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Characterization of the pyrophosphate-dependent proton transport in microsomal membranes from maize roots

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Cleared maize (*Zea mays* L. cv. LG 11) root homogenates were prepared and layered on the top of sucrose step gradients (10, 35 and 45%). The ATP- and pyrophosphate (PP_i)-dependent proton-pumping activities were recovered almost completely at the 10%/35% interface, corresponding to the microsomal fraction (Golgi, tonoplast and endoplasmic reticulum). The PP_i-dependent proton pump was characterized by the fluorescence quenching of quinacrine. The pH optimum was 7 to 8. The H^+-PPase was Mg^{2+}-dependent and the K_m for PP_i (in the presence of 3 m*M* $MgSO_4$) was 28 μ*M*. The pump was electrogenic, K^+-dependent and a permeant anion was necessary to dissipate the membrane potential ($NO_3^- = I^- > Br^- > Cl^-$). No activity was detected in the presence of electroneutral proton ionophores or, when valinomycin was added, with electrogenic ionophores. The H^+-PPase was insensitive to vanadate, oligomycin and molybdate. Diethylstilbestrol (DES) and N,N'-dicyclohexylcarbodiimide (DCCD) were strongly inhibitory at 100 μ*M*.

Key words – ATPase, maize, proton pump, pyrophosphate, pyrophosphatase, tonoplast, *Zea mays*

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Introduction

In 1975, Karlsson reported for the first time the presence of a K^+-stimulated pyrophosphatase (PPase), associated with microsomal membranes of sugar beet roots and cotyledons. Walker and Leigh (1981) showed that it was associated with the tonoplast of red beet storage roots. Several authors (Dupont et al. 1982, Churchill and Sze 1983, Bennett et al. 1984), working with the tonoplast-type H^+-ATPase, reported some proton transport in the presence of pyrophosphate (PP_i). However, it was not known if this activity was due to the H^+-ATPase or a different pump. In 1985, two independent studies (Rea and Poole, Chanson et al.) showed that the PP_i-dependent proton pumping activity was not driven by the H^+-ATPase, but by the PPase described earlier by Karlsson (1975) and Walker and Leigh (1981). The two pumps were solubilized and separated physically by gel filtration (Rea and Poole 1986, Wang et al. 1986). Such H^+-PPase was detected in different higher plants species (Blumwald 1987, Rea and Sanders 1987) and in *Nitella* (Shimmen and MacRobbie 1987a).

The H^+-PPase was found to be more active (or more abundant) in the tip than in the other parts of maize roots (Chanson and Pilet 1987). Moreover, its activity was higher than that of the H^+-ATPase, suggesting that it might be related to growth processes (Taiz 1986, Chanson and Pilet 1987).

In the present paper, the PP_i-dependent proton pumping activity of maize roots is characterized for the first time. The method of fluorescence quenching of quinacrine was used to measure the initial rate of proton pumping (Chanson and Taiz 1985).

Abbreviations – BTP, bis-tris propane; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCCD, N,N'dicyclohexylcarbodiimide; DES, diethylstilbestrol; DIDS, 4,4'-diisothiocyanostilbene-2,2'-disulfonate; DTT, dithiothreitol; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; NEM, N-ethylmaleimide; IDA, iminodiacetate; MES, 2-(N-morpholino)ethanesulphonic acid; Oxonol V, bis(3-phenyl-5-oxoisoxa-

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zol-4-yl)penthamethine oxonol; SITS, 4-acetamido-4'-isothiocyano-2,2'-stilbenesulfonic acid; Tris, tris-(hydroxymethyl) aminomethane; $\Delta\psi$, membrane potential; ΔpH, proton gradient; 1 KS, 1000 *g* supernatant.

dient buffer without DTT. Sucrose concentrations were measured by refractometry. Protein concentration was determined as previously described (Chanson and Pilet 1987).

