Inhibition of the yeast V-type ATPase by cytosolic ADP

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Abstract **The activity of the vacuolar H^+-ATPase has been characterized in isolated vacuoles of the yeast *Saccharomyces cerevisiae* by means of the patch-clamp technique. With cytosolic calcium at virtually zero ($<10^{-9}$ M), Mg-ATP induced a transient, bafilomycin A_1-sensitive current corresponding to the flow of positive charges from the cytoplasmic surface to the vacuolar lumen. The Mg-ATP-dependent current reached its maximum amplitude (30 ± 8 mA m^{-2} with 5 mM Mg-ATP, $n=34$) within 15–20 s and declined slowly over a period of about 15–20 min even in the continuous presence of Mg-ATP. This decline of pumping activity was independent of the cytosolic KCl concentration, suggesting an inhibitory mechanism different from the high salt-induced dissociation of V_0 and V_1 reported for the V-ATPase of plants and fungi. Cytosolic ADP was found to modulate the pump activity since Mg-ATP-induced pump current was smaller if monitored in the presence of 5 mM ADP and addition of 5 mM ADP in the presence of 5 mM Mg-ATP reduced the pump current by more than 50%. Furthermore, reduction of the cytosolic ADP concentration by the ATP-regenerating system creatine phosphate/creatine kinase partially relieved the endogenous inhibition of the V-ATPase, confirming that interaction of cytosolic ADP with the V-ATPase is the reason for the transient nature of the pump current in yeast vacuoles.**

Key words: V-ATPase; Vacuole; Patch-clamp; Creatine kinase; Bafilomycin; H^+ pump

1. Introduction

Vacuolar type H^+-ATPases (V-ATPase) are best known as H^+-translocating enzymes located in the vacuolar membrane of plants and fungi. However, V-ATPases have been identified in all eukaryotic cells and have been shown to play key roles in diverse physiological processes such as receptor-mediated endocytosis, protein degradation and processing, protein sorting and targeting and other processes that require intraorganellar acidification and energization of organelle membranes. In plants and fungi, including yeast, vacuolar acidification mediated by V-ATPases is essential for driving proton-coupled cotransport of ions and metabolites across the vacuolar membrane and therefore for the accumulation of Ca^{2+}, amino acids, carbohydrates, and phosphates in the vacuole [1,2].

V-ATPases from fungi, plants, and animals share a high degree of structural similarity. Like F-type ATPases in mitochondria, chloroplasts and bacteria and A-type ATPases from archaea, V-type ATPases are heteromeric protein complexes consisting of more than 10 different subunits assembled into two functional domains. A membrane-associated domain (V_0) defines the proton channel and a soluble catalytic domain (V_1) is involved in ATP hydrolysis. Considering the importance of the cellular functions that depend on V-ATPase-mediated intraorganellar acidification, this ion translocation system would be expected to be essential for survival of the cell. This seems to be true for all eukaryotic cells including *Neurospora* [3,4], but not for yeast, since yeast cells devoid of any detectable V-ATPase activity are viable, at least under certain circumstances. These yeast mutants seem to be capable of acidifying the vacuolar lumen via endocytotic pathways under acidic external conditions [5,6]. For this property, yeast has become an important experimental tool in the V-ATPase research which has revealed important insights into subunit composition, targeting and assembly, as well as into the function and regulation of the V-ATPase (for review see [7]).

Because of the already mentioned importance of this enzyme for various cellular processes, its activity should be tightly regulated. One level of regulation is the dissociation and reassembly of the V_1 and V_0 subunits, as repeatedly reported from plant cells [8–11]. Dissociation and reassembly depend on various stress factors, including cytosolic salt concentrations, temperature, and energy status of the cell [2,8,9,12]. In *Saccharomyces cerevisiae* dissociation of V_1 and V_0 can be induced by shifting from glucose to galactose or raffinose as carbon source. Reassembly is then readily achieved by switching back to glucose feeding [13].

Another way of regulating V-ATPase activity has been reported from higher plants, where mRNA and protein amount of V-ATPase subunits change depending on metabolic conditions and in response to salt stress [14]. ATPase activity might also be modulated by interaction with regulatory proteins, as shown for the V-ATPase in the kidney [15], or for the plasma membrane H^+-ATPase, Pma1, in yeast [16].

