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Sorption of Copper and Zinc to the Plasma Membrane of Wheat Root

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Abstract. Sorption of Cu^{2+} and Zn^{2+} to the plasma membrane (PM) of wheat root (*Triticum aestivum* L cv. Scout 66) vesicles was measured at different pH values and in the presence of organic acids and other metals. The results were analyzed using a Gouy-Chapman-Stem model for competitive sorption (binding and electrostatic attraction) to a negative binding site. The binding constants for the two investigated cations as evaluated from the sorption experiments were 5 M^{-1} for Zn^{2+} and 400 M^{-1} for Cu^{2+}. Thus, the sorption affinity of Cu^{2+} to the PM is considerably larger than that of Ca^{2+}, Mg^{2+} or Zn^{2+}. The greater binding affinity of Cu^{2+} was confirmed by experiments in which competition with La^{3+} for sorption sites was followed. The amount of sorbed Cu^{2+} decreased with increasing K^+, Ca^{2+}, or La^{3+} concentrations, suggesting that all these cations competed with Cu^{2+} for sorption at the PM binding sites, albeit with considerable differences among these cations in effectiveness as competitors with Cu^{2+}. The sorption of Cu^{2+} and Zn^{2+} to the PM decreased in the presence of citric acid or malic acid. Citric acid (as well as pH) affected the sorption of Cu^{2+} or Zn^{2+} to PM more strongly then did malic acid.

Key words: Copper — Zinc — Plasma membrane — Root — Organic acid — Sorption

Introduction

Copper and zinc are essential elements for higher plants, but in excess they can be toxic. Excessive amounts of Cu^{2+} and Zn^{2+} inhibit root and shoot growth, retard developmental processes and reduce crop yield (Galli, Schuepp & Brunold, 1996; Edds & Kochian, 1997). The fact that Ca^{2+} alleviates the toxicity of Al^{3+}, H^+ and Na^+ (Yermiyahu et al., 1997*b*; Kinraide, 1998), Ni^{2+} (Gabrielli & Pandolfini, 1984), Pb^{2+} (Jowett, 1964), and Mn^{2+} (Robson & Loneragan, 1970) as well as Cu^{2+} and Zn^{2+} (Parker et al., 1998; Pedler, Kinraide & Parker, 2004) suggests that these cations may compete with Ca^{2+} for cell-surface binding sites (Campbell, 1995).

Calcium plays a role in maintaining the structural soundness of cell membranes and is essential for preventing excessive leakage (Clarkson & Hanson, 1980). Excess Cu^{2+} and Zn^{2+} affect adversely plasma membrane (PM) permeability and integrity (Murphy et al., 1999; Berglund et al., 2002). Thus, alleviation of Cu^{2+} and Zn^{2+} toxicity by Ca^{2+} addition may be, at least in part, through the restoration of Ca^{2+} at the cell surface. Since cations other than Ca^{2+}, such as Mg^{2+} and H^+, also alleviate Cu^{2+} and Zn^{2+} toxicities (Kinraide, Pedler & Parker, 2004; Pedler et al., 2004; Parker et al., 1998), alleviation is not entirely due to a specific effect of Ca^{2+} addition, but may result from the displacement (e.g., by exchange) of toxic cations. Recently, Silva et al. (2001) and Pedler et al. (2004) noted large differences between the ameliorative effectiveness of Ca^{2+} and of Mg^{2+}. Kinraide et al. (2004) compared the ameliorative effects of Ca^{2+} and Mg^{2+} on Al^{3+}, Cu^{2+}, Zn^{2+}, Na^+ and H^+ rhizotoxicity and proposed three mechanisms of alleviation.

Most biomembranes are negatively charged, mainly due to the presence of acidic phospholipids, but also of other membrane components such as proteins (Møller, Lundborg & Bérczi, 1984). Studies with PM vesicles or protoplasts have provided mutually consistent results for cation interactions with

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biomembranes. The techniques used in these studies include surface potential measurements by electrophoresis (ζ-potential measurements), fluorescent dye accumulation (Chow & Barber, 1980; Møller et al., 1984; Obi et al., 1989; Brauer et al., 2000), and cation sorption (Yermiyahu et al., 1994, 1997*a*,*c*). In most of the studies the Gouy-Chapman-Stern model, which takes into account both electrostatic interactions and specific binding, was invoked to explain the sorption of cations to the PM.

The Gouy-Chapman contribution to the model is based on the electrostatic theory that relates surface charge density (σ) to surface electrical potential (ψ_0) and ion concentrations in the bulk phase in which the surface is bathed (Tatulian, 1999). Because σ is altered by solute ion binding to membrane surfaces, occupation of surface sites by ions, as described by the Stem model, has to be taken into consideration and is commonly expressed with mass action equations. Each such equation has a characteristic binding constant. Knowledge of the binding parameters allows the computation of σ, provided σ_0 (the σ in the absence of any solute binding) is known. For artificial membranes, there are, characteristically, few types of binding sites, but for PMs there are numerous types of such sites. Yet, it is found that overall ion-membrane interactions can be modeled reasonably by assuming that the PM contains only two representative types of binding sites, negatively charged and neutral (Kinraide, Yermiyahu & Rytwo, 1998).