Materials and methods

Plant material

Roots of maize (*Zea mays* L. cv. LG 11, Association Suisse des Sélectionneurs, Lausanne) were grown for 48 h in the dark (21±1°C), after 24 h of caryopse imbibition (tap water), as previously described (Pilet 1977). Primary roots of 10–20 mm in length were harvested and stored on ice under room lights. Homogenization and subsequent treatments were performed at 0–4°C (Chanson and Pilet 1987).

Homogenization

Roots (3–6 g) were chopped by hand with razor blades in 6 ml of homogenization medium (containing 250 m*M* sucrose, 2 m*M* EDTA, 1 m*M* dithiothreitol (DTT), 0.1% BSA, 50 m*M* tris-(hydroxymethyl)aminomethane (Tris), adjusted to pH 7.8 with solid 2-(N-morpholino) ethanesulphonic acid (MES). The tissue was then ground lightly (1 min) in a mortar and strained through nylon (pore diameter 0.25 mm). The remaining tissue was lightly reground (1 min) in an additional 6 ml of homogenization buffer. The second homogenate was filtered through nylon and combined with the first homogenate. Unbroken cells, wall fragments, starch grains and nuclei were removed by a 5-min centrifugation at 1000 *g* (Beckman J2-21, JA-20 rotor) and the supernatant (1 KS) was collected.

Sucrose gradients

The 1 KS fraction was diluted with homogenization buffer (21 ml, final volume) and layered on the top of a step gradient consisting of 5 ml of 45% (w/w) sucrose, 5 ml of 35% sucrose and 5 ml of 10% sucrose (each in gradient buffer containing 0.5 m*M* EDTA, 1 m*M* DTT, 2.5 m*M* Tris, adjusted to pH 7.5 with solid MES). The gradients were centrifuged for 3 h at 80000 *g* (Kontron Centrikon T-2080, TST 28.38 rotor) and fractionated into 13 portions of 1.5 ml. Fractions 7 and 8, corresponding to a peak of protein (10/35% sucrose interface) were pooled and diluted to 10% sucrose with gradient buffer. The different tubes were frozen in liquid nitrogen and stored at −70°C. For some experiments [effect of DCCD and N-ethylmaleimide (NEM)], the membranes were sedimented (1 h at 150000g, TST 28.17 rotor) and resuspended in 10% sucrose, in gradient buffer without DTT.

Quinacrine fluorescence quenching

Membrane vesicles (400 µl, 10% sucrose in gradient buffer), the appropriate salt or inhibitors and quinacrine (5 µ*M* final concentration) were added to an assay buffer of bis-tris propane (BTP)-MES (pH 7.5) to a final volume of 0.6 ml (the final total concentration of BTP plus MES was 25 m*M*). Fluorescence was measured at room temperature with a Perkin Elmer LS-5 Luminescence Spectrometer (excitation, 430 nm; emission, 500 nm). After temperature equilibration, the reaction was initiated by a mixture of $MgSO_4$ and ATP (3 m*M* each, final concentration) or by PP_i (at different concentrations) (Chanson et al. 1985). At the end of the experiment, 3 µl of monensin (1 m*M*) dissolved in ethanol (5 µ*M* final concentration of monensin) was added to collapse the proton gradient. The initial rate of fluorescence quenching was measured (% min^{-1}) for each assay. The linearity of the quinacrine fluorescence quenching response with the concentration of membrane protein was verified for quantities lower than 100 µg (80–100 µg was routinely used). All experiments were repeated on at least two separate preparations of vesicles.

Oxonol V fluorescence quenching

The formation of a membrane potential ($\Delta\psi$) was measured at room temperature, using the fluorescence quenching of bis(3-phenyl-5oxoisoxazol-4-yl)penthamethine oxonol (Oxonol V) (excitation, 580 nm; emission, 650 nm) (Wang et al. 1986). The reaction mixture (0.6 ml) contained 25 m*M* BTP-MES (pH 7.5), 2 µ*M* of Oxonol V, 0.1 m*M* PP_i and 100 µg of membrane protein (400 µl, 10% sucrose in gradient buffer). The reaction was initiated by the addition of 3 m*M* $MgSO_4$ (final concentration), in the presence or absence of 50 m*M* K-iminodiacetate (IDA). At the end of the experiment, 6 µl of gramicidin (dissolved in ethanol, 10 µ*M* final concentration of gramicidin) was added to collapse the $\Delta\psi$ gradient.