Besides its activity of hydrolyzing ATP, the V-ATPase exhibits H^+ transport activity, which can be directly measured using electrophysiological recording techniques, provided the ATPase or the membrane harboring this enzyme is accessible to these techniques. Electrophysiological studies on V-type ATPases were until very recently restricted to plants. Mg-

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ATP-dependent electrical current mediated by a V-type ATPase has been demonstrated in isolated vacuoles from *Chenopodium rubrum* [11,17] and sugar beet (*Beta vulgaris*, [18]). This *trans*-tonoplast current was shown to result in a vacuolar acidification, just as expected from the operation of a Mg-ATP driven H^+-ATPase in the vacuolar membrane. More detailed studies have been carried out by Davies and colleagues, who examined the operational transport coupling ratio of the V-ATPase from red beet vacuoles and the pH-dependency thereof [19–21]. The feasibility of applying electrophysiological techniques to study the activity of the vacuolar H^+-ATPase in isolated yeast vacuoles has been demonstrated in vacuoles from a tetraploid strain [22] and in vacuoles isolated from giant haploid yeast cells [23].

Here, we used the patch-clamp techniques to directly study the transport activity of the yeast vacuolar H^+-ATPase and to elucidate the mechanism that leads to the spontaneous inactivation of this enzyme.

2. Materials and methods

General methods for growing, handling, and protoplasting yeast and for isolating vacuoles have been previously described (for details see [22]).

A tetraploid strain of *S. cerevisiae* (Y588; [24]) was used throughout the experiments because of its larger cells as compared to haploid cells. It should be noted that patch-clamp experiments are usually not limited by the size of the cells. Nevertheless, large cells remain more convenient and easier to work with than small cells (see also [22,25]).

Current recordings from isolated yeast vacuoles were performed in the whole-vacuole mode as described [22]. For continuous data recording we used the HEKA software package Pulse/pulsefit 8.0 for Macintosh, supplemented with the chart recorder extension X-Chart, in combination with the EPC-9/ITC-16 amplifier/data acquisition system (HEKA, Lamprecht, Germany). Data were filtered at 100 Hz, using a built-in eight-pole Bessel filter, sampled at 1 kHz (for current–voltage (*I–V*) analysis) or 30 Hz (for continuous current recordings), and stored on the computer hard drive.

The sign convention proposed by Bertl et al. [26] for membrane voltage and current was used throughout.

Standard composition of the pipette solution, representing the vacuolar content in the whole-vacuole recording configuration, contained 150 mM KCl, 5 mM $MgCl_2$, 0.5 mM EGTA, buffered to pH 7.5 with Tris/MES. Experiments were started under symmetrical conditions, i.e. pipette solution equaled bath (cytosolic) solution, with continuous perfusion of the recording chamber.

ATP and ADP, respectively, were added to the bath solution as its potassium salt (K_2-ATP) and KCl concentration were adjusted to compensate for the additional potassium. In the tests designed to determine the effect of an ATP-regenerating system the disodium salt of creatine phosphate (5 mM Na_2-CP) was used. Consequently, the pipette solution was supplemented by 10 mM NaCl in these experiments and the same concentration of NaCl was added to the bath solution during the CP-free period of current recording to ensure symmetrical ionic conditions.

3. Results and discussion

3.1. Mg-ATP induces bafilomycin-sensitive currents in yeast vacuoles

The patch-clamp technique has been employed in the whole-vacuole configuration to study the function of the V-type ATPase in isolated yeast vacuoles. Experiments were started under symmetrical conditions and the vacuolar lumen was allowed to equilibrate with the pipette solution for several minutes after breakthrough. Perfusion of the recording chamber (cytosolic face of the vacuolar membrane) with 5 mM Mg-ATP induced an electrical current of about 14 pA in a vacuole