Binding constants for Al^{3+}, La^{3+}, Ca^{2+}, Mg^{2+}, Na^+, K^+, and H^+ for the investigated PMs were determined using sorption data, or estimated on the basis of published results (Yermiyahu et al., 1994; 1997*c*). These experimental values of the binding constants have been substantially confirmed by testing their compatibility with published ζ-potential measurements of PM vesicles and protoplasts extracted from several plant species and tissues (Kinraide et al., 1998). Values of the binding constants of Cu^{2+} and Zn^{2+} for biomembranes as well as for artificial phospholipid membranes are, however, lacking (Tatulian, 1999).

Knowledge of the model parameters allows for the computation of metal activities at the PM surface. These activities, rather than those in the bulk-phase, determine the toxic (or ameliorative) effects of the metals. The application of the Gouy-Chapman-Stem model for the computation of ψ_0, the extent of ion binding and the activity of ions at the PM surface, has been used successfully for the interpretation of plant-mineral interactions (Yermiyahu et al., 1997*b*; Kinraide, 1998; Zhang et al., 2001; Kinraide 2003).

The rhizosphere is rich in organic solutes and in particular in organic acids, prominent among which are polycarboxylic species, such as citric and malic acids (Ryan & Delhaize, 2001). These acids are efficient ligands for metal cations and hence are likely to strongly affect the interaction of the cations with root membranes. The effect of addition of such acids to the PM-metal reaction mixtures was therefore examined.

In the present study, Cu^{2+} and Zn^{2+} sorption to the PM of wheat root vesicles was measured at different pH values and in the presence of organic acids and other metals. The results were analyzed using a competitive sorption model previously used in studies of other cations (Nir, 1984; Yermiyahu et al., 1994, 1997*c*), and the binding constants of Cu^{2+} and Zn^{2+} were determined.

Materials and Methods

Plant Material and Isolation of Plasma Membrane Vesicles

Caryopses of wheat (*Triticum aestivum* L cv. Scout 66) were surface-sterilized with 1% NaOCl for 5 min and thoroughly rinsed with distilled water. The seeds were then placed between sheets of germination paper kept saturated with 1 mM $CaCl_2$ and held on glass plates placed in a slanted position inside a covered container in an incubator at 25 °C. After 4 d the primary roots were 8–10 cm long.

Right-side-out PM vesicles were isolated from whole roots using the procedure of Larsson, Widell and Sommarin (1988) with slight modifications. The final wash solution was adjusted to pH 7.2 with 1 M KOH and included 0.25 M sucrose and 5 mM KCl. The vesicles were kept at 2 °C overnight as a pellet, washed again with the above solution and finally resuspended to yield a protein concentration of 1 mg mL^{-1}. Protein concentration was determined using BSA as a protein standard (Bradford 1979). The pH of the vesicle suspension was 6.0 ± 0.2, and in each experiment some of the final wash solution was adjusted with HCl to the same pH as the vesicle suspension and used as a vesicle-free control.

Sorption Experiments

A detailed description of the sorption procedure was given by Yermiyahu et al. (1997*c*). In these experiments, a known quantity of the isolated PM vesicles was added to isotonic sucrose solutions adjusted for initial ion concentration and pH. After equilibrium was achieved (≈30 min), the pH of the suspensions was measured (henceforth denoted 'actual pH'), and then the PM vesicles were separated from the equilibrium solution using microfilter centrifuge tubes. The amount of a given ion sorbed to the vesicles was calculated from the reduction in ion concentration in the equilibrium solution (Yermiyahu et al., 1997*c*). In all the sorption runs the reaction solution contained 50 ± 2 μg protein in a reaction volume of 500 μl. Sorption experiments, which were designed to evaluate the binding constant of Cu^{2+}, were performed in five initial concentrations of the ion (0, 20, 35, 60 and 100 μM) at two pH values (4.0 and 5.2), while the Zn^{2+} sorption experiments were performed in six initial concentrations (0, 25, 50, 100, 200 and 300 μM) at two pH values (4.5 and 5.0).

La^{3+} was used as the competing cation in the experiments to compare the affinities of divalent cation for the root PM. La^{3+} sorption was determined in the presence of Zn^{2+}, Mg^{2+}, Ca^{2+} and Cu^{2+}. The La^{3+} concentration in the reaction solution was 21.9 μM, the divalent cation concentrations were 100 and 500 μM, and the pH was 5.0. The competitive sorption of Cu^{2+} was also tested

in the presence of other cations at a Cu^{2+} concentration of 50 μM and three concentrations of K^+ (12.5, 50 and 100 mM), Ca^{2+} (0.25, 0.5 and 1.0 mM) or La^{3+} (4, 8 and 20 μM). The treatment pH was 5.2.