Chemicals

Oxonol V was bought from Molecular Probes Inc, Junction City, OR, USA and $Na_4P_2O_7$ from Merck, Darmstadt, FRG. Monensin, nigericin, Tris, MES and BTP were obtained from Calbiochem-Behring and Na_2ATP, imidodiphosphate, DES, NEM, bovine serum albumin

(BSA), quinacrine and gramicidin from Sigma Chemical Company. All other chemicals were purchased from Fluka AG Buchs.

Results and discussion

The PP_i-dependent proton pump has been found recently in several different plant species: maize roots (Dupont et al 1982, Taiz 1986, Chanson and Pilet 1987), oat roots (Churchill and Sze 1983, Wang et al. 1986, Kaestner and Sze 1987, Pope and Leigh 1987, 1988), red beet roots (Bennett et al. 1984, Rea and Poole 1985, Pope and Leigh 1988), maize coleoptiles (Chanson et al. 1985, Hager et al. 1986), sugar beet roots (Briskin et al. 1985), *Nitella* (Shimmen and MacRobbie 1987a, b), barley roots (Dupont 1987), wheat seedlings (Maslowski and Maslowska 1987), bean hypocotyls (Rogers and Anderson 1987), pea stems (Macrì and Vianello 1987), tomato fruits (Oleski et al. 1987) and leaves of the CAM plant *Kalanchoë daigremontiana* (Marquardt and Lüttge 1987). This wide-spread occurrence has increased the interest in the complete characterization of the H^+-PPase.

So far, the H^+-PPase from only a few plant species have been studied: maize coleoptiles (Chanson at al. 1985, Hager et al. 1986), red beet roots (Rea and Poole 1985, Pope and Leigh 1988), oat roots (Wang et al. 1986, Pope and Leigh 1987, 1988), wheat seedlings (Maslowski and Maslowska 1987), pea stems (Macrì and Vianello 1987), *Nitella* (Shimmen and MacRobbie 1987b) and *Kalanchoë* (Marquardt and Lüttge 1987).

Moreover, the recent observation that the use of acridine orange as a pH probe may lead to artefacts (Pope and Leigh 1988), renders some results questionable (Rea and Poole 1985, Macrì and Vianello 1987).

Preparation of the membrane fraction

Light membrane fractions were prepared routinely from maize root homogenates, using sucrose step gradients (10, 35 and 45%). The subcellular localization of the H^+-PPase has already been presented and discussed in a previous paper (Chanson and Pilet 1987). Here again, the distribution of the ATP-and PP_i-dependent proton pumps were found to be similar (data not shown). Most of the two proton pumping activities were recovered in the 10–35% sucrose interface, corresponding to the microsomal fraction (Golgi, tonoplast and ER; Chanson and Pilet 1987).

pH Dependence

The H^+-PPase activity, measured by the method of fluorescence quenching of quinacrine, presented a slightly alkaline pH optimum (pH 7–8; Fig. 1). Similar results were found when PP_i hydrolysis was measured in the uncoupled condition (data not shown). pH 7.5 was chosen for characterizing the pump.

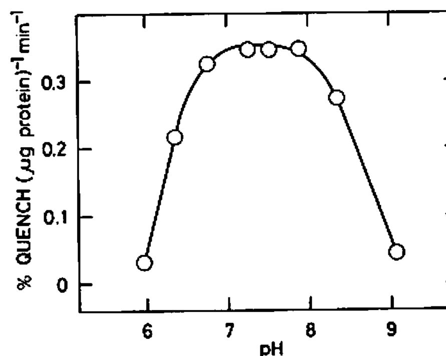

Fig. 1. Effect of pH on the PP_i-dependent fluorescence quenching of quinacrine, in the presence of 0.1 m*M* PP_i, 3 m*M* $MgSO_4$ and 50 m*M* KNO_3. The pH of the medium was adjusted by altering the proportions of BTP and MES (25 m*M*, final concentration).