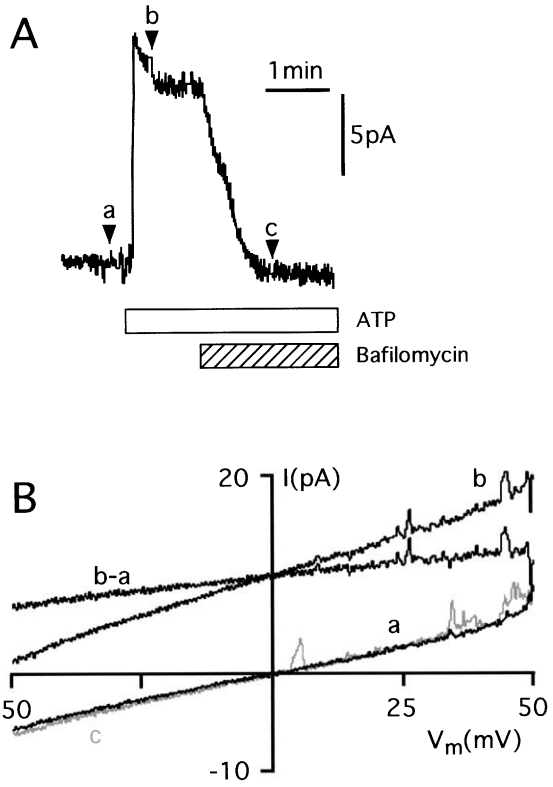

Fig. 1. ATP-induced, bafilomycin-sensitive current recorded from an isolated yeast vacuole with the membrane clamped to 0 mV. A: Mg-ATP (5 mM) induced an outward current of about 14 pA. Note that the current starts to decline slowly, even in the continuous presence of ATP. Addition of 100 nM bafilomycin A_1 caused a fast decline of the current to the zero current level. At the times indicated by the arrows, *I–V* curves were taken using voltage ramps. B: *I–V* characteristics, in the absence of Mg-ATP (**a**) and in the presence of Mg-ATP (**b**), taken at the times indicated in A. The difference of the two curves represents the *I–V* characteristics of the pump (**b**−**a**). Curve **a** is almost identical to curve **c** (gray curve), which represents the membrane characteristics in the presence of both Mg-ATP plus bafilomycin A_1.

of 12 μm in diameter (Fig. 1A). The direction of this current corresponds to the flow of positive charges from the cytosolic side to the vacuolar lumen. Current amplitudes elicited by 5 mM Mg-ATP were in the range of 7–30 pA for vacuoles of 8–17 μm in diameter. Cytosolic GTP was also able to induce currents of the same direction, an observation also reported for plant vacuoles [27]. In isolated yeast vacuoles, currents induced by 5 mM cytosolic Mg-GTP reached on average 46% the current amplitude induced by 5 mM Mg-ATP in that same vacuole (data not shown, $n = 6$).

Considering the different sizes of the vacuoles and relating the current amplitudes to the membrane area, the mean current densities in isolated yeast vacuoles induced by 5 mM Mg-ATP were 30 ± 8 mA m^{-2} ($n = 34$), which is in the same range as the current densities reported from plant vacuoles. In 5 mM Mg-ATP, the current densities observed in patch-clamp experiments in plant vacuoles were 23 mA m^{-2} [28] and 5 mA m^{-2} [29] in sugar beet, and 10 mA m^{-2} in red beet [19]. From giant yeast vacuoles Mg-ATP induced currents as high as 100 pA have been reported [23], corresponding to a current density of 100 mA m^{-2}. However, in that study all figures, but fig. 5, show much smaller currents in the range of 15–20 pA (with mM concentrations of Mg-ATP). With a size of 20–30

