 5.0. In isolated vacuoles a maximal vacuolar pH (pH_v) of 4.74 ± 0.1 is generated in the presence of 0.1 millimolar PP_i, but only 4.93 ± 0.13 in the presence of 2.5 millimolar ATP. Both substrates added together approximate the value for PP_i. Cl^--stimulates the H^+-transport driven by the tp-ATPase, but has no effect on the tp-PPase. The transport activity of the tp-ATPase approximates saturation kinetics ($K_{1/2} \approx 0.5$ millimolar), whereas transport by the tp-PPase yields an optimum around 0.1 millimolar PP_i. The transtonoplast pH-gradient is dissipated slowly by weak bases, from which a vacuolar buffer capacity of roughly 300 to 400 millimolar/pH_v unit has been estimated. From the free energy (-11.42 kilojoules per mole) for the hydrolysis of PP_i under the given experimental conditions, we conclude that the PPase-stoichiometry (transported H^+ per hydrolyzed substrate molecule) must be 1, and that *in vivo* this enzyme works as a H^+-pump rather than as a pyrophosphate synthetase.

The generation and maintenance of a steep transtonoplast electrochemical proton gradient is essential for plant cells, because many transport processes across the tonoplast are energetically linked to proton movements. It is therefore of general interest to investigate the primary active systems which are involved in these processes and to study their potential role in cellular pH-regulation. From *in vitro* studies, carried out mainly on tonoplast vesicles and in rarer cases on intact vacuoles, we know of two tonoplast located phosphohydrolases which both actively transport H^+ from the cytosol into the vacuole: an anion-sensitive ATPase and a cation-sensitive PPase[3] (23). Both enzymes are different as to their sensitivity toward inhibitors and effectors. Whereas the tp-

ATPase is stimulated by Cl^- and inhibited by NO_3^-, the tp-PPase is essentially insensitive to anions but requires high K^+ for proper action.

Taking these characteristics into account, we analyze the two electroenzymes with respect to their stoichiometries and their role in cellular pH-regulation using proton transport across the tonoplast as a measured parameter.

MATERIALS AND METHODS

Plants and General Conditions

Thalli of *Riccia fluitans* L. were grown under sterile conditions in a 12 h light/dark regime as previously described (7, 14). The media for electrophysiological tests comprised 1 mM KCl, 0.1 mM NaCl, 0.1 mM $CaCl_2$, 5 mM (MES + Tris), adjusted to the pH given in the text or legends. The media for the preparation of the protoplasts and the vacuoles are given in Table I.

Electrophysiology

The pH-sensitive microelectrodes for intracellular tests were prepared as described in Felle and Bertl (8a). Intracellular measurements were carried out using a high impedance amplifier (FD 223, WP-Instruments, New Haven, CT). Otherwise, standard electrophysiology was applied.

Preparation of Protoplasts and Vacuoles

The procedure has been described in detail in Johannes and Felle (15). Briefly, the thalli were cut into 0.5 cm pieces and thoroughly rinsed with medium A (Table I) to prevent inhibition of the cell wall-digesting enzymes. The enzyme cocktail comprised 0.5% (w/v) Driselase (Sigma), 0.5% (v/v) β-glucuronidase H2 (Sigma), and 1% (w/v) BSA dissolved in medium A. After incubation of the thalli in the enzyme solution for 14 to 16 h at 27°C the enzymes were replaced by medium B (Table I). Gentle shaking and vacuum infiltration set the protoplasts free. Purification was carried out by flotation through a discontinuous Ficoll gradient (media D, C, B; bottom to top) at 100 g for 12 min at 10°C. The protoplasts gathered in the top layer (medium B, no Ficoll).

The isolation of vacuoles was carried out as described by Kreis and Reinhard (16) for *Digitalis lanata* and modified by Johannes and Felle (15). The lysis of the protoplasts was induced by a sudden decrease in the osmotic strength of the medium, a change in pH from 5.7 to 7.2, and through the

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[2] Present address: Department of Biology, University of York, Heslington, York YO1 5DD, England.

