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The action of divalent Zn, Cd, Hg, Cu and Pb ions on the ATPase activity of a plasma membrane fraction isolated from roots of *Zea mays*

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Abstract

The effects of the divalent metal ions Zn, Cd, Hg, Cu and Pb on the ATPase activity of a plasma membrane fraction isolated from roots of *Zea mays* have been investigated. When Mg-ions (3 m*M*), with or without K-ions (50 m*M*) are included in the reaction medium, inhibition of ATPase activity was found in all cases, the relative order of the inhibitors over the concentration range 10 to 100 μM, being Hg > > Cu ~ Cd > Zn ~ Pb. Below 1.0 μM only Hg caused substantial inhibition. In the absence of Mg ions, Zn and to a lesser extent Cd, activated the enzyme up to a concentration of 1 m*M*, activity being further stimulated in the presence of K-ions (50 m*M*). No activation of ATPase activity was found for Hg, Cu or Pb. It was concluded that Zn-ATP and Cd-ATP are both alternative substrates for the enzyme. Further experiments showed that both K_m and V_{max} for the substrates Zn-ATP and Cd-ATP are very much lower than for the usual substrate Mg-ATP.

These present results are discussed in relation to the known actions of these divalent cations on the trans-root potential and H-ion efflux in excised maize roots (Kennedy and Gonsalves, 1987).

Abbreviations. DTT, DL-dithiothreitol; EDTA, Ethylene diamine tetra-actetic acid, disodium salt; MES, 2-[N-morpholino] ethanesulphonic acid; TES, 2([-hydroxyl-1, 1-bis(hydroxymethylethyl)] amino) ethanesulphonic acid; TRIS, 2-amino- 2-(hydroxymethyl) 1, 3-propandiol; TRP, trans-root potential.

Introduction

The presence in the soil solution of high concentrations of the divalent metal ions Zn, Cd, Hg, Cu and Pb often gives rise to toxicity symptoms in plants (Woolhouse, 1983). Also some plants can act as accumulators for one or more of these ions (Marschner, 1983; Ure and Berrow, 1982). Whatever the ultimate site(s) of action, these ions must cross the plasma membranes of the root before reaching the symplast. As the transport systems within these membranes mediate nutrient uptake, it is pertinent to enquire whether these ions may interact with membrane components in such a way as to alter the acquisition of nutrients. The plasma membrane ATPase responsible for electrogenic pumping is a potential site of interaction of these metal ions. These interactions are reported and discussed here.

It has recently been reported that the cations Zn, Cd, Hg, Cu and Pb can affect trans-membrane and trans-root potentials and also H-ion efflux associated with excised roots of *Zea mays* (Kennedy and Gonsalves, 1987). Changes in H-ion efflux would be expected to alter the proton gradient across the root cell plasma membranes. The nature and extent of these effects vary considerably with the cation concerned and with its concentration in the solution bathing the roots. Hg, Cu and Pb-ions, and also Zn-ions, when uptake is not limited by the

presence of Mg in the bathing solutions, depolarise the TRP and inhibit H-ion efflux. However, lower concentrations of Cd (II) (10 μM) hyperpolarise the TRP as do Zn (II) ions when uptake is not limited by the presence of Mg in the solutions bathing the roots.

Depolarisation of the membrane potential difference across root plasma membranes would reduce the uptake of catonic nutrients, while reductions in both proton and potential gradients would inhibit the uptake of amino acids (Jung *et al.*, 1982; Kinraide and Etherton, 1980) and mono-saccharides (Kennedy and Stewart, 1982). Lowered proton gradients would also inhibit the uptake of phosphate (Bowling and Dunlop, 1978; Dunlop and Bowling, 1978). On the other hand, hyperpolarisation of membrane potentials, which is observed when Zn or Cd ions are present in the bathing solution under the conditions indicated above, would be expected to improve conditions for the uptake of some nutrients.

Proton and potential gradients across plant plasma membranes are maintained, at least in part, by an electrogenic proton pump mediated by a K-stimulated, Mg-activated ATPase in the membrane (Sze, 1983, 1984). Inhibition or stimulation of this enzyme by the range of metal ions examined here would therefore be expected to affect changes in both potential and proton gradients across root plasma membranes. In this paper the measured effects of these cations on maize root plasma membrane-bound ATPase activity are presented and compared with their reported action (Kennedy and Gonsalves, 1987) on membrane potential, TRP and H-ion efflux. This has allowed an assessment as to what extent their actions on this enzyme can account for the observed changes in potential and H-ion efflux.