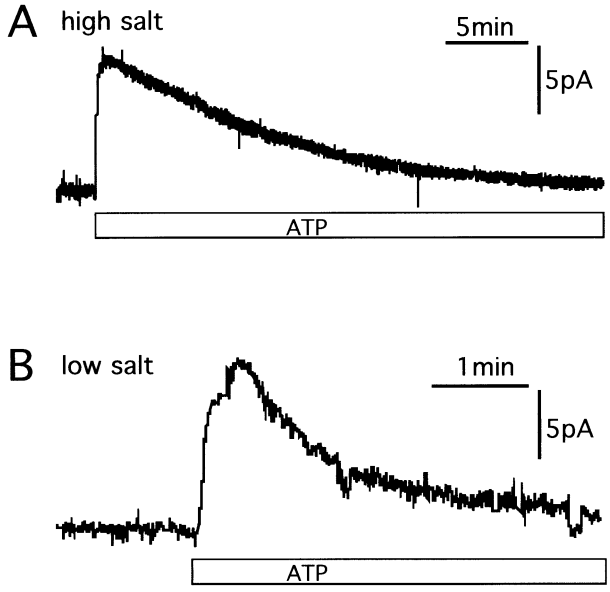

Fig. 2. A: Whole-vacuole current recorded at 0 mV membrane voltage. Under standard conditions (symmetric, 150 mM KCl, 5 mM $MgCl_2$, 0.5 mM EGTA, pH 7.5 with Tris/MES) an outward current of about 10 pA was induced by perfusion of the recording chamber (cytosolic side of the membrane) with 10 mM Mg-ATP. The current reached its maximum within less than 20 s and declined slowly, returning nearly to the zero current level within 15–20 min, even in the continuous presence of ATP (bar at the bottom of the current trace). B: Time course of the pump current recorded in symmetric low KCl (15 mM KCl in pipette and bath solution compared to 150 mM in standard solutions). Osmolality of the solutions was adjusted with sorbitol (270 mM). Note that even in low salt concentration, the ATP-induced current (ATPase activity) declined in the continuous presence of cytosolic ATP, suggesting that the transient nature of the pump current, observed in Fig. 2A, is not due to a high salt-induced dissociation of the V_1 and V_0 subunits.

μm as reported for these vacuoles, the current densities for most of the experiments shown (except Fig. 5) would result in current densities of maximum 16 mA m^{-2} (for 20 pA and 20 μm), which is well within the range of current densities reported here and reported from plant vacuoles.

In order to identify the Mg-ATP-induced current with the activity of the V-ATPase and to distinguish the pump current from possible other ATP-induced ionic currents, we tested this current for bafilomycin A_1 sensitivity. Bafilomycin A_1 and other bafilomycin-derived agents are known to be highly specific and potent inhibitors of V-type ATPases, whilst F-type and P-type ATPases are only slightly inhibited or completely insensitive [30,31]. Therefore, bafilomycin A_1 is well suited to distinguish between V-ATPase activity and the activity of other ATP-dependent transport systems. The Mg-ATP-induced current was sensitive to the specific V-type ATPase inhibitor bafilomycin A_1. Addition of 100 nM bafilomycin A_1 to the cytosolic solution resulted in a fast and complete inhibition of the ATP-induced current (Fig. 1A), which is consistent with the observation of Yabe et al. [23], who showed 50% inhibition with 10 nM and >90% inhibition with 40 nM bafilomycin A_1. The complete inhibition of the ATP-induced current in isolated yeast vacuoles by bafilomycin A_1 indicates that the observed current is mediated by a V-type H^+-ATPase and that under our experimental conditions Mg-ATP does not induce any other ionic current at the vacuolar membrane.

From the current density of 30 mA m^{-2}, corresponding to 3×10^{-2} C s^{-1} m^{-2}, and assuming a transport rate of about 100 H^+ s^{-1}, as reported from plants [11,32,33], the density of V-ATPases in the yeast tonoplast can be estimated to be about 1900 μm^{-2}. From electronmicrographs visualizing the ball-and-stalk structure of the peripheral V_1 domain of the *Neurospora crassa* V-ATPase [34], a density of about 1500 μm^{-2} can be determined and the V-ATPase densities in plant vacuolar membranes have been reported to be in the same range (1100–3000 μm^{-2}; [35]).

It should be mentioned that plant vacuolar membranes are equipped with two types of proton pumping enzymes [36,37], a V-ATPase and a pyrophosphatase (PPase). No homolog to the plant PPase can be found in the yeast genome and consistent with this, cytosolic pyrophosphate does not induce any electrical current in isolated yeast vacuoles (data not shown).

3.2. Current–voltage (*I–V*) characteristics of the V-ATPase

In order to gain more detailed information of the properties of the V-ATPase, a *I–V* analysis of the vacuolar membrane has been performed under symmetrical ionic conditions.

For *I–V* analyses (Fig. 1B), currents were recorded in response to voltage ramps, clamping the vacuolar membrane from −50 to +50 mV within 2.5 s and the resulting currents were plotted against the applied voltage. Voltage ramps were applied before addition of ATP, in the presence of ATP when the current reached its maximum, and in the presence of ATP plus bafilomycin A_1.