[3] Abbreviations: PPase, pyrophosphatase; DCCD, *N,N'*-dicyclohexylcarbodiimide; FCCP, *p*-trifluoromethoxycarbonyl cyanide phenylhydrazone; KIDA, potassiumiminodiacetate; pH_c, cytosolic pH; pH_v, vacuolar pH; $\Delta\mu H^+/F$, electrochemical proton gradient.

Table I. *Media Used for the Preparation of Protoplasts (A-D), for Protoplast Lysis (E), and for the Isolation and Purification of Vacuoles (F-H)*

All concentrations are given in mM, except for Ficoll (% w/w).

Component	A	B	C	D	E	F	G	H
KCl	10	10	10	10		50	50	50
MgCl₂	2	1	1	1		0.5	0.5	0.5
CaCl₂	0.5							
DTT	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
MES	20	20	20	20				
Hepes/Tris					20	20	20	20
KOH	5.6	5.6	5.6	5.6				
Na₂EDTA					4			
Mannitol	400	400	400	400		150	300	
Sucrose					200	150		
Betaine								300
Ficoll			5	25	5.5			
pH	5.7	5.7	5.7	5.7	8.6	7.2	7.2	7.2

presence of EDTA. Two minutes of such treatment were sufficient to lyse 95% of the protoplasts. After centrifugation at 100g for 7 min at 10°C, the bulk of vacuoles collected at the betaine/mannitol interface. The yield was 5 to 20% of the lysed protoplasts. The volume of the vacuoles was estimated during the assays by measuring the diameters of about 700 vacuoles in a Fuchs-Rosenthal chamber.

H⁺-Transport Assay

For probing the vacuolar pH, acridine orange absorbance changes were continuously monitored at 490 nm and 20°C using a photometer (Gilford Response, Corning Giessen, FRG). Unless otherwise stated, the assay medium comprised 50 mM KCl, 0.5 to 3 mM MgCl₂, 0.1 mM EGTA, 0.5 mM DTT, 300 mM mannitol, 20 mM (Hepes + Tris, adjusted to pH 7.2), and 30 μM acridine orange. To work under comparable conditions the vacuoles were resuspended in this medium to a final density of 5 × 10⁴ vacuoles mL⁻¹ of a homogeneous suspension, and were divided into 5 samples of 1 mL each to be assayed simultaneously. The test substances were added from stock solutions in 2 μL (FCCP) or 10 μL (Na₄PPᵢ, MgATP, KCl, Pi) aliquots.

When assayed this way, the vacuoles reacted to standard tests as described in the literature (20, 28), *e.g.* ATP-driven

H⁺-transport was sensitive to DCCD and NO₃⁻, but was insensitive to vanadate and azide, PPᵢ-driven H⁺-transport was insensitive to NO₃⁻ but partly sensitive to DCCD. These data will not be shown.

The calculation of the vacuolar pH was carried out using the procedure of Gogarten-Boekels *et al.* (9) and were brought into relation with the data derived from the microelectrode tests.

RESULTS

Electrochemical Proton Gradient Across the Tonoplast of *Riccia fluitans*

Direct measurements with pH-sensitive microelectrodes within the vacuole of *Riccia fluitans* rhizoid cells yield a pHᵥ of 4.83 ± 0.39 (n = 21). This means that under control conditions with a pHc of 7.3 (3, 8a) an *in vivo* pH-gradient of 2.1 to 2.9 exists across the tonoplast (Fig. 1A).