The range of concentrations used here was chosen to overlap those found in soil solution with toxic concentrations of these metals. The concentrations of these cations will vary with pH and with the concentrations of the major cations in the soil solution. (Gerritse and Van Driel, 1984; Sanders and Adams, 1987), and also with soil/solution volume ratio (Sanders and Adams, 1987). In an analysis of a selection of 33 soils from the Netherlands, Great Britain and France, Gerritse and Van Driel (1984) found soil water concentrations as high as 34 μM Zn, 0·70 μM Cd, 0.40 μM Pb and 10 μM Cu when extracted with water and 230 μM Zn, 15 μM Cd, 0.33 μM Pb and 5.7 μM Cu for the same soil samples when the extraction medium included $CaCl_2$ (0.015 M), NaCl (0.01 M) and KCl (0.01 M). Heavy metals in soil solution from sewage sludge-treated soils also tend to be high *e.g.* 210 μM Zn and 9.4 μM Cu in a soil extracted with 0.01 M $CaCl_2$ at pH 5.5. (Sanders and Adams, 1987). Also, concentrations of 16.5 μM Pb, 110 μM Zn, 7.4 μM Cu and 1.2 μM Cd have been found in soil solutions from roadside extracts in a minor agricultural area (Wong and Lau, 1983).

Reports of soil water concentrations of Hg are rare, but the high total concentrations found in some areas, *e.g.* up to 260 mg kg^{-1} Hg near a mercury mine in Spain (Lindberg, *et al.*, 1979), suggest they can be quite high. In view of the above evidence, the concentrations of divalent metal ions used were from 0.1 to 500 μM for Cu, Pb and Hg and 1.0 to 500 μM for Zn and Cd.

Materials and methods

Plant material

Zea mays (Vars: Pioneer 131 and Fronica) seeds were washed for 2 h in running tap water and then with de-ionised water before germination.

Plasma membrane isolation

A sucrose density centrifugation procedure was chosen in order to separate the plasma membrane fraction as this has been reported to yield inside out or unsealed vesicles, thus facilitating ATPase assays. Partitioning techniques using aqueous two-phase systems would appear to yield mainly right-side out vesicles (Larsson *et al.*, 1984) which would be less appropriate for this investigation.

The method was adapted from Leonard and Hotchkiss (1976). 250 g of seeds were germinated on a plastic mesh resting on a glass tank (13 dm^3) containing $CaSO_4$ (1.0 mM) solution. Paper towels kept the seeds moist during germination, 2d, and the seedlings were then grown a further 5d in the aerated $CaSO_4$ solution, in the dark at 25 °C. Solutions were changed every two days. Roots were

harvested by cutting them directly from the mesh into de-ionised water for surface washing.

120 cm^3 of ice cold homogenising medium (0.25 *M* sucrose, 25 m*M* TES, 1.0 m*M* DTT, 4.0 m*M* EDTA at pH 7.9) was added to 40 g of washed roots in a chilled mortar. The roots, 10–12 cm in length, were cut into 1 cm segments and homogenised for 10 min. with a pestle to a pulpy mass. The pale yellow homogenate was filtered through muslin and centrifuged at 13,000 × *g* for 15 min. All extraction procedures were at 4 °C. The supernatant was centrifuged at 80,000 × *g* for 30 min. and the pellet retained. Initially, extraction procedures included a pellet wash, the pellet being resuspended in a solution containing sucrose (0.25 *M*) and TES (1.0 m*M*) at pH 7.5, and then re-centrifuged at 80,000 × *g* for a further 30 min. Only a marginal gain in specific activity was obtained by re-washing and this step was therefore omitted.

The pellet was re-suspended in 1 cm^3 of buffered solution (0.25 *M* sucrose and 1.0 m*M* TES) and 0.5 cm^3 of this layered onto a two step discontinuous sucrose gradient (6.22 cm^3 of 45% sucrose and 1.78 cm^3 of 34% sucrose). These solutions were centrifuged at 80,000 × g for 2 h. Three regions of material were evident, a pellet, a layer at the 34%–45% interface and a slight streak of material on the wall between pellet and interface. The band at the interface was removed with a 'U' tipped Pasteur pipette to give 2–4 cm^3 of solution. Dilutions of this fraction were made with TRIS-MES buffer (1 m*M*, pH 7.5) containing DTT (1.0 m*M*).