In *Kalanchoë* (Marquardt and Lüttge 1987), a pH optimum around 8–9 was found, whereas in pea stems (Macrì and Vianello 1987), a value of 6.5 was determined. When the hydrolysis of PP_i was measured, a broad pH optimum around 8–9 was found in red beet roots (Walker and Leigh 1981, Rea and Poole 1985), tulip petals (Wagner and Mulready 1983) and wheat seedlings (Maslowski and Maslowska 1987).

Salts effects

Divalent salts: The pump had an absolute requirement for Mg^{2+}, and no other divalent cation could replace it (data not shown). The same results were found for the H^+-PPase of *Kalanchoë* (Marquardt and Lüttge 1987) and the PPase activity of red beet roots (Walker and Leigh 1981). As already discussed by Wang et al. (1986), the ratio between PP_i and Mg^{2+} concentrations was important for the activity of the pump. In maize roots, a concentration of 3 m*M* Mg^{2+} gave the higher initial rate of quenching, when 0.1 m*M* PP_i was used to

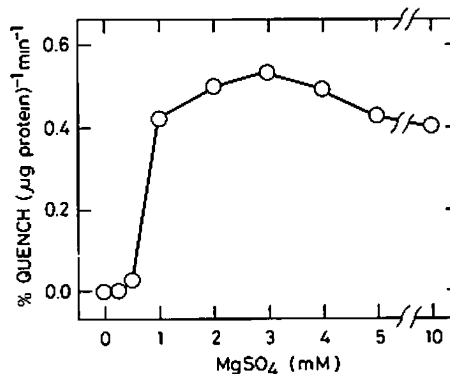

Fig. 2. Effect of the concentration of $MgSO_4$ on the PP_i-dependent fluorescence quenching of quinacrine, in the presence of 0.1 m*M* PP_i, 50 m*M* KNO_3 and 25 m*M* BTP-MES (pH 7.5).

start the H^+-pumping (Fig. 2). This concentration of $MgSO_4$ was used for all the other experiments. No activity was detectable at Mg^{2+} concentrations lower than 0.5 m*M* (probably due to the presence of EDTA from the gradient buffer) and some inhibition was observed at high concentrations (6–10 m*M*). Further experiments will be needed to explain the complicated effects of Mg^{2+} and PP_i on the activity of the pump.

Monovalent salts: The PP_i-dependent proton pump was most active with KNO_3, followed by KI, KBr and KCl (Tab. 1). The more permeant anions were giving the higher rates of fluorescence quenching, by dissipation of the $\Delta\psi$ (Tab. 1; Pope and Leigh 1987). Experiments with Oxonol V showed that the pump was electrogenic and confirmed its strict K^+-dependency (Fig. 3). No $\Delta\psi$ or ΔpH formation was observed in its absence. Rb^+ and Cs^+ were able to replace partly K^+ (Tab. 1), as already shown for other materials (Hager et al. 1986, Wang et al. 1986, Macrì and Vianello 1987, Marquardt and Luttge 1987). Wang et al. (1986) proposed that K^+ binds to a cation-sensitive site, causing a conformational change from an inactive to an active form of the protein. The results obtained with maize roots are in accordance with this hypothesis.

When KNO_3 and KCl were tested at different concentrations (Fig. 4), the PP_i-dependent fluorescence quenching was always higher with KNO_3 than with KCl. Contradictory results were found during the first characterizations of the H^+-PPase. The pump of maize coleoptiles was more active in presence of KNO_3 than KCl (Chanson et al. 1985, Hager et al. 1986). In contrast, in red beet and oat roots, the proton gradient formation was lower in presence of KNO_3 than KCl (Rea and Poole 1985, Wang et al. 1986), whereas, the PPase hydrolytic activity was simllar. These last data were taken as indirect evidence for an outwardly directed H^+/NO_3^- symport system, dissipating the proton gradient. However, these results must be considered with caution. The experiments with oat roots were done with high concentrations of PP_i (1.5 m*M*), conditions in which the ΔpH formation (measured by the method of fluorescence quenching of quinacrine) was inhibited by an excess of substrate (Wang et al. 1986). When low concentrations of PP_i were used (90 μ*M*), KNO_3 induced a ΔpH comparable or larger than that found with KCl. In the experiments of Rea and Poole (1985), the fluorescence quenching of acridine orange was used as a pH