In the absence of ATP, the *I–V* curve of the vacuolar membrane is nearly linear over the voltage range of ±50 mV with a conductance of 100 pS, representing an unspecific leak conductance caused by the seal between membrane and patch pipette. The *I–V* curve intersects with the voltage axis at 0 mV (trace a, obtained at the arrow marked **a** in Fig. 1A), just as expected for a non-energized membrane under symmetrical ionic conditions. Addition of 5 mM Mg-ATP resulted in a parallel shift of the *I–V* curve towards negative voltages (trace b, obtained at the arrow marked **b** in Fig. 1A),

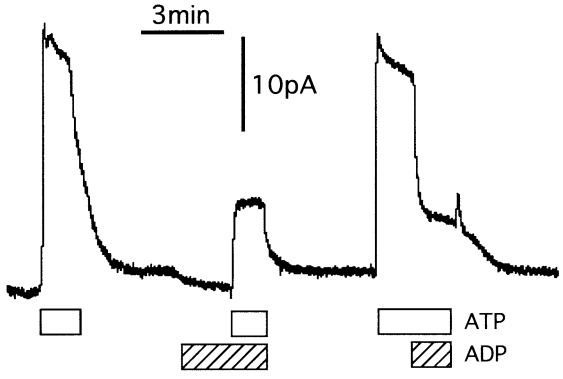

Fig. 3. Modulation of ATP-induced current by cytosolic ADP. Current recording from a vacuole which has not been treated either with ATP or with ADP before. In the presence of 5 mM ADP, the ATP-induced outward current is just about 40% of the current seen initially with 5 mM ATP. The small downward deflection observed upon ADP addition is due to complete removal of residual ATP. After a washing period of about 4 min to remove both ATP and ADP, the subsequent ATP application induced a current which reached almost the initial magnitude. Addition of 5 mM ADP on top of ATP reduced the current to roughly the level observed before with ATP in the presence of ADP.

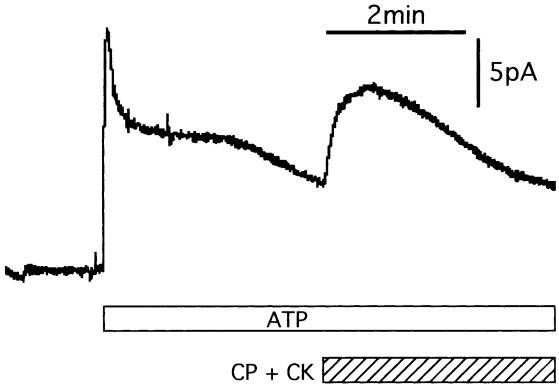

Fig. 4. Spontaneous inactivation of the vacuolar ATPase is partly relieved by the addition of CP/creatine-phosphokinase. Addition of 5 mM Mg-ATP to the cytosolic solution induced an outward current, which spontaneously declined as already observed in Fig. 3. After the current has declined to about 70% of the maximum, 5 mM CP and 1 mg ml^{-1} CK were added to the bath solution with ATP still present. The current recovered to almost the initial maximum before it declined again. Upon CP/CK addition, the fast components in both current activation and current decline are no longer observed, which probably reflects the velocity of the rate limiting CK-mediated phosphorylation reaction.

displaying a short circuit current (current at 0 mV) of about 12 pA. The difference of the curves representing the energized membrane (**b**) and the non-energized membrane (**a**), respectively, describes the *I–V* characteristics of the V-ATPase (**b**−**a**), which is nearly linear, almost voltage-independent and does not intersect the voltage axis within the voltage range of ±50 mV (Fig. 1B).

The observation here, that the *I–V* curve of the V-ATPase is rather voltage-independent within the voltage range tested, i.e. ±50 mV, indicates that this voltage range is rather far from equilibrium. This however is not surprising given the experimental conditions used. With only Mg-ATP present at the cytosolic side and no ADP and P_i supplied, the reversal voltage, at which the current reverses the sign or the ATPase reaction cycle reverses direction, should become rather negative (tend towards $-\infty$), because [ADP]·[P_i]/[ATP] becomes very small (tends towards 0). Using similar conditions with only Mg-ATP present and no ADP and P_i supplied, Yabe et al. [23] found that the *I–V* curve of the ATP-dependent pump does not become negative even at sufficiently low potentials (−70 mV) and they concluded from this observation that the H^+ pump does not work in the negative direction [23]. Nevertheless, a H^+/ATP ratio of 3.5 was estimated from these experiments. Experimental determination of the reversal voltage, which is the intersection of the pump *I–V* curve with the voltage axis, is crucial for calculating the ratio of transported H^+ per ATP hydrolyzed. This, however, is only possible with all three components Mg-ATP, ADP, and P_i present at the cytosolic side, because only then, the reaction cycle of the V-ATPase can reverse direction and the *I–V* curve will intersect with the voltage axis, as shown previously in yeast vacuoles [22] and in plants [19,20,29].