Measurement of ATPase activity

The assay was adapted from Hodges and Leonard (1972). ATPase activity was determined from the release of inorganic phosphate (Fiske and Subbarow, 1925). The assay mixture consisted of 1.5 cm^3 of a solution containing TRIS-MES (30 m*M*) and $MgSO_4$ (3 m*M*) at pH 6.0, 0.1 cm^3 of KCl solution (1.0 *M*), when required, and 0.2 cm^3 of the diluted membrane fraction containing between 10 and 50 μg of protein. Volume changes due to addition of test cation solutions were compensated by varying the volume of TRIS-MES buffer. The reaction was started by the addition of 0.2 cm^3 of ATP-TRIS salt (30 m*M*) to give a final concentration of 3.0 m*M* ATP. The reaction temperature was 38 °C. 4 cm^3 of ice-cold 1% ammonium molybdate in H_2SO_4 (1 *M*) was added to stop the reaction, followed immediately by 0.8 cm^3 of the reducing agent, 1-amino-2-napthol-4-sulphonic acid (previously prepared, filtered and stored in the dark). The absorbance of solutions was determined at 660 nm.

Protein was determined (Lowry *et al.*, 1951) after dilution of the medium to reduce sucrose interference (Gerhardt and Beevers, 1968).

The effects of the various cations on enzyme activity were carried out by addition of the cation solution to the complete assay mixture before addition of the substrate. Experiments were also performed by pre-incubating the enzyme preparation for 15 minutes in the presence of the added divalent cation before addition of the substrate. However there was no significant difference in the levels of inhibition observed.

Results

ATPase activity of the plasma membrane fraction

Membrane fractions showed similar levels of ATPase activity, both with and without added KCl, to those recorded elsewhere (Leonard and Hotchkiss, 1976; Zocchi and Hanson, 1983). As the variety of maize used initially (Pioneer 131) became unavailable it was necessary to change to another (Fronica). The ATPase activities and behaviour towards added cations were very similar. However, a slight but reproducible difference in pH optimum was observed, being 6.0 for Pionneer 131 and 6.2–6.3 for Fronica, both obtained in the presence of added KCl (50 m*M*). Therefore, as the assay conditions were identical there may be slight varietal variants of this enzyme. Both values are similar to those found previously (Beffagna *et al.*, 1979; Leonard and Hotchkiss, 1976).

In the absence of Mg and K ions, a small but reproductible maximum in activity was observed at pH 8, and a further one at or below pH 5 (Fig. 1; A, d). However, in the presence of $MgSO_4$ (3 m*M*), the ATPase activity had characteristics consistent with those reported for the plasma membrane enzyme and different from those in the tonoplast (DuPont *et al.*, 1982; Churchill and Sze, 1984). This was