The tonoplast electrical potential difference (Eₜₒ) is more difficult to determine, because of the very negative plasma membrane potential of −220 to −250 mV (measured under standard conditions, external pH = 7.2, [K⁺]ₒ = 0.1 to 1 mM). Since both voltages are measured in series, the much smaller
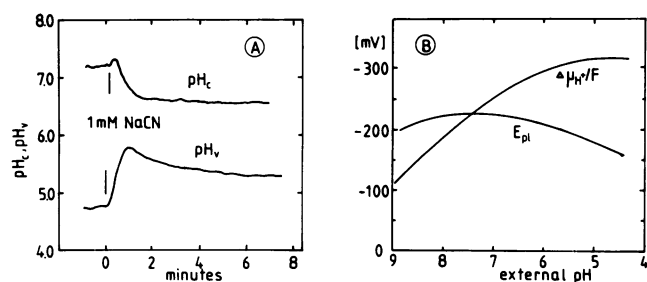

Figure 1. A, pHᵥ and pHc before and after the addition of 1 mM NaCN; B, electrochemical protongradient (ΔμH⁺/F) and membrane potential (Eₚₗ) across the plasma membrane of *R. fluitans*, as a function of external pH.

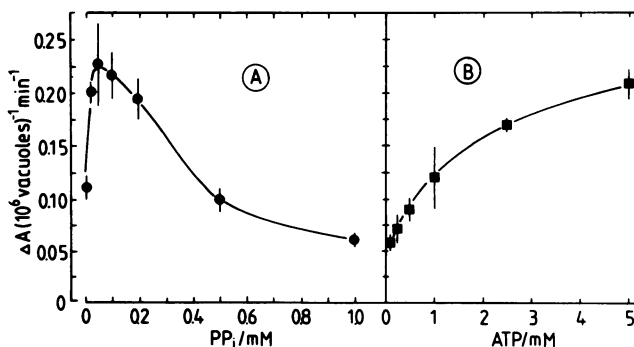**Figure 2.** Initial rates of the acridine orange absorption decrease (A × min⁻¹), measured at 490 nm as a function of (A) externally added PPᵢ in the presence of 3 mM Mg₂Cl₂, and (B) ATP in the presence of equimolar MgCl₂. Mean values (±SE) from 5 to 9 measurements.

Table II. *Vacuolar pH of Intact Vacuoles of R. fluitans Before and After Energization with MgATP and Na4PP$_i$, respectively*

External pH 7.2. pH$_v$ denotes the substrate dependent pH gradient across the tonoplast with respect to the nonenergized, freshly isolated vacuoles.

Substrate	pH$_v$ ± SE	n	ΔpH$_v$
mM			
	5.34 ± 0.12	258	
ATP (1)	4.95 ± 0.12	20	0.39
ATP (2.5)	4.93 ± 0.13	7	0.41
PP$_i$ (0.1)	4.74 ± 0.10	30	0.60

tonoplast portion is almost within the error margin: the tonoplast potential of *R. fluitans* is low and slightly positive, viz. +20 ± 5 mV (n = 16), which is in agreement with reports on *Nitella* (25) and sugar beet (12). This brings the tonoplast electrochemical proton gradient to −139 to −196 mV (cytosolic side) which is considerably less than the transplasma-membrane $\Delta\mu H^+/F$ of −250 to −300 mV (Fig. 1B). Addition of 1 mM NaCN, known to deactivate the ATP-driven pumps by inhibiting the oxidative phosphorylation, rapidly increases vacuolar pH by 0.4 to 0.6 unit, whereas at the same time it decreases the cytosolic pH by about the same amount (Fig. 1A). This reduces the electrochemical proton gradient across the tonoplast by roughly 60 mV.

Generation of the ΔpHv by the tp-ATPase and tp-PPase

In intact vacuoles the proton gradient across the tonoplast, built up and maintained in the presence of ATP or PP$_i$, can be demonstrated most conveniently photometrically, using acridine orange as vacuolar pH probe (2, 9).