Tab. 1. Effect of different monovalent salts on the initial rate of PP_i-dependent relative fluorescence quenching of quinacrine. Membrane vesicles (400 μl, 10% sucrose in gradient buffer), $MgSO_4$ (3 m*M*) and quinacrine (5 μ*M*) were added to an assay buffer of BTP-MES (25 m*M*, pH 7.5) to a final volume of 0.6 ml. The reaction was initiated by PP_i (0.1 m*M*). The concentration of monovalent salt was 50 m*M* except for 25 m*M* K_2SO_4 and Na_2SO_4.

Additions	% quenching (μg protein)$^{-1}$ min^{-1}	% control
None	0	
KNO_3 (control)	0.286	100
KI	0.276	96
KBr	0.213	75
KCl	0.184	64
K_2SO_4	0.055	19
K-IDA	0.029	10
K-MES	0.012	4
$KHCO_3$	0.012	4
KNO_2	0.000	0
KF	0.000	0
RbCl	0.169	59
CsCl	0.104	36
NaCl	0.013	5
LiCl	0.000	0
BTP-Cl	0.000	0
BTP-NO_3	0.000	0
Na_2SO_4	0.000	0
K-MES + BTP-NO_3	0.156	55
K-IDA + BTP-NO_3	0.123	43

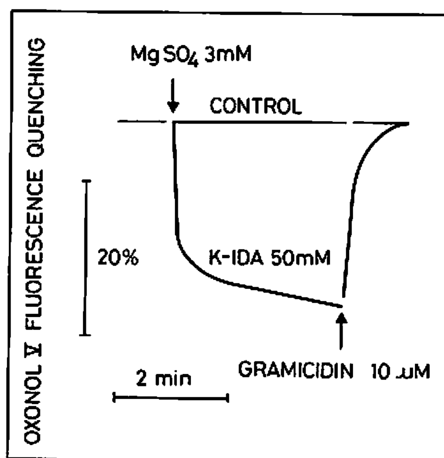

Fig. 3. PP_i-dependent fluorescence quenching of Oxonol V, with and without 50 m*M* K-IDA, in the presence of 0.1 m*M* PP_i, 3 m*M* $MgSO_4$ and 2 μ*M* Oxonol V.

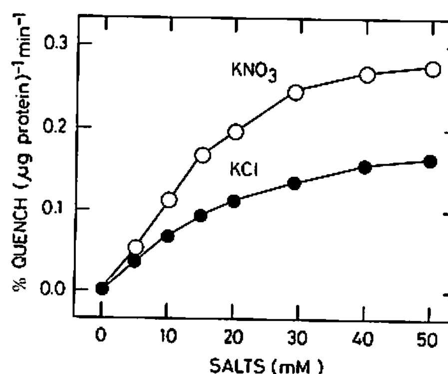

Fig. 4. Effect of the concentration of KCl and KNO_3 on the PP_i-dependent fluorescence quenching of quinacrine, in the presence of 0.1 m*M* PP_i, 3 m*M* $MgSO_4$ and 25 m*M* BTP-MES (pH 7.5).

