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Regulation of plasma membrane H^+-ATPase from corn-root by Mg^{2+} and pH

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Abstract

The plasma membrane H^+-ATPase of corn-root is activated by free Mg^{2+} at pH 6.0 (K_s = 2.9 mM) but not at pH 7.0. As a result, the pH dependence of the enzyme varies depending on the Mg^{2+} concentration of the medium. The activation by Mg^{2+} is promoted by an increase in V_{max} with no effect on the apparent K_m for Mg · ATP. Different from Mg^{2+}, free Mn^{2+}, Co^{2+} and Ni^{2+} do not activate at pH 6.0 and inhibit the H^+-ATPase at pH 7.0. The effects of divalent cations on the corn ATPase observed in this report are different from those previously described for the yeast enzyme (Brooker, R.J. and Slayman, C.W. (1983) J. Biol. Chem. 258, 8833–8838), suggesting different mechanisms of regulation for the isoforms of yeast and corn H^+-ATPase.

Keywords: ATPase, H^+-; pH; Plasma membrane; Magnesium ion activation; Corn root; (*Zea mays* L.)

1. Introduction

The plant plasma membrane H^+-ATPase is a P-type ATPase that plays a key role in nutrition and development of plants [1–4]. The physiological substrate of this enzyme is the complex Mg · ATP, with free ATP being a weak competitive inhibitor [5–7]. Free Mg^{2+} modulates the H^+-ATPase in different manners depending on the enzyme source used. The H^+-ATPase purified from yeast plasma membrane [5,8,9] is activated by low concentrations of free Mg^{2+} (K_s = 5 μM) and is inhibited by higher concentrations of free Mg^{2+} (K_i = 3.5 mM). The H^+-ATPase found in the roots of *Avena sativa* L. is also inhibited by high Mg^{2+} [7], but in this higher plant the inhibition is much less pronounced than that observed in yeast and the degree of inhibition varies depending on the K^+ concentration in the medium.

This report shows that the H^+-ATPase of corn-root is activated rather than inhibited by high Mg^{2+} concentrations. The degree of activation varies depending on the pH value of the medium.

2. Materials and methods

Maize seeds (*Zea mays* L.) were germinated in the dark at 28°C on wet filter paper. The roots were harvested after the 4th or 5th day. Vesicles derived from the plasma membrane were isolated from the maize roots using differential centrifugation and a sucrose gradient, as previously described [10]. The vesicles were resuspended in a 10 mM Tris-HCl buffer (pH 7.6) containing 20% (v/v) glycerol, 1 mM dithiothreitol and 1 mM EDTA and stored frozen under liquid nitrogen until use. Protein concentration was determined by the method of Lowry et al. [11], with bovine serum albumin as the standard. The ATPase activity was determined by measuring the release of P_i, either colorimetrically [12] or using [γ-^{32}P]ATP, as previously described [13]. In all experiments, the ATPase activity was measured with and without 0.1 mM vanadate, an inhibitor of the H^+-ATPase [14], and the difference between these two activities was attributed to the H^+-ATPase. The inhibitory activity of vanadate did not vary in the pH range of 5.5 to 8.0 The vanadate sensitive activity was not impaired by 50 mM KNO_3, an inhibitor of tonoplast ATPase or by 1 mM molybdate, an inhibitor of phosphatases [14]. The accumulation of protons by the vesicles was determined by measuring the fluorescence quenching of ACMA with an Hitachi F-3010 fluorimeter [2]. The excita-

Abbreviations: Mops, 4-morpholinopropanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; ACMA, 9-amino-6-chloro-2-methoxyacridine; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone.

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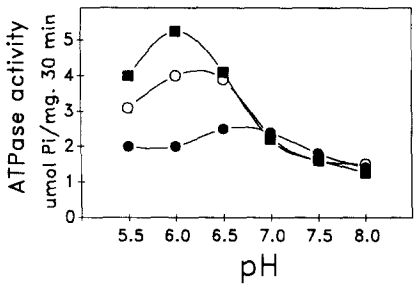

Fig. 1. Mg^{2+} and pH dependence. The composition of the reaction medium was 50 mM MOPS-Tris buffer adjusted to different pH values, 1 mM ATP and either (●) 1 mM, (○) 4 mM or (■) 10 mM $MgCl_2$. The reaction was started by the addition of plasma membrane protein to a final concentration of 30 μg/ml and arrested after 30 min incubation at room temperature by the addition of trichloroacetic acid (10%, w/v).