The *I–V* characteristics of the vacuolar membrane in the presence of both Mg-ATP and the specific V-ATPase inhibitor bafilomycin A_1 (Fig. 1B, trace **c**, gray curve) are superimposable to the *I–V* curve of the non-energized vacuolar membrane, before addition of Mg-ATP. This indicates that under our experimental conditions there is no ATP-inducible current in isolated yeast vacuoles other than that mediated by the V-type ATPase. Moreover, Mg-ATP does also not induce any bafilomycin-insensitive conductance, which is evident from the virtually identical *I–V* curves of the non-energized vacuolar membrane and the membrane in the presence of both Mg-ATP and bafilomycin A_1.

3.3. Spontaneous inactivation of V-ATPase activity

V-ATPase activity, monitored as Mg-ATP-induced electrical current from isolated yeast vacuoles, displays spontaneous inactivation. The first signs of such an inactivation can be seen in the current trace of Fig. 1A, but the full extent becomes evident in longer lasting recordings such as Fig. 2A. Upon addition of 10 mM Mg-ATP, the pump current reached a maximum of about 10 pA (∅ = 11 μm) within 10–15 s and declined slowly over a period of 15–20 min even in the sustained presence of a constant Mg-ATP concentration. Similar spontaneous inactivation of V-ATPase activity was also observed in a patch-clamp study from plant vacuoles [11], but the mechanism underlying this inactivation has not been investigated or discussed. It was recently reported that in plants and fungi bafilomycin-sensitive ATPase transport activity is lost in high (≥150 mM) salt solution when ATP is simultaneously present [1,8–10,13]. This inactivation has been shown to result from the dissociation of the V_1 and V_0 domains, which could potentially also account for the transient nature of the observed pump currents in yeast vacuoles.

In order to test this hypothesis, we compared the magnitude and time courses of the ATP-induced currents in symmetric high (150 mM) and low (15 mM) KCl. An example of a current recording in low KCl is shown in Fig. 2B. Like in high KCl, the current elicited by 5 mM Mg-ATP in low KCl (15 mM) is transient with a peak current similar to that in high KCl concentration (Fig. 2A).

Overall, current densities in the peak produced by 5 mM Mg-ATP were 29 ± 3 mA m^{-2} ($n = 3$) in 15 mM KCl and 30 ± 8 mA m^{-2} ($n = 34$) in 150 mM KCl, respectively. The reduction of the cytosolic KCl concentration from 150 to 15 mM did neither affect the peak current induced by 5 mM Mg-ATP, nor prevent the current from declining in the continuous presence of Mg-ATP. In fact, the current decay in low salt appears to be even faster than in high salt. However, for the lack of sufficient repeats ($n = 3$), we would not decide on the statistical significance of this difference in time course. Hence, for the observed transient nature of the pump current in isolated yeast vacuoles an inhibitory action has to be postulated which is different from the high salt-induced V_1/V_0 dissociation reported from plants.

3.4. Inhibition of the V-ATPase by cytosolic ADP

A first clue on the nature of the current inhibition might be obtained from the data in Fig. 3. Mg-ATP (5 mM) added to the bath solution induced an outward current of about 28 pA (∅ = 16 μm), which showed the previously observed spontaneous decline in the presence of ATP. Upon removal of ATP, the outward current showed a fast decay, similar to that observed by inhibition of the V-ATPase with bafilomycin A_1 (compare with Fig. 1).

No measurable outward current was observed upon perfusion of the recording chamber with 5 mM Mg-ADP, but addition of 5 mM ATP on top of Mg-ADP elicited a clear outward current. However, the magnitude of this ATP-in-

duced current was only about 40% of that initially induced by Mg-ATP in the absence of ADP. Nearly the full response was elicited by 5 mM Mg-ATP after ADP and ATP had been washed out for about 4 min. Addition of 5 mM ADP on top of the Mg-ATP resulted here in a fast reduction of the current to a level comparable to that observed with ATP in the presence of 5 mM Mg-ADP. This suggests that ADP interacts with the V-ATPase and inhibits H^+ transport. Modulation of V-ATPase activity through binding of nucleoside diphosphates has been reported from the chromaffin granule enzyme [38] and V-ATPase-mediated proton transport in chicken kidney and osteoclasts is inhibited by submillimolar concentrations of ADP [39]. Since these findings have been reported from experiments performed in different systems with different experimental approaches, the inhibition of the V-ATPase by cytosolic ADP does not seem to be an experimental artifact, but rather an important physiological process for saving energy at times of energy shortage, when the concentration of ADP is expected to be high.