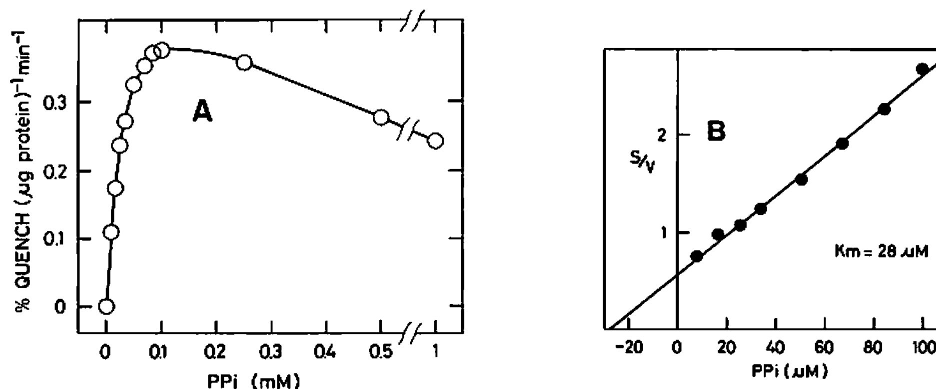

Fig. 5. A, Effect of the concentration of PP_i on the fluorescence quenching of quinacrine, in the presence of 3 mM $MgSO_4$, 50 mM KNO_3 and 25 mM BTP-MES (pH 7.5). B, Hanes-Woolf plot (using the 8 lower concentrations of PP_i) that gave an apparent K_m for PP_i of 28 µM.

probe. But, Pope and Leigh (1988) showed that the reduction of pH-gradient formation with NO_3^-, was an artefact induced by the acridine orange.

Recent results confirmed that NO_3^- did not inhibit the activity of the PP_i-dependent proton pump in pea stem (Macrì and Vianello 1987) and in *Nitella* (Shimmen and MacRobbie 1987b). Under these conditions, the presence of a nitrate/proton symport is unlikely or is restricted to some plant species only.

Effect of PP_i at different concentrations

The activity of the PP_i-dependent proton pump was tested at different PP_i-concentrations, in the presence of 3 mM $MgSO_4$ and 50 mM KNO_3 (Fig. 5A). A Hanes-Woolf plot of the data (Fig. 5B) gives an apparent K_m for PP_i of 28 µM.

Similar values were reported for red beet (Rea and Poole 1985) and oat roots (Wang et al. 1986), pea stem (Macrì and Vianello 1987), leaves of *Kalanchoë* (Marquardt and Lüttge 1987) and wheat seedlings (Maslowski and Maslowska 1987). The high K_m value (500 µM) obtained in maize coleoptiles (Chanson et al. 1985) might be explained by the equimolar concentrations of Mg^{2+} used for these experiments. In contrast to the strong inhibition observed at high concentrations of PP_i, in the case of the H^+-PPase from oat roots (Wang et al. 1986), the activity of the maize pump was only slightly decreased. Similar results were found with red beet roots (Rea and Poole 1985) and wheat seedlings (Maslowski and Maslowska 1987). In *Kalanchoë*, the inhibition at high PP_i concentrations was present only at high concentrations of $MgSO_4$ (3 and 6 mM) (Marquardt and Lüttge 1987).

Effect of different ionophores

The capacity of the H^+-PPase to form a ΔpH in presence of different ionophores was tested (Tab. 2). Nigericin (an electroneutral H^+-K^+ ionophore) and monensin (an electroneutral H^+-Na^+ ionophore) completely inhibited the formation of a proton gradient at 10 µM. Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) (two electrogenic H^+ ionophores) and gramicidin (an electrogenic channel-forming quasi-ionophore), only partly collapsed the proton gradient at 10 µM, arguing for a tight coupling between the transport of protons and the electroneutral symport of monovalent anions (Cl^-, NO_3^-) through an anion channel (Pope and Leigh 1987). Valinomycin (an electrogenic K^+ ionophore) increased the proton gradient formation at low concentrations (0.1–10 µM), whereas at 100 µM it was inhibitory (data not shown), perhaps due to a loss of ionophore specificity (Chanson and Taiz 1985). The membrane potential of the vesicles equilibrated with K^+ was more rapidly decreased in the presence of valinomycin. Rea and Poole (1985) found only a non-significant effect of valinomycin on PP_i-dependent quenching,

Tab. 2. Effect of different ionophores (at 10 µM) on the initial rate of PP_i-dependent fluorescence quenching of quinacrine. Membrane vesicles (400 µl, 10% sucrose in gradient buffer), $MgSO_4$ (3 mM), KNO_3 (50 mM) and quinacrine (5 µM) were added to an assay buffer of BTP-MES (25 mM, pH 7.5) to a final volume of 0.6 ml. The reaction was initiated by PP_i (0.1 mM). All the experiments were done in the presence of 1% ethanol. Control: 0.315% quenching (µg protein)$^{-1}$ min^{-1}.