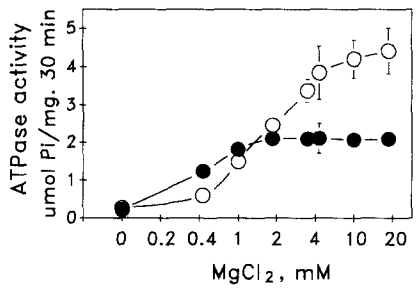

Fig. 2. Effects of $MgCl_2$ at pH 6.0 and pH 7.0. The assay medium composition was 50 mM Mops-Tris buffer adjusted to either pH 6.0 (○) or pH 7.0 (●), 1 mM ATP, 30 mg/ml plasma membrane protein and the $MgCl_2$ concentrations shown in the figure. Values are means ± S.E. of five experiments.

tion wavelength was set at 415 nm and emission wavelength at 485 nm. The free Mg^{2+}, free ATP and Mg · ATP concentrations were calculated using the absolute association constants and computer program described by Fabiato and Fabiato [15].

3. Results

3.1. Effects of pH and free Mg^{2+} on the ATPase activity

The pH dependence of the corn-root H^+-ATPase varies depending on the $MgCl_2$ concentration used (Fig. 1). With 1 mM ATP and in the pH range of 5.5 to 6.5, an increase of the ATPase activity was observed as the $MgCl_2$ concentration was raised from 1 to 10 mM. This activation was not observed at neutral and alkaline pH values (Figs. 1 and 2). At equal ATP and $MgCl_2$ concentrations, the activity varied little as the pH was raised from 5.5 to 8.0 with a modest optimum between pH 6.5 to 7.0 (Fig. 1). In the presence of 10 mM $MgCl_2$, the pH dependence became more pronounced and the maximal activity was displaced toward pH 6.0. Earlier reports have shown that in the pH range of 6.7 to 7.5 the yeast H^+-ATPase is inhibited by free Mg^{2+} concentrations higher than 2 mM [5,6,8,9]. This was not observed with the use of the corn-root ATPase (Fig. 2). At pH 7.0 the rate of ATP hydrolysis did not vary as the $MgCl_2$ concentration in the medium was raised from 1 to 20 mM and at pH 6.0, high $MgCl_2$ concentrations activated the enzyme. The effect of Mg^{2+} did not vary when the vesicles were washed with KI (Table 1) in order to decrease a possible contamination of the vesicles with non-specific phosphatases [16,17]. Lysophosphatidylcholine decreased the activation promoted by high Mg^{2+} at pH 6.0 (Table 1). Lysophosphatidylcholine is known to decrease the autoinhibition promoted by the C-terminal region of the ATPase molecule [18–20]. The effect of Mg^{2+} was not modified by monovalent cations. The same results were obtained at both pH 6.0 and pH 7.0 when the $MgCl_2$ dependence shown in Fig. 2 was measured in the absence of monovalent cations or in the presence of either 100 mM KCl or 100 mM NaCl (data not shown). Using the experimental values of Fig. 2, the different ionic species available in the medium were calculated and the rate of ATP hydrolysis was correlated with the concentrations of free Mg^{2+} (Fig. 3). At pH 7.0, increasing the free Mg^{2+} concentration from 0.25 mM up to 20 mM had practically no effect on the rate of ATP hydrolysis. This was confirmed in Fig. 4B and Table 2 where the apparent V_{max} and K_m of the H^+-ATPase for the complex Mg · ATP were measured in the presence of different free Mg^{2+} concentrations. Earlier reports [5–7] have shown that the

Table 1
Effects of potassium iodide and lysophosphatidylcholine

Conditions	μmol P_i/mg per 30 min					
	pH 6.0		% increment	pH 7.0		% increment
	1 mM	10 mM		1 mM	10 mM	
Control	1.36	3.90	187	1.85	2.22	20
KI washed	1.19	3.06	157	1.46	1.58	8
Lysophosphatidylcholine	3.96	7.51	90	4.02	4.76	18

The composition of the control reaction medium was 50 mM Mops-Tris buffer adjusted to either pH 6.0 or pH 7.0, 1 mM ATP, 1 mM or 10 mM $MgCl_2$ and when referred, 60 μg/ ml lysophosphatidylcholine. The reaction was started by the addition of plasma membrane protein to a final concentration of 30 μg/ml and arrested after 30 min incubation at room temperature by the addition of trichloroacetic acid (10%, w/v). Washed with KI refers to vesicles that were resuspended in 250 mM KI and after 15 min incubation in ice were sedimented by centrifugation (30 min at 100000 × g), the pellet was resuspended in ice-cold water and immediately used. The values are the average of two experiments.

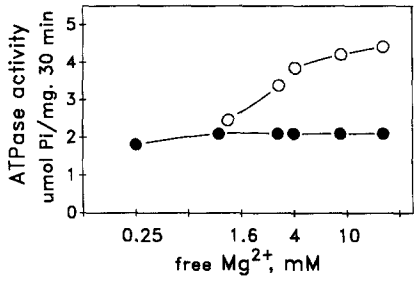

Fig. 3. Effect of free Mg^{2+} at pH 6.0 (○) and pH 7.0 (●). Free Mg^{2+} dependence was calculated using the experimental values of Fig. 2 obtained with $MgCl_2$ concentrations varying from 1.0 to 20 mM. The concentrations of Mg.ATP and of free ATP in the different points shown in the figure varied from 0.74 to 0.99 mM and from 0.255 to 0.005 mM, respectively.