Finally, the sorption of Cu^{2+} in the presence of organic acids was tested at a Cu^{2+} concentration of 100 μM and two concentrations of citric acid (60 and 300 μM) or malic acid (200 and 1500 μM) at two treatment pH values (4.5 and 5.5). Zn^{2+} sorption was examined at a Zn^{2+} concentration of 100 μM and two concentrations of citric acid (100 and 500 μM) and malic acid (1000 and 3000 μM) at a pH value of 5.5. The concentrations of the acids were chosen so as to yield a similar range of free metal concentrations in the various acid-metal systems. The equilibrium concentrations of the various species in those systems were computed by the GEOCHEM-PC program (Parker, Norvell & Chaney, 1995).

In all the sorption experiments, the cations were added as their Cl^- salts. Final Cu^{2+}, Zn^{2+}, and La^{3+} concentrations in the bulk solution were determined by inductively coupled plasma emission spectrometry ([ICP] Spectro Ciros CCD) and the amounts of Cu^{2+}, Zn^{2+} and La^{3+} sorbed to the root PM vesicles were calculated from the difference between the metal concentrations in the corresponding vesicle and vesicle-free bulk solutions. Triplicate measurements were made for each treatment and each sorption experiment was done with at least two PM isolates.

Evaluation of Binding Constants

Binding constants for Cu^{2+} and Zn^{2+} sorption to the PM were determined from the data obtained in the sorption experiments. These constants were evaluated by employing a previously described procedure (Yermiyahu et al, 1991*c*) while taking into account the hydrolysis constants of the metals. The hydrolysis constants were $\log K_1 = -7.70$ and $\log K_2 = -13.78$ for Cu^{2+}, and $\log K_1 = -7.69$ and $\log K_2 = -16.80$ for Zn^{2+} (Lindsay, 1979).

Abbreviations

ψ_0, surface electrical potential; σ, surface charge density; σ_0, the σ in the absence of any solute binding; pH_{Eq}, equilibrium pH; $[I^Z]_{Eq}$, concentration of ion *I* with charge *Z* in the bulk-phase medium at equilibrium; K_I, equilibrium constant for binding of ion *I* at a negative site on the plasma membrane; PM, plasma membrane; *RMSE*, root mean square error.

Results

Sorption of Cu^{2+} and Zn^{2+} to PM Vesicles

The amount of Cu^{2+} sorbed to PM vesicle as a function of $[Cu^{2+}]_{Eq}$ at two treatment pH values is presented in Fig. 1*A*. The amount of sorbed Cu^{2+} increased with increasing $[Cu^{2+}]_{Eq}$ and with increasing treatment pH (in the range of 4.0 to 5.2). The term 'treatment pH' refers to the pH in the solution before vesicles were added. The actual pH in the equilibrium solution ($[pH]_{Eq}$) as a function of $[Cu^{2+}]_{Eq}$ is presented in Fig. 1*B*. $[pH]_{Eq}$ decreased with increasing $[Cu^{2+}]_{Eq}$. This occurs because of the displacement of bound H^+. The amount of Zn^{2+} sorbed to the PM vesicles and $[pH]_{Eq}$ as a function of $[Zn^{2+}]_{Eq}$ at two treatment pH values are presented in Fig. 2*A* and 2*B*, respectively. Similarly to Cu^{2+}, the amount of Zn^{2+} sorbed in-

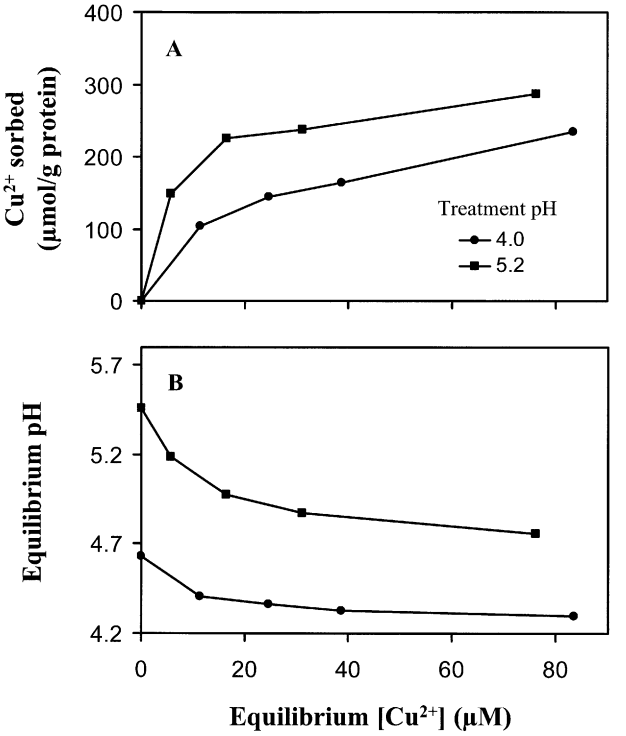

Fig. 1. Cu^{2+} sorbed to PM vesicles (*A*) and equilibrium pH of the vesicle suspension (*B*) as a function of equilibrium Cu^{2+} concentration in the bulk phase. Treatment pH refers to the pH of the solution before the addition of the PM vesicles.