In Figure 2 the rates of absorption decrease are shown as a function of PP$_i$ and ATP concentrations. Some differences are conspicuous: (a) both enzymes work in a different concentration range of their substrates; (b) the proton transport activity of the tp-ATPase approximates saturation kinetics ($K_{1/2} \approx 0.5$ mM), whereas proton transport by the tp-PPase yields an optimum around 0.1 mM PP$_i$, a problem which has been dealt with in an earlier paper (15). There we concluded that this behavior depends critically on the total amount of Mg^{2+} and PP$_i$ in the assay medium. In calculating the different complexes, formed of Mg^{2+} together with PP$_i$, we could demonstrate that MgPP$_i^{2-}$ and/or MgHPP$_i^-$ were the substrate(s), whereas Mg$_2$PP$_i$ was an inhibitor. For this reason we do not give a K_m value for PP$_i$ in this paper.

Comparing the effectiveness of both enzymes with respect to their ability to generate a transtonoplast ΔpH, we find that the final vacuolar pH is lower after the addition of PP$_i$ than in the presence of optimal ATP (Fig. 3): starting with an initial vacuolar pH 5.34 ± 0.12 of the freshly prepared vacuoles which is equivalent to a Δ pH of about 1.9 across the tonoplast, in the presence of 2.5 mM ATP a maximal acidification of 0.41 pH units is generated, but 0.6 with 0.1 mM PP$_i$ (Table II). It appears, therefore, that under the given experimental conditions the PPase is more effective in building up a transtonoplast proton gradient. When both substrates are added together, the generated pH gradient is not different from the tests where solely PP$_i$ was added. This is directly

demonstrated with the kinetics of Figure 3, where the single and cumulative effects of both enzymes are shown on the same batch of a homogeneous suspension of vacuoles.

Influence of Chloride

With tonoplast vesicles chloride is often used for charge compensation to prevent the locking of proton translocation by the rapid increase in membrane potential due to primary active charge translocation (H$^+$). But chloride is also known to directly stimulate the ATPase-mediated H$^+$-transport, as shown in Figure 4A. In intact vacuoles of *Riccia* no or only little effect of Cl$^-$ is found on H$^+$-accumulation carried out by the tp-PPase (Fig. 4B) which confirms the conclusion that in the *Riccia* vacuoles the effect of Cl$^-$ on the tp-ATPase is clearly direct and not caused by any electrical interactions. The small stimulation of the PPase activity in the presence of higher Cl$^-$ concentrations (Fig. 4B) may be due to the increase of the background K$^+$.

Dissipation of the Δ pHv

Apart from the natural 'proton-leak' which slowly reduces the transtonoplast proton gradient, addition of pump inhibitors, protonophores, and weak bases lead to an acceleration of the equilibration with the surrounding assay medium. In case the vacuoles contain high amounts of nonpermeant compounds, Donnan phenomena may prevent a total equilibration, as reported for *Beta vulgaris* (19) and for *Chenopodium rubrum* (13). No such phenomena were noticed in the *Riccia* vacuoles, because the addition of FCCP completely and rapidly abolished the transtonoplast pH gradient (data not shown). Whereas the action of FCCP is based on the fact that both the protonated species and the anion are membrane
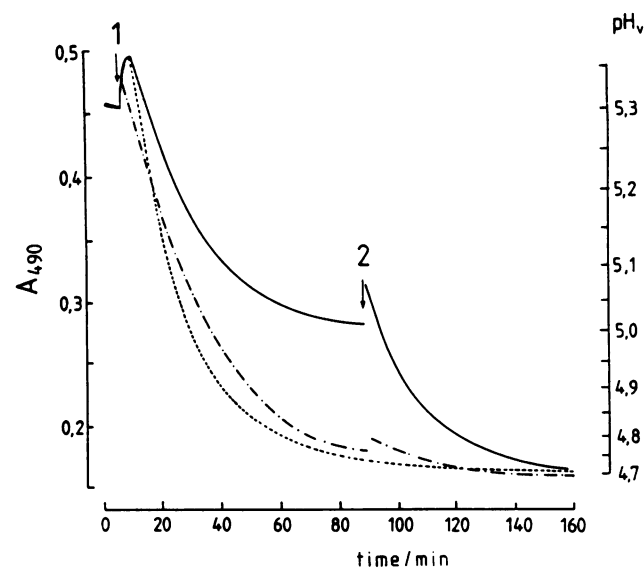