Additions	% control
Control	100
Nigericin	0
Monensin	0
CCCP	47
FCCP	29
Gramicidin	44
Valinomycin	123
+CCCP	0
+FCCP	0
+Gramicidin	0

possibly explained by the small activity of their H^+-PPase.

When an electrogenic ionophore (CCCP, FCCP or gramicidin) was added to the medium in the presence of valinomycin (K^+ and H^+ could be exchanged without changing the $\Delta\psi$), ΔpH formation was completely prevented.

Effect of different inhibitors

Several inhibitors were tested on the activity of the H^+-PPase (Tab. 3). Vanadate (100 μM), a potent inhibitor of the H^+-ATPase of the plasmalemma, was without effect as reported previously (Chanson et al. 1985, Rea and Poole 1985, Wang et al. 1986, Macrì and Vianello 1987, Maslowski and Maslowska 1987, Shimmen and MacRobbie 1987b). The pump was not inhibited by oligomycin (5µg ml^{-1}) and only slightly by azide (1 mM) (two inhibitors of the F_1F_0-ATPase of the mitochondria) (Rea and Poole 1985, Wang et al. 1986, Maslowski and Maslowska 1987, Macrì and Vianello 1987). The pump was insensitive to molybdate (1 mM), an inhibitor of non-specific phosphatases (Walker and Leigh 1981, Rea and Poole 1985, Wang et al. 1986, Maslowski and Maslowska 1987). Surprisingly, the H^+-PPase from pea stem was inhibited 54% by 100 μM molybdate (Macrì and Vianello 1987). Imidodiphosphate, a PP_i analog, strongly inhibited the H^+-PPase (I_{50} 12 μM; Fig. 6; Chanson et al. 1985).

The effect of DES, on the activity of the pump, was difficult to evaluate. The inhibition was strong at 100 μM (Macrì and Vianello 1987), but took place only around 30 s after the beginning of the experiment (data not shown). Some quenching was observed just after the addition of the PP_i. Under these conditions, it was not possible to measure the initial rate of fluorescence and the total quench after 5 min (in %) was used (Tab. 3).

Tab. 3. Effect of different inhibitors on the initial rate of PP_i-dependent fluorescence quenching of quinacrine. Membrane vesicles (400 µl, 10% sucrose in gradient buffer), $MgSO_4$ (3 mM), KNO_3 (50 mM) and quinacrine (5 μM) were added to an assay buffer of BTP-MES (25 mM, pH 7.5) to a final volume of 0.6 ml. The reaction was initiated by PP_i (0.1 mM). Control: 0.334% quenching (µg protein)$^{-1}$ min^{-1}. The experiments with oligomycin and DES were done in the presence of 1% ethanol. Control: 0.315% quenching (µg protein)$^{-1}$ min^{-1}. [a]), % of total reversible quench after 5 min.

Additions		% control
Control		100
Vanadate	100 μM	100
Molybdate	1 mM	101
Oligomycin	5 µg ml^{-1}	98
Azide	1 mM	83
SITS	100μM	86
DIDS	100μM	58
DES[a]	100μM	18

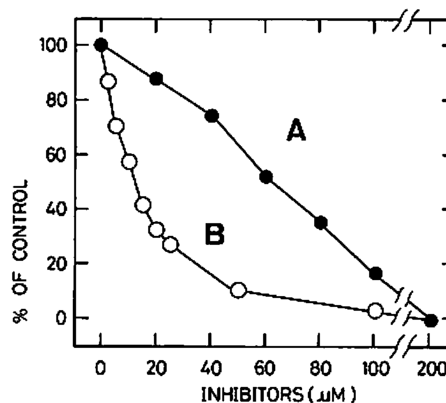

Fig. 6. Inhibition of the PP_i-dependent fluorescence quenching of quinacrine by DCCD (A) and imidodiphosphate (B), in the presence of 0.1 mM PP_i, 3 mM $MgSO_4$, 50 mM KNO_3 and 25 mM BTP-MES (pH 7.5).