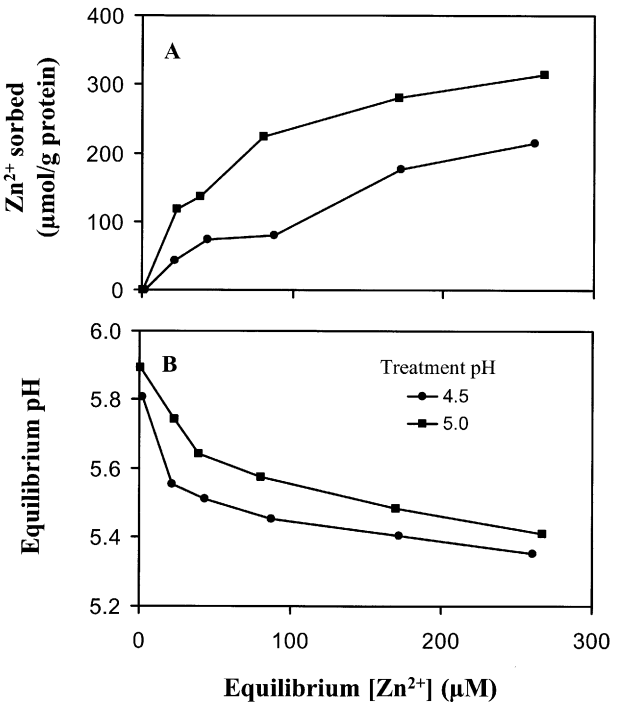

Fig. 2. Zn^{2+} sorbed to PM vesicles (*A*) and equilibrium pH of the vesicle suspension (*B*) as a function of equilibrium Zn^{2+} concentration in the bulk phase. Treatment pH refers to the pH of the solution before the addition of the PM vesicles.

creased with increasing $[Zn^{2+}]_{Eq}$ and with increasing treatment pH (Fig. 2*A*), while $[pH]_{Eq}$ decreased with increasing $[Zn^{2+}]_{Eq}$ (Fig. 2*B*).

Evaluation of Cu^{2+} and Zn^{2+} Binding Constants

To evaluate K_{cu}, the sorption data were analyzed using parameter values previously obtained (Yermiyahu et al., 1997*c*), namely, σ_0 (0.3074 $\mu mol_c\ m^{-2}$, where the subscript *c* denotes charge), K_K (1 M^{-1}), K_{Ca} (30 M^{-1}) and K_H (21,500 M^{-1}). In addition, it was necessary to determine for the reaction mixture the concentrations of potentially negative binding sites (120 μM, which is a cation binding capacity of 1200 $\mu mol_c\ g^{-1}$ protein) computed on the basis of measured negative sites per unit protein in the reaction mixture (Yermiyahu et al., 1997*c*), and the concentration of Ca^{2+} (25 μM), and H^+ (65 μM) introduced with the vesicle suspension. Once all the required parameters and values, other then K_{Cu}, were set, values for K_{Cu} were determined by maximizing R^2 and minimizing the root mean square error (*RMSE*) in fitting the model to all data points presented in Fig. 1. The best fit between measured and model-computed values was obtained for K_{Cu} = 400 M^{-1}. The drawn lines in Fig. 3 were produced by the sorption model, using the above value for K_{Cu}. For the correlation between measured and calculated values for $[Cu^{2+}]_{Eq}$ R^2 = 0.980 and *RMSE* = 15.5 μM, and for pH_{Eq} R^2 = 0.986 and *RMSE* = 0.05 pH units. The same procedure was used to evaluate a value of 5 M^{-1} for K_{Zn} (Fig. 4). For the correlation between measured and calculated values for $[Zn^{2+}]_{Eq}$ R^2 = 0.999 and *RMSE* = 5.5 μM, and for pH_{Eq} R^2 = 0.922 and *RMSE* = 1.18 pH units.

