

H^+ /ion antiport as the principal mechanism of transport systems in the vacuolar membrane of the yeast *Saccharomyces carlsbergensis*

L.A. Okorokov, T.V. Kulakovskaya, L.P. Lichko and E.V. Polorotova

Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences, Pushchino, Moscow Region 142292, USSR

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The secondary transport systems of the yeast vacuolar membrane have been investigated by (i) the method of radioactive isotopes ($[^{14}C]$ arginine); (ii) activation of H^+ -ATPase by cations (Cat^+), when the enzyme is under H^+ control and (iii) measurement of changes in the proton gradient (ΔpH) and membrane potential (E_m) due to the supposed substrates of the transporters. The main mechanism of cation transport across the yeast tonoplast is probably H^+/Cat^+ antiport. The apparent K_m of antiporters for Ca^{2+} , Mg^{2+} , Mn^{2+} , Zn^{2+} and P_i are 0.06, 0.3, 0.8, 0.055–0.17 and 1.5 mM, respectively.

H^+ -ATPase Cation H^+ Antiporter Vacuolar membrane (Yeast)

1. INTRODUCTION

Several groups have reported on the localization of basic amino acids, vitamins, derivatives of purine bases [1] and inorganic ions [2] in fungal vacuoles. The existence of the transport systems of basic amino acids (arginine, lysine, etc.) and purine bases in the tonoplast has also been proved [1,3–5].

The presence of a specific ATPase in the vacuolar membrane, other than the ATPases of mitochondria and plasmalemma, was demonstrated by its solubilization, partial purification [6,7] and reconstitution into liposomes [8]. It was also found that yeast vacuolar membranes possessed the ATP-dependent H^+ pump [9] identified as H^+ -ATPase [10] which supported the $\Delta\mu H^+$ -dependent transport of arginine [11], Ca^{2+} [12,13] and other ions [10,13].

We present here new evidence of the presence of H^+ /ion antiporters in the vacuolar membrane of the yeast *Saccharomyces carlsbergensis* operating at the expense of $\Delta\mu H^+$ formed by the tonoplast ATPase.

2. MATERIALS AND METHODS

Yeasts were grown, vacuoles isolated, and protein and enzyme activities determined as described [7,8,10].

Measurement of fluorescence quenching of ACMA was described earlier [10], however, the protein content was 30–50 $\mu g/ml$ incubation medium. The membrane potential (E_m) was qualitatively determined by fluorescence-quenching measurement of the dye oxanol V (4.5 μM) [10,13]. The incubation medium consisted of 100 mM sorbitol, 10 mM Mes, adjusted with NaOH to pH 7.2 (ΔpH measurement) or pH 6.5 (E_m measurement). Fluorescence of oxanol V was measured in a Hitachi MPF-4 fluorescence spectrophotometer; excitation and emission wavelengths were 580 and 640 nm, respectively.

Arginine transport into isolated vacuoles was demonstrated by the accumulation of $[^{14}C]$ -arginine. 20 μl of 30 mM MgATP, pH 6.5, and 500 μl vacuolar suspension (270–470 μg protein) were introduced into conical test-tubes (Eppendorf). The suspension obtained was incubated at room

temperature (22°C) for 3 min, supplemented with 10–20 μ l [14 C]arginine up to a concentration of 40–50 μ M and again incubated for 3 min with mixing. After the addition of cold 10 mM Mes-Na buffer and 0.1 M sorbitol (pH 6.5) up to 1 ml, the suspension was shaken and centrifuged ($28\,000 \times g$) for 5 min at 0°C. The supernatant was removed and the test-tube walls dried with a paper filter. To each pellet, 200 μ l of 2% Triton X-100 were added. 100- μ l samples were dried on Whatman GF/B, and the radioactivity determined in a liquid scintillation spectrometer.

3. RESULTS AND DISCUSSION

3.1. Ion activation of H^+ -ATPase when the enzyme is under H^+ control

H^+ translocation catalyzed by ATPase located on the cytoplasmic surface of vacuolar membranes acidified the intravacuolar space. The generation of ΔpH (acid inside) and E_m (positive inside) inhibited the ATPase, indicating that the enzyme was under H^+ control [10,13]. Measurement of the activity of the vacuolar H^+ -ATPase being under H^+ control revealed its stimulation by free Mn^{2+} (fig.1). The increase in concentration of free Mg^{2+} or K^+ also stimulated ATPase when the substrate was MgATP (not shown). Mg^{2+} activated the ATPase released from the H^+ control (measurements with 50 mM Tris-Pipes) [14,15] by no more than 10–15%, while Mn^{2+} even inhibited it. The apparent K_m of the H^+/Mn^{2+} antiporters determined from the data on ATPase activation

were 0.8 and 0.3 mM for Mn^{2+} and Mg^{2+} , respectively. ATPase activation by Zn^{2+} and arginine (Arg^+) ions allowed for estimation of the apparent K_m of the corresponding antiporters (55 and 7 μ M, respectively).