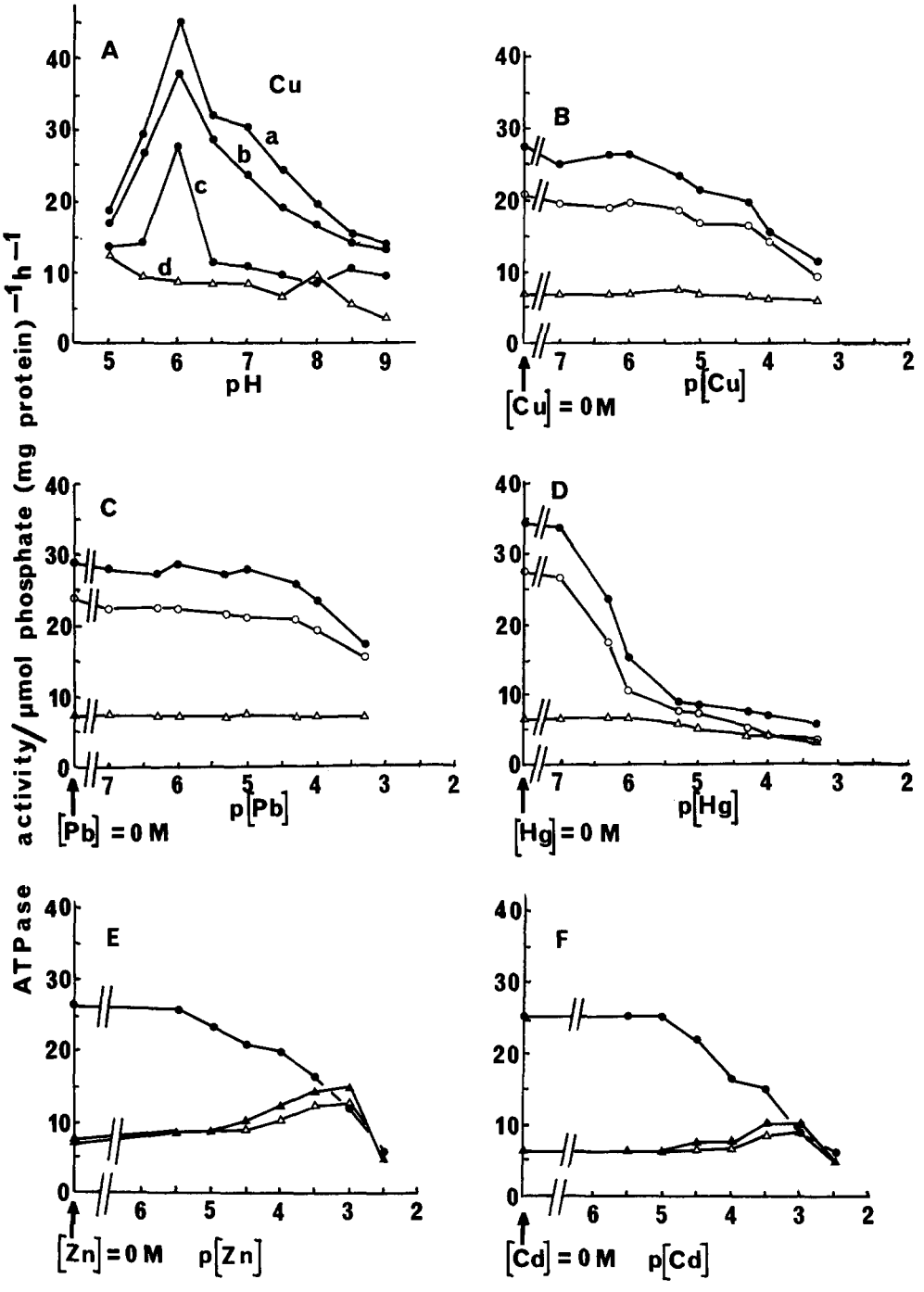

Fig. 1. Action of divalent cations on plasma membrane-bound ATPase activity. Assay protocol as in Materials and Methods. **1A**: assay solutions for (**a**), (**b**) and (**c**) contained $MgSO_4$ (3.0 m*M*), KCl (50 m*M*) and 5, 50 and 500 μM Cu respectively. Solution (**d**) contained no added $MgSO_4$, KCl nor Cu. **1B, 1C, 1D, 1E** and **1F**: ●, $MgSO_4$ (3 m*M*) and KCl (50 m*M*) included in the assay solution; ○, $MgSO_4$ (3 m*M*) included in the assay solution; ▲, KCl (50 m*M*) included in the assay solution; △, no $MgSO_4$ nor KCl added.

shown by (a), identical stimulation of activity by KCl (50 m*M*) and K_2SO_4 (25 m*M*) and (b) only slight (12%) inhibition when KCl was replaced by KNO_3 compared to over 60% found for tonoplast ATPase from corn roots (DuPont *et al.*, 1982).

K, Mg-ATPase activity was unaffected by oubain (100 μM) and by the mitochondrial ATPase inhibitor oligomycin (100 μM). DCCD (100 μM) reduced activity by 20 to 30 percent: both plasma membrane and tonoplast ATPase activity are known to be inhibited at this concentration (Sze, 1984).

Apparent K_m and V_{max} constants were calculated using Hanes plots. The mean values and standard deviations from 5 experiments obtained in the presence of KCl (50 m*M*) in reaction media at pH 6.0 were $K_m = 1094 \pm 102\,\mu M$ Mg.ATP and $V_{max} = 52.4 \pm 18.5$ μmol phosphate (mg protein)$^{-1}$ h^{-1}. These constants were obtained for substrate concentrations up to or just above 3 m*M* Mg.ATP.