Comparison between the Affinities of Cu^{2+} and Other Cations to the Root PM

To verify the considerable difference between K_{Cu} and K_{Zn} determined above, La^{3+} was used as a competitive cation and its sorption was determined in the presence of four divalent cations. La^{3+} was chosen, because its binding constant was established in a previous study and the measurement of La^{3+} concentration is relatively easy (Yermiyahu et al., 1997*c*). The $[La^{3+}]_{Eq}$ as a function of divalent cation type and concentration are presented in Fig. 5. The treatment pH was 5.0 and the actual pH values in the vesicle suspension, in the absence of any divalent cation, were 5.17 and 5.76 with and without La^{3+}, respectively. $[La^{3+}]_{Eq}$ without addition of any divalent cation was 3.34 μM, which indicated that 85% of the total La^{3+} (21.9 μM) was sorbed under these conditions. Addition of 100 μM Zn^{2+}, Mg^{2+} or Ca^{2+} desorbed La^{3+} a little or not at all, and addition of 500 μM of these ions caused a greater but still slight desorption of La^{3+} (Fig. 5). In contrast, additions of

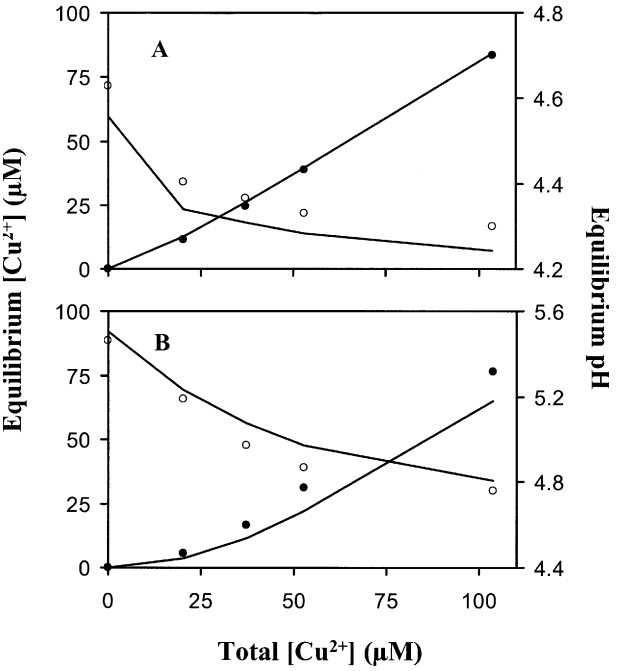

Fig. 3. Equilibrium pH (*empty circles*) and Cu^{2+} concentration (*filled circles*) in the vesicle suspension as a function of the Cu^{2+} added at treatment pH 4.0 (*A*) and 5.2 (*B*). The solid lines were generated by the sorption model. Treatment pH refers to the pH of the solution before the addition of the PM vesicles.

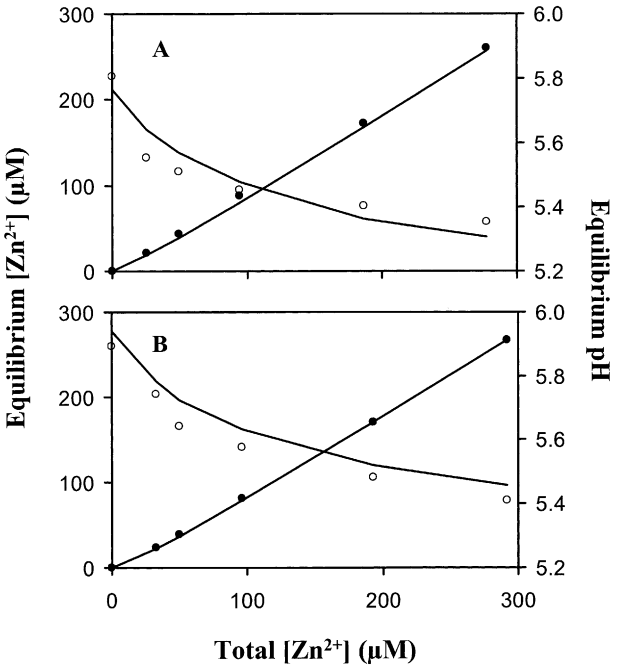

Fig. 4. Equilibrium pH (*empty circles*) and Zn^{2+} concentration (*filled circles*) in the vesicle suspension as a function of the Zn^{2+} added at treatment pH 4.5 (*A*) and 5.0 (*B*). The solid lines were generated by the sorption model. Treatment pH refers to the pH of the solution before the addition of the PM vesicles.