3.2. $\Delta\mu H^+$ -dependent transport of [14 C]arginine into isolated vacuoles

Isolated vacuoles of *S. carlsbergensis* retained their radioactivity after incubation with [14 C]-arginine. The introduction of MgATP into the incubation system increased [14 C]arginine accumulation 5-fold. Such MgATP-stimulated accumulation of the amino acid was inhibited by DCCD, DES and combination of the protonophore FCCP with valinomycin (table 1). Potassium sulphate inhibited the ATP-stimulated ($\Delta\mu H^+$ -dependent) accumulation of [14 C]arginine by isolated vacuoles by less than 50%. Its effect was hardly different from KSCN, though the permeant anion SCN^- converts E_m to ΔpH [16]. Evidently, arginine transport into vacuoles occurs at the expense of both E_m and ΔpH , and is partially inhibited by K^+ competing with arginine for intravacuolar protons.

3.3. Changes in ΔpH and E_m by ions when ATPase is operating or switched off

It is logical to expect that, when ions are transported into vacuoles at the expense of ΔpH or E_m , their flux should change the value of these components of $\Delta\mu H^+$. The changes are easier to observe when the ATPase is switched off or when the rates of ΔpH and E_m creation by the ATPase are lower than those of their consumption. Ca^{2+} added before MgATP decreases the ΔpH (fig.2A) and induces the dissipation of the H^+ gradient, being added after the creation of ΔpH (fig.2B). It is noteworthy that the membrane potential increases under such conditions (fig.2C) [13]. Such an effect of Ca^{2+} on ΔpH and E_m agrees well with the idea of the electroneutral $Ca^{2+}/2 H^+$ antiporter when ATPase is activated by 60% [13]. The effect of K^+ and Zn^{2+} on E_m differs from that of Ca^{2+} . These ions decrease the formation of E_m when added before MgATP (fig.3). Taking into account that Zn^{2+} decreases both ΔpH (fig.2A) and E_m (fig.3) one can assume that the Zn^{2+}/H^+ antiport is electrogenic. According to another interpretation, Zn^{2+} increases the unspecific proton permeability

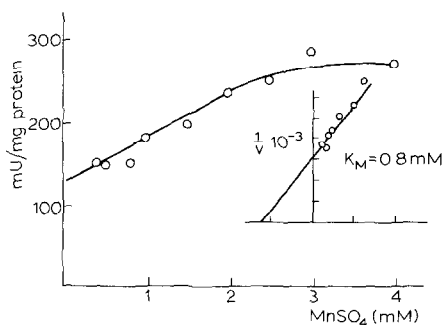


Fig.1. Relation between the specific activity of the ATPase of isolated intact vacuoles and concentration of free Mn^{2+} . (Inset) Lineweaver-Burk plot.

Table 1

Effect of various reagents on the accumulation of [14 C]arginine by isolated vacuoles of the yeast *S. carlsbergensis*

Additions	None	1.25 mM MgADP	1.25 mM MgATP	1.25 mM MgATP						
				1% ethanol	50 μ M DCCD	50 μ M DES	1 μ M FCCP + 1 μ M valinomycin	1 mM cold arginine	10 mM KSCN	10 mM K_2SO_4
[14 C]Arginine accumulation* (%)	100	104	416	360	124	111	117	100	176	200

*Mean values of 3 experiments

of the tonoplast and is transported by the electroneutral $Zn^{2+}/2 H^+$ antiporter.

To evaluate the kinetics of the H^+ /ion antiporters quantitatively we determined the initial velocities of ΔpH dissipation depending on the ion concentrations. For this purpose we selected condi-

tions for blocking the H^+ -ATPase when the main part of ΔpH had been created. On addition of DCCD under such conditions, the ΔpH created was not dissipated and did not increase further (fig.4) for more than 10 min. However, the addition of Ca^{2+} or Zn^{2+} results in the dissipation of ΔpH (fig.4). The data on the relation between the initial rate of ΔpH dissipation and Ca^{2+} concentration make it possible to determine the apparent K_m for the $Ca^{2+}/2 H^+$ antiporter: 60 μM Ca^{2+} . The K_m of the corresponding antiporter for Zn^{2+} thus determined is equal to 170 μM (vs 55 μM determined from the enzyme activation) and 1.5 mM for orthophosphate.