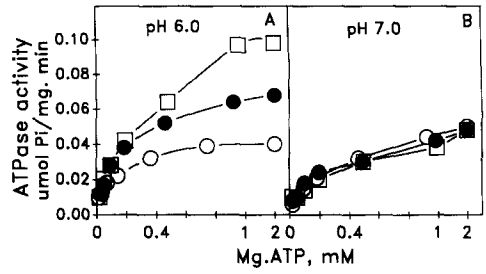

Fig. 4. Apparent K_m for ATP at pH 6 (A) and pH 7 (B). The assay medium composition was 50 mM Mops-Tris buffer adjusted to either pH 6.0 or pH 7.0, 30 μg/ml plasma membrane protein and the ATP and $MgCl_2$ concentrations needed to obtain the Mg·ATP concentrations shown in the figure in the presence of free Mg^{2+} concentrations varying between (○) 1.0 and 1.4 mM, (●) 5.0 and 5.2 mM or (□) 10.0 and 10.8 mM. At pH 6.0 and with 1.0–1.4 mM free Mg^{2+} the concentration of free ATP varied between 23 and 29% of the total ATP pool. This decreased to the range of 4 to 8% when the free Mg^{2+} concentration was raised to 10.0–10.8 mM. At pH 7.0 and with 1.0–1.4 mM free Mg^{2+} the concentration of free ATP varied between 7 and 8% of the total ATP pool and decreased to 0.9% when free Mg^{2+} concentration was raised to 10 to 10.8 mM.

true substrate of the H^+-ATPase is the complex Mg · ATP and that free ATP is a weak competitive inhibitor. Both Fig. 4 and Table 2 show that the apparent affinity of the ATPase for Mg · ATP at pH 6.0 and pH 7.0 was practically the same and that the activation promoted by Mg^{2+} at pH 6.0 (Fig. 4A) was related to an increase in V_{max} with no effect on the apparent affinity of the enzyme for Mg · ATP. Double reciprocal plots of the data of Fig. 3 revealed that the concentration of free Mg^{2+} needed for half maximal activation of the ATPase activity measured at pH 6.0 was 2.9 mM.

3.2. Proton transport

During the hydrolysis of ATP the vesicles accumulate protons and both an electrical gradient ($\Delta\Psi$) and a pH gradient (ΔpH) are formed across the vesicle membranes. The $\Delta\Psi$ is practically abolished when 100 mM KCl is present [2]. High Mg^{2+} concentrations promoted an increase in ΔpH at pH 6.0, but not at pH 7.0 (Fig. 5A). The fluorescence quenching measured in Fig. 5B reflects the formation of a ΔpH across the vesicle membranes. Raising the $MgCl_2$ concentration from 2 to 10 mM promoted a 2-fold increase in fluorescence quenching at pH 7.0 and a 5-fold increase at pH 6.0 (Fig. 5B). In the absence of permeable ions, $\Delta\Psi$ inhibits the generation of ΔpH. Thus, in Fig. 5B, part of the activation by high magnesium is probably promoted by the Cl^- introduced in the medium with Mg^{2+}.

Table 2
Effect of Mg^{2+} on the kinetics of the H^+-ATPase

Free Mg^{2+} (mM)	V_{max} (nmol P_i /mg per min)		K_m (μM)	
	pH 6.0	pH 7.0	pH 6.0	pH 7.0
1.0	58±4	49±2	156±6	191±29
5.0	89±7	52±8	164±8	192±29
10.0	89±7	49±9	164±8	192±29

The assay medium composition and experimental conditions are the same as in Fig. 5. Values are means±S.E. of three experiments.

3.3. Divalent cation specificity

It has been shown previously that the divalent cations Mn^{2+}, Co^{2+}, and to a lesser extent Ni^{2+}, mimic the behavior of Mg^{2+} in both corn-root [21] and yeast H^+-ATPase [9]. With these two enzymes, the rates of hydrolysis measured with complexes of ATP with Mg^{2+}, Mn^{2+} and Co^{2+} were similar, and several fold higher than those measured with Ni^{2+}. As observed for Mg^{2+}, at pH 6.7 the yeast ATPase is also strongly inhibited by free Mn^{2+}, Co^{2+} and Ni^{2+} [9]. We now show that for the corn-root ATPase, the effect of high concentrations of free divalent cations varies depending on the pH value of the assay medium (Fig. 6). At both pH 6.0 and pH 7.0 the rates of hydrolysis measured using equal concentrations of ATP and either Mg^{2+} (Fig. 2), Mn^{2+} or Co^{2+} (Fig. 6) were similar. At pH 6.0 however, high concentrations of Mn^{2+} and Co^{2+} did not activate the ATPase as observed with Mg^{2+} (compare Fig. 2 and 6) and at pH 7.0, free Mn^{2+} and Co^{2+}, different from Mg^{2+}, inhibited the corn enzyme in a manner similar to that described for the yeast ATPase [9]. The rate of ATP hydrolysis measured with Ni^{2+} was