3.5. *Partial relief of the inhibition by an ATP-regenerating system*

If ADP binding to the V-ATPase was indeed the reason for the transient nature of the pump current and the reduced current amplitude in the presence of ADP, this inhibition should be prevented and/or reversed by reduction of the ADP concentration via the ATP regeneration system creatine phosphate/creatine kinase (CP/CK). Fig. 4 shows an experiment where the ATPase activity has been elicited by addition of 5 mM Mg-ATP to the bath solution, resulting in the typical transient pump current. The current reached a maximum of 15 pA ($\varnothing = 12$ μm) within about 15 s and declined in the continuous presence of Mg-ATP.

This pump inhibition could be partially relieved by the addition of 5 mM CP plus 1 mg ml^{-1} CK, confirming that ADP binding is involved in the inhibition of the V-ATPase, which leads to the transient nature of proton pumping activity even in the continuous presence of Mg-ATP. However, the reactivation or release of pump inhibition by CP/CK treatment was not complete and sustained, indicating that the V-ATPase inactivation consists of a CP/CK-sensitive and an CP/CK-insensitive component. In giant yeast vacuoles an overshoot of the pump current was observed upon addition of 1 mM Mg-ATP, which was absent if studied in the presence of CP/CK [23]. In that study, pump current was nearly constant over the recording periods of about 5 min in the presence of CP/CK, however, the current amplitudes were just about 15% of those observed with Mg-ATP only. Since these experiments have been performed on different vacuoles of (probably) different size, it is hard to judge whether the observed difference in pump current is due to the different experimental conditions or simply the result of a different size of the vacuoles. Our data in Figs. 3 and 4 showing temporary relief of the spontaneous V-ATPase inhibition by CP/CK suggest that ADP exhibits a dual inhibitory effect on the yeast V-ATPase. This could be explained by the existence of two different types of binding site for ADP, which was found in the chromaffin granule enzyme by analyzing binding of radiolabeled Mg-ADP to the enzyme [38]. The two components (fast and slow) of current decline observed in yeast vacuoles might reflect the involvement of two ADP-binding sites, one displaying high affinity and one displaying low affinity for ADP binding. Phosphate was also found to inhibit V-ATPases in membrane preparations from chicken osteoclast and kidney with a K_i of 10.5 and 5.5 mM, respectively [39], and more than 10 mM phosphate was required to decrease the ATPase activity in tonoplast vesicles from plant cells significantly [40]. Since no extra phosphate was supplied in our experiments and phosphate is not expected to accumulate to these high concentrations during continuous perfusion of the recording chamber, phosphate is not very likely to account for the CP/CK-insensitive component of spontaneous ATPase inhibition.

In conclusion, the activity of yeast vacuolar H^+-ATPase is inhibited by cytosolic ADP, probably involving two different binding sites. In vivo, the cytosolic ATP/ADP ratio might be an important parameter for controlling V-ATPase activity.