Effects of the divalent cations of Zn, Cd, Hg, Cu and Pb on ATPase activity

In the presence of $MgSO_4$ (3.0 m*M*) all these cations inhibited ATPase activity to different extents both with and without added KCl (50 m*M*) (Fig. 1). In the presence of Mg and K ions and for heavy metal ion concentrations between 10 and 100 μM, the order of decreasing inhibitions was Hg > > Cd ~ Cu > Zn ~ Pb. Only Hg inhibited the enzyme at concentrations of 0.5 and 1.0 μM to a significant extent (Fig. 1).

In the absence of Mg, Zn and, to a lesser extent Cd, activated the enzyme, the induced activity being greater in the presence of 50 m*M* KCl (Fig. 1E and F). This was not evident for Cu, Pb, or Hg (Fig. 1B, C and D). For Zn and Cd the highest concentration employed (3.0 m*M*) was inhibitory, maximum activation occurring from 0.3 to 1.0 m*M* (Fig. 1E and F).

Pb had little effect on the residual ATPase activity in the absence of Mg ions, Cu was slightly inhibitory, and only Hg inhibited to any extent (fig. 1C, B and D). The resistance to high concentrations of Hg implies that there are no catalytically important sulphydryl groups associated with this residual activity, which could suggest the presence of an acid phosphatase at this pH (Dixon and Webb, 1979a).

The inhibitory actions of Cu (Fig. 1A) and Cd (not shown) were studied over a pH range from 5 to 9. In both cases, although the activity at the pH optimum was depressed, there was also significant suppression of the shoulder around pH 7.

Apparent K_m and V_{max} constants for the activation of ATPase activity by Zn and Cd

Apparent K_m and V_{max} values (Table 1) were estimated for the activation of ATPase activity above that in the absence of added divalent ions, using both Hanes and Eadie-Hofstee transforms of the data. The ATP concentration in all experiments was 3.0 m*M*, while Zn or Cd ion concentrations were increased from zero to 0.5 m*M*, except in one experiment (Zn, no K) where the highest concentrations employed was 1.0 m*M*. Higher concentrations of Zn or Cd were not used here to avoid complications due to apparent substrate inhibition (Fig. 1; E and F). Only one experiment with Cd had activities sufficiently far above basal level to allow a reasonable estimate of K_m and V_{max}, although all experiments (4) clearly showed activation.

Discussion and conclusions

Zinc and cadmium

Effects on ATPase activity. Of the five metal ions examined only Zn and Cd are able to activate the membrane ATPase in a similar manner to Mg. As with Mg, the additional presence of K-ions (50 m*M*) stimulates further activity. Both metals become inhibitory at a concentration of 3 m*M*. These results for Zn are in agreement with previous work, 1.5 m*M* Zn causing activation of ATPase activity in oat root plasma membrane fractions (Leonard and Hodges, 1973) while inhibiting activity at 3.0 m*M* in maize root membranes (Leonard and Hotchkiss, 1976). It would appear, therefore, that Zn-ATP and Cd-ATP can act as alternative substrates for this enzyme.

Both Zn-ATP and Cd-ATP have much lower K_m and V_{max} values (Table 1) than those of the usual

Table 1. K_m and V_{max} for ATP-Zn and ATP-Cd as substrates for the plasma membrane- bound ATPase, in the absence of Mg. Estimated from Hanes Plots

Divalent cation	K mM	K_m μM	V_{max}	Number of experiments
Zn	0	137(35)	8.5(2.6)	4
Zn	50	115	7.4	1
Cd	0	32	4.2	1

The figures in parentheses are standard deviations of the constants from 4 experiments. Assay conditions as in Materials and Methods. V_{max} in μmol phosphate (mg protein)$^{-1}$h^{-1}.4 or 5 concentration values were used in each experiment. The ATP concentration in all experiments was 3.0 m*M*, while Zn or Cd ion concentrations were increased from zero to 0.5 m*M*, except for one experiment (Zn, no K) where the highest concentration was 1.0 m*M*.