4,4'-Diisothiocyanostilbene-2,2'-disulfonate (DIDS) and 4-acetamido-4'-isothiocyano-2,2'-stilbenesulfonic acid (SITS) (100 μM) only partly decreased the proton pumping activity. The literature concerning DIDS effects contains conflicting reports. In pea stems, the H^+-PPase was almost completely inhibited by 50 μM DIDS (Macrì and Vianello 1987), whereas only a slight inhibition was observed (at 10 μM) in oat roots (Wang et al. 1986). In maize roots (Hager et al. 1986) DIDS (at 50 μM) was without effect.

When the DTT was removed from the membrane fraction, DCCD, a proton channel blocker, was inhibiting the maize root H^+-PPase (I_{50} 65 μM; Fig. 6), in agreement with the results found with maize coleoptiles (Chanson et al. 1985) and pea stems (Macrì and Vianello 1987). In *Nitella* cells (Shimmen and MacRobbie 1987b) and oat roots (Wang et al. 1986), DCCD was active only at high concentrations (>100 μM). Moreover, in maize seedlings, the PPase inhibition due to 1 mM DCCD was only 15% (Maslowski and Maslowska 1987).

On the basis of these conflicting results, it is difficult to conclude that the H^+-PPase represents a new class of proton pump, insensitive to DCCD, as proposed by Wang et al. (1986). When DTT was present in the assay medium, the inhibition by DCCD was completely prevented (data not shown). It can not be excluded that traces of DTT were responsible for these conflicting results.

KF, assayed at different concentrations, inhibited the pump (I_{50} 1.1 mM; Fig.7), as previously reported (Wang et al. 1986, Maslowski and Maslowska 1987).

Effect of different growth factors

One of the main objective of the present study was to test several substances – controlling root growth processes – on the activity of the H^+-PPase.

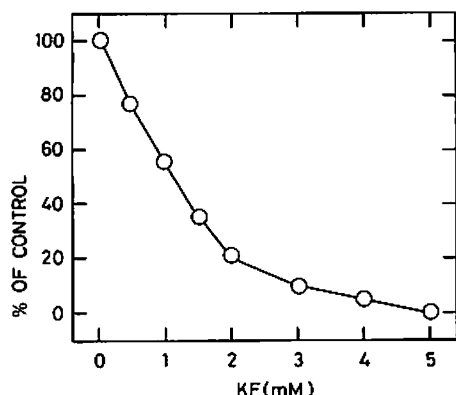

Fig. 7. Inhibition of the PP_i-dependent fluorescence quenching of quinacrine by KF, in the presence of 0.1 m*M* PP_i, 3 m*M* $MgSO_4$, 50 m*M* KNO_3 and 25 m*M* BTP-MES (pH 7.5).

FC stimulated the growth of maize roots (Pilet 1976, Gabella and Pilet 1979), whereas ABA and IAA were inhibitory (at the concentrations tested in this paper) (Pilet 1976, Pilet and Chanson 1981, Pilet and Saugy 1987). Zeatin was a strong root growth inhibitor in wheat, flax and cucumber (Stenlid 1982).

Different growth factors (IAA, ABA and zeatin) and FC were tested on the activity of the H^+-PPase in vitro. None of these substances were active at the concentrations tested (data not shown), arguing against a direct effect on the PP_i-dependent proton pump. In some future experiments, the maize roots will be pretreated with these different chemicals, before membrane fractionation and analysis of the activity of the H^+-PPase.

The physiological importance of the H^+-PPase has already been discussed (see Taiz 1986, Blumwald 1987, Rea and Sanders 1987). The presence of two different proton pumps on the tonoplast is puzzling and new experiments are needed to explain their respective roles in the plant cell metabolism. The complete characterization of the maize root H^+-PPase could be considered as an important step towards an understanding of its function.

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