Figure 3. Kinetics of the acridine orange absorption measured at 490 nm after energization with 0.1 mM PP$_i$ and 1 mM ATP, respectively. The substrates were added either successively or together (· · · · · ·). 1, Addition of the first substrate (——ATP; – · –PP$_i$); 2, addition of the second substrate (——PP$_i$; – · –ATP).
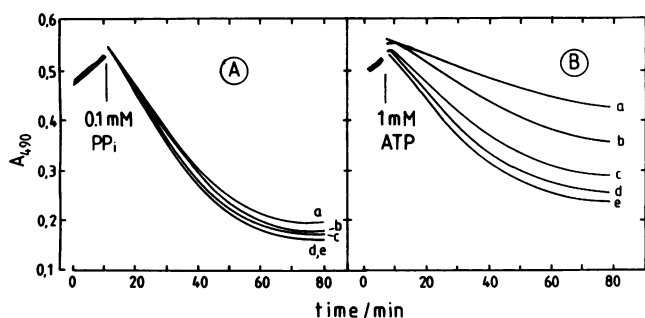
Figure 4. Kinetics of the acridine orange absorption measured at 490 nm after energization with PP$_i$ (A) and ATP (B) at the indicated concentrations and in the presence of different Cl⁻ concentrations. a, Control without KCl; b, 10 mM KCl; c, 25 mM KCl; d, 50 mM KCl; e, 75 mM KCl. The test medium contained 1 to 3 mM MgSO$_4$, the potassium concentration was kept constant by exchange with KIDA.

permeant, the weak base methylamine (pK$_a$ = 10.65) acts solely through the pH-dependent distribution of its unprotonated species across the tonoplast. This is because, unlike for the plasma membrane (6), no uniport for the protonated methylamine exists at the tonoplast of *Riccia*. As shown in Figure 5A, millimolar methylamine concentrations increase the pH$_v$ according to the vacuolar buffer capacity and to the amount of unprotonated methylamine present in the assay solution.

For the reasons given above methylamine cannot be used as *in vivo* agent to quantitatively manipulate vacuolar pH. Therefore the weak base procaine (pK$_a$ = 9.05) has been chosen, using pH-sensitive microelectrodes on intact cells. Two mM procaine (pK$_a$ = 9.05) rapidly increase the vacuolar pH from 4.62 to 5.05 (Fig. 5B).

DISCUSSION

Methodology

We are in the fortunate position of being able to measure the vacuolar pH of *Riccia fluitans* with two independent methods, *viz.* the pH sensitive microelectrode and the acridine orange accumulation. The nonlinear calibration scale of the acridine orange absorption change depends critically on the acridine orange concentration and on the amount of vacuoles in the batch, but also suffers from the lack of fixpoints to start the calibration; therefore, the *in vivo* vacuolar pH serves as an estimate for the maximal *in vitro* acidification measured in the presence of optimal ATP and PP$_i$ concentrations.

Stoichiometry of the PPase

H⁺-translocating PPases at the tonoplast appear to be ubiquitous in plants (21). So far, many studies have been carried out under energetically favorable conditions: (a) on tonoplast vesicles, where no or only little electrochemical proton gradients exist *a priori* and hence favor proton-transport driven by the tp-PPase. (b) Usually, no additional inorganic phosphate is added to the test medium. Under physiological *in vivo* conditions, however, P$_i$ is present in excess, and the protons have to be transported against an electrochemical H⁺-