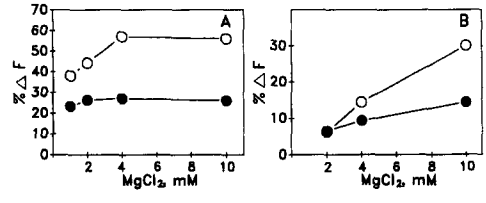

Fig. 5. H^+ transport. The assay medium composition was 10 mM Mops-Tris buffer adjusted to either pH 6.0 (○) or pH 7.0 (●), 100 μg/ml plasma membrane protein, 2 μM ACMA, 1 mM ATP, and the $MgCl_2$ concentrations shown in the figure, (A) with 100 mM KCl; (B) without KCl. The same result was observed in three experiments.

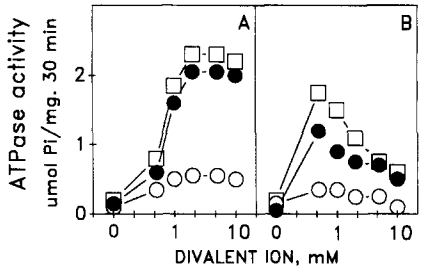

Fig. 6. Effects of different divalent cations on the ATPase activity. The assay medium composition was 50 mM Mops-Tris buffer adjusted to either pH 6.0 (A) or pH 7.0 (B), 1 mM ATP, 30 μg/ml plasma membrane protein and different concentrations of either $CoCl_2$ (□), $MnCl_2$ (●) or $NiCl_2$ (○).

slower than that measured with the other divalent cations, but for concentrations of Ni^{2+} higher than those of ATP, the pattern of no activation at pH 6.0 and inhibition at pH 7.0 was the same as observed for Mn^{2+} and Co^{2+}.

4. Discussion

Magnesium is the second most abundant cation in the cell, trailing only potassium, and it is known to activate a large number of enzymes, either directly or by forming complexes with substrates [22]. The concentration of free Mg^{2+} in the cytosol of corn-root cells is not known. The total magnesium concentration found in oat roots is 8.5 mM [23]. Notice in Fig. 2 that maximal activation is attained with 5 mM $MgCl_2$.

At neutral pH the effect of millimolar Mg^{2+} concentrations seems to vary depending on the enzyme source used. It has no effect on the corn-root enzyme (Fig. 2), but strongly inhibits the yeast H^+-ATPase. This difference seems to be specific for Mg^{2+}, since Co^{2+}, Mn^{2+} and Ni^{2+} inhibit both the corn enzyme (Fig. 6) and the yeast enzyme [9].

In yeast, the H^+-ATPase activity varies depending on the physiological state of the cell, increasing during glucose metabolism [24] and during acidification of the growth medium [25]. Enhancement of the ATPase activity results from a combined effect on the K_m, V_{max} and a shift of the pH optimum toward alkaline values. An increase in V_{max} and a shift of the pH optimum toward more alkaline values, but no effect on the K_m, were observed when oat roots were treated with the fungal phytotoxin fusicoccin [26]. The molecular mechanism of glucose regulation in yeast and of the fusicoccin effect in oat roots is not known, but different evidence indicates that it might be related to conformational changes of the enzyme which would interfere with the inhibitory interaction between the carboxyl terminus and the ATP-binding domains of the enzyme [26,27]. In fact, mutations that lead to modest changes in the amino acid sequence of these two regions of the H^+-ATPase yield isoforms with different V_{max} and different pH dependencies: depending on the mutation, maximal activity can be shifted either to the alkaline pH range, as observed with the use of glucose or fusicoccin, or to the acidic pH range, as observed in Fig. 1 with high Mg^{2+} concentrations [27]. The autoinhibition promoted by the carboxyl terminus of the ATPase is abolished by lysophosphatidylcholine [18–20]. In Table 1 it was noted that the activation at pH 6.0 by high Mg^{2+} was not abolished in presence of lysophosphatidylcholine. Thus, it seems that at pH 6.0, the binding of Mg^{2+} to the corn-root ATPase does not interfere with the autoinhibitory effects promoted by the carboxy-terminal regions of the enzyme.

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