substrate Mg-ATP (K_m, 1094 μM; V_{max}, 52.4 μmol P_i (mg protein)$^{-1}$ h^{-1}), measured in the presence of 50 m*M* KCl. Using a Mg-ATP concentration of 3.0 m*M*, and the K_m and V_{max} values found here, in the equation for the total velocity for an enzyme reaction with two competing substrates (Dixon and Webb, 1979b), it can be shown that both Zn-ATP and Cd-ATP at concentrations of 0.5 m*M* will reduce the total velocity of the reaction by a significant amount. Thus at least a part of the inhibition due to Zn or Cd ions (Fig. 1E and F) is likely to be due to this factor. The values obtained for K_m and V_{max} using Mg-ATP as substate are very similar to those found by other workers for maize root plasma membrane (Briskin and Leonard, 1982, DuPont et al., 1981; Leonard and Hotchkiss, 1976). A similar method to that employed here for Zn and Cd in which ATP (3.00 m*M*) and KCl (50 m*M*) concentrations were held constant while increasing the divalent metal ion concentration, gave K_m and V_{max} as 720 μM and 69.4 μmol P_i (mg protein)$^{-1}$ h^{-1} respectively for an oat root plasma membrane fraction with Mg as the divalent cations. In the absence of KCl the K_m and V_{max} were 840 μM and 38.6 μmol P_i (mg protein)$^{-1}$ h^{-1} (Leonard and Hodges, 1973). These authors found that when the Mg concentration was maintained at 3.0 m*M*, while increasing the ATP concentration, the V_{max} values were very similar to those experiments where the ATP concentration was held at 3.0 m*M* but where the Mg-ATP concentration was varied by changing the Mg-ion concentration. However in this latter case the K_m value obtained, whether or not KCl was present, was 380 μM. This suggests that the method employed here would give rather higher values of K_m but similar V_{max} values to experiments where the ATP concentration is varied while keeping the Zn or Cd concentration constant. Therefore the major inference that both K_m and V_{max} are substantially less than for the substrate Mg-ATP is upheld.

Comparison with actions on TRP and H-ion efflux. As Mg-ATP is present in the cytoplasm in vivo, its concentration at the enzyme site could determine whether or not Zn (Cd)-ions will further activate or inhibit activity. This concentration is not known and would be difficult to determine. Nevertheless if further acivation by Zn (Cd)-ATP occurred, it would be expected to increase electrogenic proton pumping, with a concomitant hyperpolarisation of membrane potential. However, Zn or Cd-ions could also change membrane potential in other ways, *e.g.* by affecting the ion permeabilities of the plasma membrane. For Zn, the maximum percentage inhibitions of H-ion efflux and the maximum depolarisation of TRP (Kennedy and Gonsalves, 1987) measured in the absence of exogenous Mg in the bathing solution, are significantly greater than the inhibition of enzyme activity at the same concentrations (Table 2), suggesting that the depolarisation is not mediated by inhibition of membrane ATPase activity. However, when Zn-ion uptake is limited by competitive inhibition by the presence of Mg in the bathing solution (Giordano *et al.*, 1974), depolarisation is prevented and a rapid but persistent hyperpolarisation of the TRP is induced instead (Kennedy and Gonsalves, 1987). Lower concentrations (10 μM) of the more slowly penetrating Cd-ion induce hyperpolarisation of the TRP even in the absence of Mg in the solution bathing the roots, but in the presence of Mg, these hyperpolarisations are more sustained and also occur at higher concentrations (Kennedy and Gonsalves, 1987). In contrast Hg, Cu or Pb-ions, which do not activate ATPase activity in the absence of

Table 2. Comparison of the percentage inhibitions of ATPase activity[a] with the maximum percentage inhibitions of H-ion efflux[b] and depolarisations of trans-root potential (TRP)[b] caused by additions of Zn, Hg and Cu ions

Concentration (μM)	Percentage ATPase activity[c]	Inhibition H-ion efflux	Depolarisation of TRP (mV)
Zinc			
0.1	n.d	n.d	n.d
1.0	1	n.d	n.d
5.0	3	37	13
10.0	6	56	11
50.0	15	63	23
100.0	21	60	35
500.0	42	n.d	n.d
Mercury			
0.1	3	n.d	10
1.0	55	n.d	27
5.0	73	62	42
10.0	74	78	41
50.0	76	83	41
100.0	79	77	57
500.0	82	n.d	n.d
Copper			
0.1	n.d	n.d	n.d
1.0	−3	n.d	7
5.0	5	90	23
10.0	8	97	32
50.0	34	100	37
100.0	52	100	37
500.0	56	n.d	n.d