The above data agree well with the idea of the availability of H^+ /ion antiporters in the vacuolar membrane of the yeast *S. carlsbergensis*. The H^+ /ion antiport is evidently the principal mechanism of transport across the tonoplast. It is used to transfer inorganic cations (Mn^{2+} , Ca^{2+} , Mg^{2+} , Zn^{2+} , K^+), organic cations (arginine, lysine,

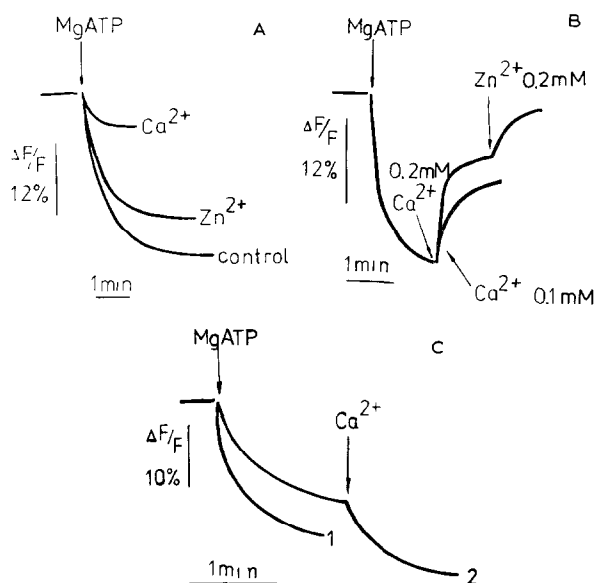


Fig.2. (A) Effect of Ca^{2+} and Zn^{2+} added before MgATP (0.75 mM) on the formation of ΔpH (fluorescence quenching of ACMA). Lower curve, control. Ca^{2+} and Zn^{2+} were added as Mes and sulphate salts accordingly. (B) Effect of Ca^{2+} and Zn^{2+} on the ΔpH created. (C) Effect of Ca^{2+} on the ATPase-dependent formation of E_m (fluorescence quenching of oxanol V). Upper curve, MgATP (0.75 mM), then Ca-Mes (2 mM); lower curve, Ca-Mes (2 mM) and then MgATP (0.75 mM).

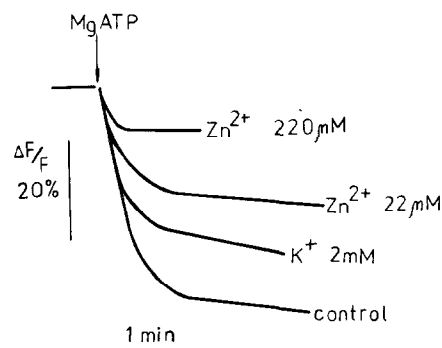


Fig.3. Effect of Zn^{2+} and K^+ , added before MgATP, upon E_m formation.

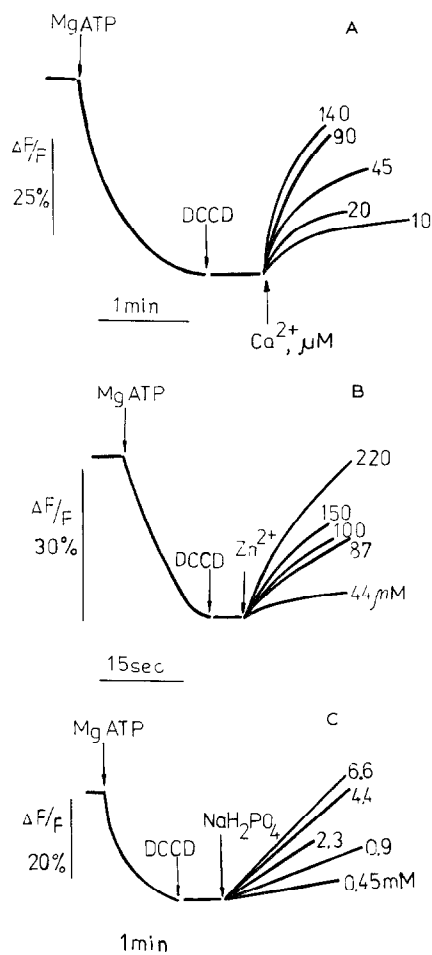


Fig.4. Dissipation of $\Delta p\text{H}$ by Ca^{2+} (A), Zn^{2+} (B) and phosphate (C) when the vacuolar ATPase is blocked by DCCD (75 μM). Phosphate added as sodium salt.

etc.) and probably phosphate in the case of E_m dissipation.

The finding of systems for the transport of various ions functioning as antiporters offers new scope for studies of the role of vacuoles and their transport systems in the regulation of the level of ions and some metabolites in the cytosol. This was demonstrated for K^+ , Mg^{2+} , Mn^{2+} and phosphate [17] and proved recently by Nicolay et al. for phosphate [18].

It is noteworthy that the apparent K_m of antiporters for Mg^{2+} , Mn^{2+} and phosphate (0.3, 0.8 and 1.5 mM) are sufficiently close to the concentrations of these ions in the cytosol evaluated earlier (1.35, 0.3 and 1 mM, respectively) [2]. One

can assume that the transport systems for the above ions operate until the ion levels in the cytosol approach the K_m of these systems. It is also noteworthy that the ATPase of the plasmalemma is inhibited by free Mg^{2+} at 0.4–0.5 mM free Mg^{2+} [19,20], i.e. within the K_m region of the $\text{Mg}^{2+}/\text{H}^+$ antiporter of the tonoplast and when the vacuolar ATPase is activated by Mg^{2+} (here).

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