gradient of roughly −160 mV in *R. fluitans*. Therefore, whether the energy from the hydrolysis of PP$_i$ is sufficient to drive protons from the cytoplasmic side across the tonoplast, may be questioned. In considering this problem, the knowledge of both the PPase stoichiometry and the ratio of pyrophosphate/P$_i$ are most important. In *Pisum sativum* (5) and *Zea mays* (4, 24) PP$_i$ concentrations of 0.05 and 0.39 mM, respectively, were found, while for *Kalanchoë* much higher values were reported (17). Rebeille *et al.* (22) determined cytoplasmic inorganic phosphate applying the ³¹P-NMR technique and found 5 mM. According to the considerations of Rea and Sanders (21), a stoichiometry of 1 is most likely, if one assumes a realistic cytosolic substrate/product-ratio of 1/100. A stoichiometry >1 would inverse the pump and a synthesis of PP$_i$ should result.

In order to get more information on this, one can either measure substrate hydrolysis with H⁺ transport-velocity or pH-gradient formation. Since hydrolysis is not restricted to intact vesicles or vacuoles, but will also take place with a much higher turnover on tonoplast fragments, this approach will lead to an overestimation. At the same time the transport rate will be underestimated, because a high vacuolar buffer capacity (see below) and 'proton leaks' retard the formation of the transtonoplast proton gradient. In our studies we therefore preferred the thermodynamic rather than the kinetic approach.

The free energy change of the pyrophosphate driven proton translocation across the tonoplast can be calculated using the following equations (21)

$$\Delta G = nFE_{to} - RT \ln \left[\frac{K_{PPi}[\text{MgPP}_i{}^*][\text{H}^+]_c^n}{[\text{P}_i]^2[\text{H}^+]_v^n} \right] \quad (1)$$

and

$$n = \frac{\Delta G - \Delta G_{PPi}}{\Delta \mu \text{H}^+} \quad (2)$$

where n is the stoichiometry and R, T, F have their usual meanings. MgPP$_i$* (= MgPP$_i{}^{2-}$ + MgHPP$_i{}^-$) = 0.063 mM (15). According to the given conditions in this study, $\Delta \mu$H⁺ is 15.9 kJ/mol, using E$_{to}$ = +20 mV, pH$_v$ = 4.74 (Table II) and pH$_o$ = 7.2; ΔG_{PPi} = −27.38 kJ/mol, using K_{PPi} = 2.5 × 10⁴ M
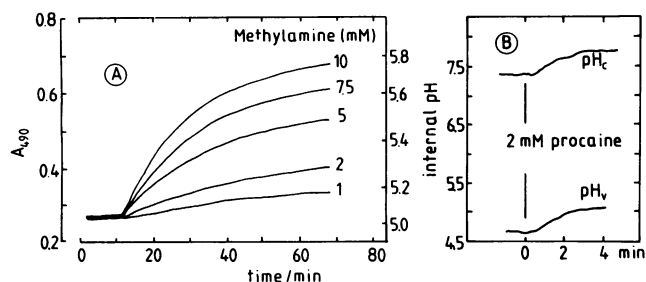

Figure 5. A, Kinetics of the acridine orange absorption measured at 490 nm in the presence of different methylamine concentrations, as indicated. The baseline at 0.27 marks the previously generated vacuolar pH of 5.05, energized by PP$_i$. B, Cytosolic and vacuolar pH before and after the addition of 2 mM procaine, measured with the pH-sensitive microelectrode in *R. rhizoid* cells. "Internal pH" refers to both pH$_c$ and pH$_v$.