[a] Measured in presence of $MgSO_4$ (3 mM) and KCl (50 mM).
[b] Bathing solution around roots contained KCl (1.0 mM) and $CaSO_4$ (0.1 mM), (Kennedy and Gonsalves, 1987).
[c] 32 percent for 0.5 μM Hg.
n.d. not done

Mg, show no tendency to hyperpolarise the TRP whether or not Mg-ions (0.1 or 1.0 mM) are present in the bathing solution. The hyperpolarisations induced by Zn and Cd may be caused by stimulation of the electrogenic proton pump mediated by the membrane ATPase. The crucial factor would appear to be the relative concentrations of Zn (Cd)-ATP and Mg-ATP at the enzyme site. It is pertinent to note that further activation of the membrane ATPase by Zn or Cd ions, with its associated increase in trans-membrane potential due to the stimulation of electrogenic proton pumping, would be beneficial for the uptake of cationic nutrients.

Mercury

Hg (II) is by far the strongest inhibitor of the membrane ATPase (Fig. 1). Plant plasma membrane ATPases are known to be inhibited by sulphydryl agents such as p-chloromercuribenzenesulphonic acid, mersalyl and 5, 5-dithiobis (2-nitrobenzoic) acid (Beffagna *et al.*, 1979). However, the inhibitory effect could be attributable to other causes, as Hg (II) is also known to bind strongly to O and N ligands.

For Hg concentrations above 5 μM inhibitions of TRP and H-ion efflux (Kennedy and Gonsalves, 1987) are similar in magnitude and occur over the same concentration range as do the inhibitions of ATPase activity (Table 2). However, Hg(II) ions are known to inhibit TRP and H-ion efflux at least down to 10 and 30 nM respectively (Kennedy and Gonsalves, 1987), whereas at 100 nM the ATPase activity is hardly affected (fig. 1D). Thus, although inhibition of the membrane ATPase is likely to be a contributing factor in the observed reductions of TRP and H-ion efflux, these results suggest that, even at these low concentrations, Hg ions may affect the plasma membrane in other ways. The much slower action of p-chloromercuribenzenesulphonic acid, a sulphydryl reagent of much lower membrane permeability than the Hg (II)-ion, indicates that the site of action for Hg is not on the membrane surface (Kennedy and Gonsalves, 1987).

Copper

The inhibitions of ATPase activity, measured in the presence of Mg (3 mM) and K (50 mM) ions, are of similar magnitude to those induced by Cd-ions (Fig. 1). However the effect of Cd-ions on TRP and H-ion efflux are of much lower magnitude, and the rate at which these effects become apparent much slower, suggesting a higher rate of penetration for Cu-ions (Kennedy and Gonsalves, 1987). On comparing the extents of the inhibitions induced by Cu-ions on the membrane ATPase with their action on TRP and H-ion efflux (Table 2), it is clear that the effects of membrane properties are greater than could be accounted for by inhibition of the membrane ATPase, implying a more potent mode of action. The leakiness of K-ions induced by

a Cu-ion concentration as low as 10 μM (Wainwright and Woolhouse, 1977) is likely to be a major cause of the membrane effects observed, rather than inhibition of membrane ATPase activity.

Lead

The percentage inhibition of ATPase activity by Pb, when measured in the presence of $MgSO_4$ (3 mM) and KCl (50 mM) is similar to that for Zn (Fig. 1), but there is no significant increase in activity in the absence of Mg (Fig. 1) indicating that, like Cu and Hg, but unlike Zn and Cd, Pb-ATP is not a substrate for the membrane ATPase.

Pb (II)-ions have a very slow action on both TRP and H-ion efflux from maize roots, indicating poor access to the site(s) of action. It is possible that the depolarisations of TRP and inhibition of H-ion efflux (Kennedy and Gonsalves, 1987) could be due to inhibition of the membrane ATPase, but the slow rates at which the inhibitions of TRP and H-ion efflux develop makes comparisons difficult.

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