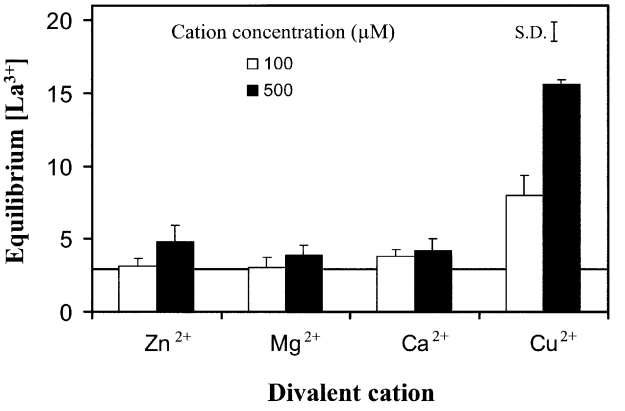

Fig. 5. Equilibrium La^{3+} concentration in the vesicle suspension in the presence of four divalent cations. The La^{3+} concentration in the reaction solution was 21.9 μM and the divalent cation concentrations were 100 or 500 μM. The horizontal line represents the La^{3+} concentration in the equilibrium solution when no divalent cation was added.

Cu^{2+} caused significant increases in $[La^{3+}]_{Eq}$ (Fig. 5). The addition of Cu^{2+} decreased the actual pH of the suspension to 4.98 at 100 μM Cu^{2+} and to 4.74 at 500 μM. These results confirm the greater affinity of Cu^{2+} to the vesicles as expected from the calculated binding constants. The highest amount of sorbed Cu^{2+} measured was over 300 μmol Cu g^{-1} (or 600 μmol_c g^{-1}) protein, namely half the binding capacity (1200 μmol_c g^{-1} protein). This sorption was, however, below the plateau in the sorption isotherm (Fig. 1). The high affinity between the PM and Cu^{2+} as compared to the other cations tested (e.g., Fig. 2) seems, therefore, to be a general property of the membrane and is not associated with some small fraction of non-phospholipid sites that might be present in the PM material.

The amount of Cu^{2+} sorbed to PM vesicles in the presence of other cations is presented in Fig. 6. The Cu^{2+} concentration was 50 μM, and the treatment pH was 5.2. The pH_{Eq} in the presence of Cu^{2+} alone was 5.56 and did not change greatly upon the addition of K^+ or Ca^{2+}. Additions of La^{3+}, on the other hand, caused a reduction in the pH to 5.26 in the presence of 20 μM of that ion. The amount of sorbed Cu^{2+} decreased with increasing K^+, Ca^{2+} or La^{3+} concentration (Fig. 6), indicating that all these cations competed with Cu^{2+} at the PM binding site to some extent. However, there is a considerable difference between the effectiveness of the various cations as competitors with Cu^{2+} (note the difference in *x*-axis scales in Fig. 6).

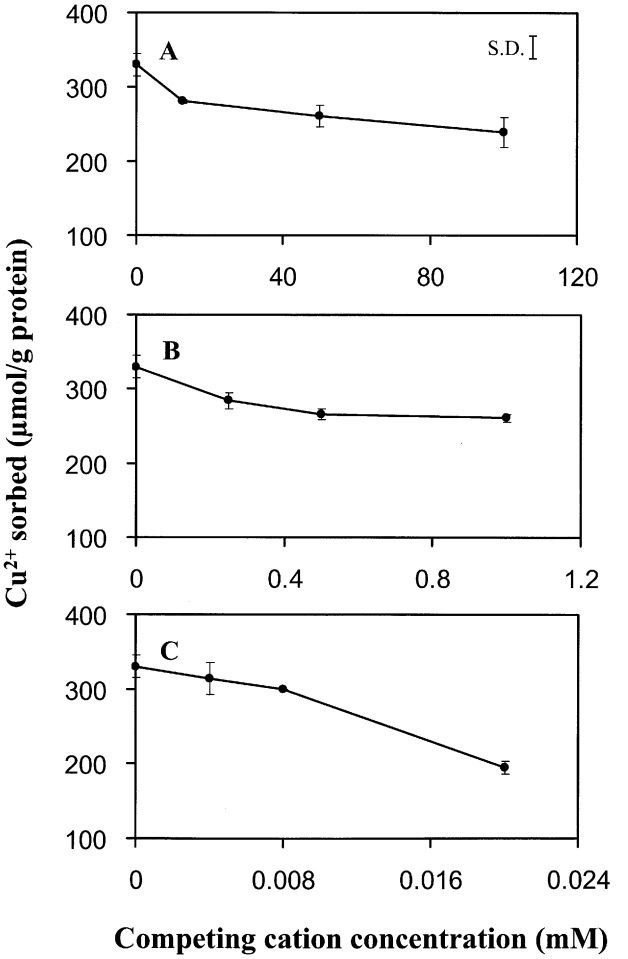

Fig. 6. Cu^{2+} sorbed to PM vesicles as a function of K^+ (*A*), Ca^{2+} (*B*) and La^{3+} (*C*) concentrations. The Cu^{2+} concentration in the reaction suspension was 50 μM, and the treatment pH was 5.2.

