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 mM), with or without K-ions (50 mM) 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 mM, activity being further stimulated in the presence of K-ions (50 mM). 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-acetic acid, disodium salt; MES, 2-[N-morpholino] ethanesulphonic acid; TES, 2(3-hydroxyl-1,1-bis(hydroxymethylene)] 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 \( \mu M \)) 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 \( \mu M \) Zn, 0-70 \( \mu M \) Cd, 0.40 \( \mu M \) Pb and 10 \( \mu M \) Cu when extracted with water and 230 \( \mu M \) Zn, 15 \( \mu M \) Cd, 0.33 \( \mu M \) Pb and 5.7 \( \mu M \) 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 \( \mu M \) Zn and 9.4 \( \mu M \) Cu in a soil extracted with 0.01 M CaCl\(_2\) at pH 5.5. (Sanders and Adams, 1987). Also, concentrations of 16.5 \( \mu M \) Pb, 110 \( \mu M \) Zn, 7.4 \( \mu M \) Cu and 1.2 \( \mu M \) 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 \( \mu M \) for Cu, Pb and Hg and 1.0 to 500 \( \mu M \) 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, 2 d, and the seedlings were then grown a further 5 d in the aerated CaSO\(_4\) solution, in the dark at 25°C. Solutions were changed every two days. Roots were