(1) and total P_i = 5 mM. For n = 1 a ΔG of -11.42 kJ/mol, for n = 2 a ΔG of $+4.56$ kJ/mol results. Since a negative ΔG signals hydrolysis of pyrophosphate, whereas a positive ΔG means its synthesis (Fig. 6), for the *Riccia* vacuoles only a stoichiometry of 1 is energetically feasible. This also holds if we take a considerable error of E_{to} into account. Furthermore, there are a number of 'proton leaks' (*i.e.* proton cotransport) which move the operating range away from the thermodynamic equilibrium. This is evident from Figure 6, where for three different stoichiometries the free energy of hydrolysis as well as for synthesis of pyrophosphate are plotted (21). The thermodynamic equilibria (at ΔG = 0) are pH 5.85 for n = 3 (pH 5.05) for n = 2, and 2.7 for n = 1. Since with both methods, pH-sensitive microelectrode and acridine orange accumulation, vacuolar pH has been found to be even slightly below the equilibrium pH calculated for n = 2, we conclude the tp-PPase in *Riccia* definitely works as a proton pump in parallel with the proton ATPase.

Some Aspects of Cellular pH Regulation

Vacuolar Buffer Capacity

Both enzymes have to transport protons not only against an energy gradient, but also against the vacuolar buffer capacity. Whereas weak acids are most suitable to manipulate cytosolic pH and to estimate the buffer capacity there (50–100 mM H^+/pH_c for *R. fluitans*), weak bases like methylamine are usually favored for acid compartments such as the plant vacuole. The uncharged and in many cases membrane permeant base readily crosses the tonoplast, is protonated there and thus shifts the vacuolar pH until a new dissociation equilibrium is reached. From the amount of accumulated
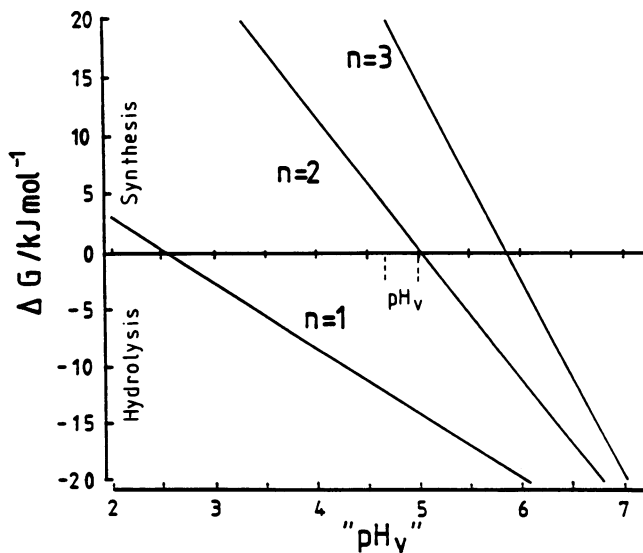

Figure 6. Influence of the transtonoplast ΔpH on the reversibility of the tonoplast-PPase for $H^+/$substrate stoichiometries of 1, 2, and 3. The relationships between ΔG and ΔpH were calculated from Equations 1 and 2 according to Rea and Sanders (21). pH_v is the interval of the actually measured vacuolar pH; 'pH_v' = values as inserted into Equation 1. See text.

protonated base (dBH^+) and the altered pH_v (dH^+) one can estimate the vacuolar buffer capacity β (8, 23).

$$\beta = dBH^+/dH^+ \qquad (3)$$

where

$$dBH^+ = \frac{B_{tot} \cdot V_{tot} \cdot 10^{pK-PH_v}}{V_o(1 + 10^{pK-PH_o}) + V_i(1 + 10^{pK-pH_v})} \qquad (4)$$

taking concentration changes due to vacuolar accumulation into account.

Figures 5 and 6 compare *in vitro-* and *in vivo-*measurements. Methylamine in the lower millimolar range concentration dependently increases vacuolar pH, the values of which are given in Table II together with the calculated buffer capacities. The numbers strongly scatter around 400 mM $H^+/$ pH unit, a value which exceeds the cytosolic buffer capacity by a factor of about five, but is far higher than the β of 1 mM/ pH unit reported for Chara (26) or the 30 to 40 mM/pH unit reported for *Catharantus roseus* (10, 18). On the other hand, it would explain the slow generation of the transtonoplast pH gradient after energization shown in Figures 3 and 4. In Figure 6 the results from the pH-sensitive microelectrode show that external addition of 2 mM procaine (pK_a = 9.02) to intact cells rapidly alters vacuolar pH from 4.62 to 5.05, *i.e.* by 0.43 pH units. According to Equation 1 and taking a measuring error of ± 0.1 pH unit into account, one can calculate a β of 300 to 400 mM/pH which is in the range of the *in vitro* estimations of Table III.