Cu^{2+} and Zn^{2+} Sorption in the Presence of Organic Acids

The amounts of Cu^{2+} sorbed to PM vesicles in the presence of organic acids at two treatment pH values are given in Fig. 7. The sorption of Cu^{2+} decreased with increasing concentrations of citric acid (Fig. 7*A*) or malic acid (Fig. 7*B*) and with decreasing treatment pH, at least in the range of 4.5 to 5.5. Changes in citric acid and H^+ concentration were more effective than changes in malic acid concentration. For example, at the treatment pH of 5.5, the amount of sorbed Cu^{2+} decreased by 75% when 300 μM citric acid was added, but sorbed Cu^{2+} decreased by only 37% when 1500 μM malic acid was added. GEOCHEM calculations indicated that the fraction of organic acid-complexed Cu^{2+} was >0.99 in the presence of 300 μM added citric acid and 0.87 in the presence of 1500 μM added malic acid at treatment pH 5.5. At a treatment pH of 4.5, the complexed fraction of Cu^{2+} was >0.99 in the presence of 300 μM citric acid and 0.73 in the presence of 1500 μM malic acid, while the decrease in sorption of the cation due to the addition of 300 μM citric acid and 1500 μM malic acid was 68% and 75%, respectively. Similar results were obtained for Zn^{2+} (Fig. 8), where at the treatment pH of 5.5 the amount of Zn^{2+} sorbed to the

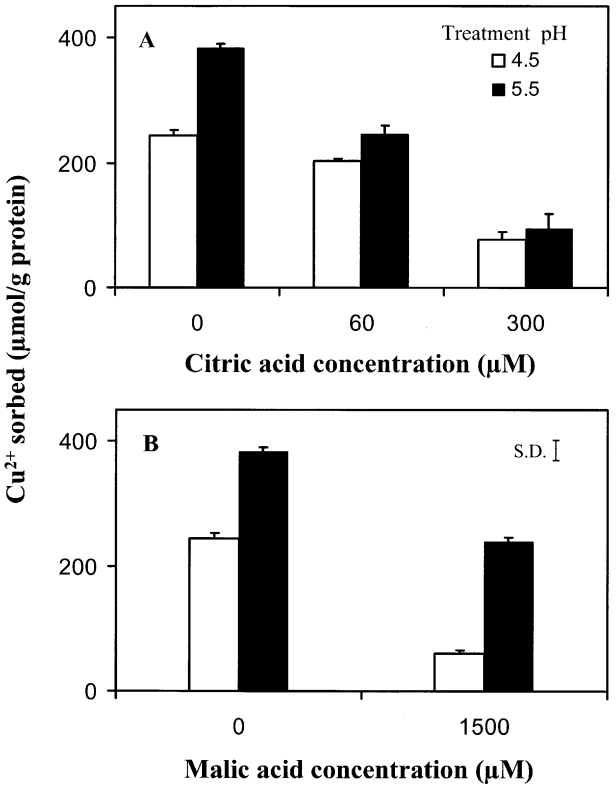

Fig. 7. Cu^{2+} sorbed to PM vesicles as a function of total citric acid (*A*) and malic acid (*B*) concentration at two treatment pH values. The Cu^{2+} concentration was 100 μM.

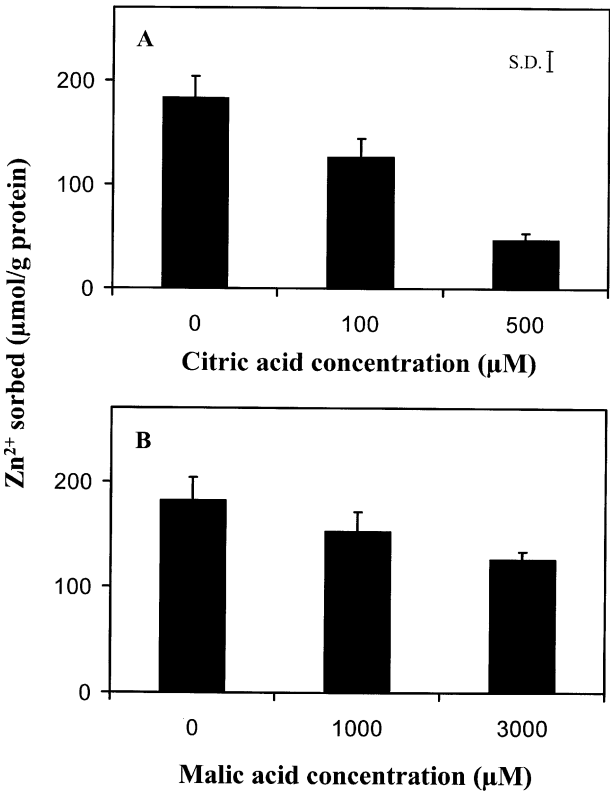

Fig. 8. Zn^{2+} sorbed to PM vesicles as a function of total citric acid (*A*) and malic acid (*B*) concentration. Zn^{2+} concentration was 100 μM, and the treatment pH was 5.5.