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References

[1] Nelson, N. (1992) J. Exp. Biol. 172, 19–27.
[2] Taiz, L. (1992) J. Exp. Biol. 172, 113–122.
[3] Dow, J.A., Davies, S.A., Guo, Y., Graham, S., Finbow, M.E. and Kaiser, K. (1997) J. Exp. Biol. 200, 237–245.
[4] Bowman, E.J., Kendle, R. and Bowman, B.J. (2000) J. Biol. Chem. 275, 167–176.
[5] Nelson, H. and Nelson, N. (1990) Proc. Natl. Acad. Sci. USA 87, 3503–3507.
[6] Munn, A.L. and Riezman, H. (1994) J. Cell Biol. 127, 373–386.
[7] Stevens, T.H. and Forgac, M. (1997) Annu. Rev. Cell Dev. Biol. 13, 779–808.
[8] Rea, P.A., Griffith, C.J., Manolson, F. and Sanders, D. (1987) Biochim. Biophys. Acta 904, 1–12.
[9] Ward, J.M., Reinders, A., Hsu, H.T. and Sze, H. (1992) Plant Physiol. 99, 161–169.
[10] Ward, J.M. and Sze, H. (1992) Plant Physiol. 99, 170–179.
[11] Weiser, T. and Bentrup, F.W. (1994) Physiol. Plant 91, 17–22.
[12] Kane, P.M. (1999) J. Bioenerg. Biomembr. 31, 49–56.
[13] Kane, P.M. (2000) FEBS Lett. 469, 137–141.
[14] Ratajczak, R. (2000) Biochim. Biophys. Acta 1465, 17–36.
[15] Zhang, K., Wang, Z.Q. and Gluck, S. (1992) J. Biol. Chem. 267, 9701–9705.
[16] Navarre, C., Catty, P., Leterme, S. and Dietrich, F. (1994) J. Biol. Chem. 269, 21262–21268.
[17] Bentrup, F.W., Gogarten-Boekels, M., Hoffmann, B., Gogarten, J.P. and Baumann, C. (1986) Proc. Natl. Acad. Sci. USA 83, 2431–2433.
[18] Hedrich, R., Flügge, U.I. and Fernandez, J.M. (1986) FEBS Lett. 204, 228–232.
[19] Davies, J.M., Hunt, I. and Sanders, D. (1994) Proc. Natl. Acad. Sci. USA 91, 8547–8551.
[20] Davies, J.M., Sanders, D. and Gradmann, D. (1996) J. Membr. Biol. 150, 231–241.
[21] Davies, J.M. (1997) J. Exp. Bot. 48, 633–641.
[22] Bertl, A., Bihler, H., Kettner, C. and Slayman, C.L. (1998) Pflüg. Arch. 436, 999–1013.
[23] Yabe, I., Horiuchi, K., Nakahara, K., Hiyama, T., Yamanaka, T., Wang, P.C., Toda, K., Hirata, A., Ohsumi, Y., Hirata, R., Anraku, Y. and Kusaka, I. (1999) J. Biol. Chem. 274, 34903–34910.
[24] Mirzayan, C. and Copeland, C.S. (1992) J. Cell Biol. 116, 1319–1332.
[25] Bertl, A. and Slayman, C.L. (1992) J. Exp. Biol. 172, 271–287.
[26] Bertl, A., Blumwald, E., Coronado, R., Eisenberg, R., Findlay, G., Gradmann, D., Hille, B., Köhler, K., Kolb, H.-A., MacRobbie, E., Meissner, G., Miller, C., Neher, E., Palade, P., Pantoja, O., Sanders, D., Schroeder, J., Slayman, C.L., Spanswick, R. and Williams, A. (1992) Science 258, 873–874.
[27] Hedrich, R., Kurkdjian, A., Guern, J. and Flügge, U.I. (1989) EMBO J. 8, 2835–2841.

[28] Coyaud, L., Kurkdjian, A., Kado, R. and Hedrich, R. (1987) Biochim. Biophys. Acta 902, 263–268.
[29] Gambale, F., Kolb, H.A., Cantu, A.M. and Hedrich, R. (1994) Eur. Biophys. J. 22, 399–403.
[30] Bowman, E.J., Siebers, A. and Altendorf, K.H. (1988) Proc. Natl. Acad. Sci. USA 85, 7972–7975.
[31] Dröse, S., Bindseil, K.U., Bowman, E.J., Siebers, A., Zeeck, A. and Altendorf, K.H. (1993) Biochemistry 32, 3902–3906.
[32] Ward, J.M. and Sze, H. (1992) Plant Physiol. 99, 170–179.
[33] Matsuura-Endo, C., Maeshima, M. and Yoshida, S. (1990) Eur. J. Biochem. 187, 745–752.
[34] Bowman, B.J., Dschida, W.J. and Bowman, E.J. (1992) J. Exp. Biol. 172, 57–66.
[35] Lüttge, U. and Ratajczak, R. (1997) Adv. Bot. Res. 25, 253–296.
[36] Rea, P. and Sanders, D. (1987) Physiol. Plant 71, 131–141.
[37] Obermeyer, G., Sommer, A. and Bentrup, F.-W. (1996) Biochim. Biophys. Acta 1284, 203–212.
[38] Webster, L.C. and Abbs, D.K. (1996) Eur. J. Biochem. 240, 156–164.
[39] David, P. and Baron, R. (1994) J. Biol. Chem. 269, 30158–30163.
[40] Dietz, K.J., Heber, U. and Mimura, T. (1998) Biochim. Biophys. Acta 1373, 87–92.