Does such a high buffer capacity make physiological sense? *Chara*, for instance, has a much lower vacuolar buffer capacity (26). A vacuole is a storage compartment and is involved in various regulation processes. Mediating between cytosol and vacuole, the electrochemical *trans*-tonoplast proton gradient provides the transport energy for many compounds and ions. Provided a cell is small, vacuoles with low buffer capacities would suffer a considerable pH_v shift following an already small pH-load and thus alter transport driving forces. A high buffer capacity prevents this.

Joint Action of the Pumps

The stoichiometry of the tonoplast ATPase has been reported to be 2 for many higher plants (21), although with very acidic vacuoles below pH 3 a stoichiometry of 1 has to be assumed, and patch-clamp studies seem to confirm this (11, 12). Calculating the free energy from cytosolic [ATP] = 3

Table III. *Estimation of the Vacuolar Buffer Capacity (β) According to Equation 1 and to the Data Given in Figure 5A.*

Bt = Total added methylamine concentration. B, HB+ free and protonated methylamine.

Bt	pH$_v$	B	HB$_v^+$	ΔpH_v	β
mM		*μM*	*mM*		*mM/pH$_v$*
1.0	5.15	0.28	89	0.15	594
2.0	5.30	0.56	126	0.30	420
5.0	5.50	1.41	199	0.50	398
7.5	5.65	2.11	211	0.65	325
10.0	5.80	2.82	199	0.80	249

mM, [ADP] = 0.5 mM, [P] = 5 mM, E_{to} = 20 mV, and K_{ATP} = 10^5 M, Equation 5 and values from Rea and Sanders (21)

$$\Delta G = nFE_{to} - RT \ln \left[\frac{K_{ATP}[ATP][H^+]^n_c}{[ADP][P_i][H^+]^n_v} \right] \qquad (5)$$

yields ΔG = 14.17 kJ/mol for n = 2, which is close to the available energy of the pyrophosphatase at n = 1 (-11.42 kJ/mol), showing that the basic capacities of the two pumps are similar. The *in vitro* finding that the pyrophosphatase generates roughly 0.2 pH units more than the ATPase (Fig. 3; Table II), could be caused by a different density of the pumps per membrane area. Since the 'proton leak' is equally effective on both pumps, this means that in the intact cell both pumps work against the same proton gradient.

As evident from the ΔG values of -14.17 kJ/mol for the ATPase (n = 2) and -11.42 kJ/mol for the PPase (n = 1), both enzymes operate far from their equilibrium (PPase: ΔG = 0 at pH_v = 2.7; ATPase: ΔG = 0 at pH_v = 3.5). This is important, because a pump working close to its equilibrium cannot be regulated reasonably well itself and hence is not able to contribute much to pH-regulation per se; thermodynamic forces would largely prevent this.

The data from Figure 1, where the action of NaCN is shown to decrease the transtonoplast $\Delta\mu H^+/F$ by about 60 mV, are interesting, because the tp-PPase alone is seemingly not able to restore either the cytosolic, or the vacuolar pH. At first glance this appears surprising, because on the short-term the PPase should not be affected by NaCN. However, taking the pH optimum (≈ 7.2) of the PPase into account (13), one must realize that the decrease of cytosolic pH to 6.6 causes a considerable drop in PPase activity by about 40%. Since the pH optimum is just around the cytosolic pH, the conclusion must be that the tp-PPase cannot contribute considerably to cellular pH regulation, regardless to which side the cytosolic pH is shifted.

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