PM decreased by 74% upon addition of 500 μM citric acid but only by 31% upon addition of 3000 μM malic acid. The GEOCHEM calculations showed that the complexed fraction of Zn^{2+} in solution was 0.98 in 500 μM citric acid and 0.80 in 3000 μM malic acid.

Discussion

In our previous studies (e.g., Yermiyahu et al., 1994, 1997*c*) we quantified the sorption of a number of metal cations by PM of root cells and showed that a Gouy-Chapman-Stern model can simulate that sorption. Because the present study used similar plant material, the previously determined model parameters, such as σ_0 and cation binding constants, could be adopted. Therefore, the only unknown parameters for the Cu^{2+} or Zn^{2+} systems were K_{Cu} or K_{Zn}. The results of the present study indicate that the sorption affinity of Cu^{2+} to the PM is considerably larger than that of Ca^{2+}, Mg^{2+} or Zn^{2+} as determined by computed binding constants and by the La^{3+}-competition experiments (Fig. 5).

The dependence of La^{3+} desorption upon the binding constant of the competing ion is presented in Fig. 9. The amount of La^{3+} sorbed was calculated using a Gouy-Chapman-Stem model for a La^{3+}

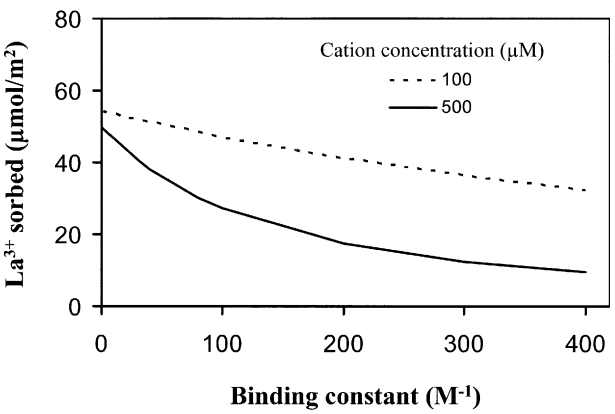

Fig. 9. The calculated sorption of La^{3+} to PM vesicles in the presence of a divalent cation as a function of its binding constant. The La^{3+} concentration in the reaction mixture was 5 μM at pH 5.0, and the divalent cation concentrations were 100 or 500 μM. La^{3+} sorbed was calculated using a Gouy-Chapman-Stern model with model parameters σ_0 = 0.3074 $\mu mol_c\ m^{-2}$ and K_{La} = 2200 M^{-1}.

concentration of 5 μM, at pH 5 and in the presence of a hypothetical divalent cation with different binding constants at concentrations of 100 and 500 μM. The other model parameters used were 0.3074 $\mu mol_c\ m^{-2}$ for σ_0 and 2200 M^{-1} for K_{La}. It can be seen from

Fig. 9, that cations with binding constants of 30 M^{-1} (e.g., Ca^{2+} [Yermiyahu et al., 1997]) or less (e.g., Zn^{2+} or Mg^{2+} [Yermiyahu et al., 1994]) have only a minor effect on La^{3+} sorption, while a cation with a binding constant of 400 M^{-1} (e.g., Cu^{2+}) will have a major effect on that sorption. Shomer et al. (2003) found that cations reduced the measured wheat root cell wall negativity in a consistent order (Cu^{2+} > Ca^{2+} > Mg^{2+} > Zn^{2+}). In general, the depolarizing effectiveness of cations (including monovalent, divalent, and trivalent cations) were similar for PMs and cell walls (Kinraide, 2004).

The amount of free (i.e., noncomplexed) Cu^{2+} in the solution containing 300 μM citric acid at a treatment pH of 5.5 was < 1 μM. Yet, the amount of Cu sorbed to PM vesicles was equivalent to that sorbed from an equilibrium concentration of 4 μM Cu^{2+} in the absence of the organic acid (Fig. 1). This may mean that the Cu-citrate complex is adsorbed onto the PM, but to a far lesser extent than free Cu^{2+}. By the same reasoning, Cu-malate, Zn-citrate and Zn-malate complexes may have been adsorbed, too. At the lower pH, proton competition resulted in both a lower organic acid-metal ion complexation and a lower sorption to the PM.

The high affinity of Cu^{2+} for the PM as compared to that of Zn^{2+} may explain, at least in part, the greater toxicity of Cu^{2+} to plants. However, Cu^{2+} is also more toxic than Al^{3+} and H^{+}, both of which have greater binding affinities than Cu^{2+} and thus binding strength alone cannot explain the great toxicity of Cu^{2+}. The mechanisms of most ion toxicities are not completely understood and may combine a variety of processes, both intracellular and extracellular.

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