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Binding and Electrostatic Attraction of Lanthanum (La^{3+}) and Aluminum (Al^{3+}) to Wheat Root Plasma Membranes

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Abstract. A general model for the sorption of trivalent cations to wheat-root (*Triticum aestivum* L cv. Scout 66) plasma membranes (PM) has been developed and includes the first published coefficients for La^{3+} and Al^{3+} binding to a biological membrane. Both ions are rhizotoxic, and the latter ion is the principal contributor to the toxicity of acidic soils around the world. The model takes into account both the electrostatic attraction and the binding of cations to the negatively charged PM surface. Ion binding is modeled as the reaction $P^- + I^Z \rightleftharpoons PI^{Z-1}$ in which P^- represents a negatively charged PM ligand, located in an estimated area of 540 $Å^2$, and I^Z represents an ion of charge Z. Binding constants for the reaction were assigned for K^+ (1 M^{-1}) and Ca^{2+} (30 M^{-1}) and evaluated experimentally for La^{3+} (2200 M^{-1}) and H^+ (21,500 M^{-1}). Al sorption is complicated by Al^{3+} hydrolysis that yields hydroxoaluminum species that are also sorbed. Binding constants of 30 and 1 M^{-1} were assigned for $AlOH^{2+}$ and $Al(OH)_2^+$, respectively, then a constant for Al^{3+} (20,000 M^{-1}) was evaluated experimentally using the previously obtained values for K^+, Ca^{2+} and H^+ binding. Electrostatic attraction was modeled according to Gouy-Chapman theory. Evaluation of parameters was based upon the sorption of ions to PM vesicles suspended in solutions containing variable concentrations of H^+, Ca^{2+} and La^{3+} or Al^{3+}. Use of small volumes, and improved assay techniques, allowed the measurement of concentration depletions caused by sorption to vesicles. Some independent confirmation of our model is provided by substantial agreement between our computations and two published reports of La^{3+} effects upon zeta potentials of plant protoplasts. The single published report concerning the electrostatic effects of Al on cell membranes is in essential agreement with the model.

Key words: Aluminum — Electrical potential — Lanthanum — Plasma membrane — Root — Surface charge

Introduction

Plasma membrane (PM) surface electrical properties appear to play an important role in mineral rhizotoxicity (Wagatsuma & Akiba, 1989; Suhayda et al., 1990; Kinraide, Ryan & Kochian, 1992; Kinraide, 1994; Yermiyahu et al., 1994, 1997). Because PM surfaces are usually negatively charged, the ion concentrations at root PM surfaces can differ significantly from the concentrations in the rooting medium. Treatments that alter PM surface negativity, such as changes in the ionic strength of the rooting medium, alter the effectiveness of ionic toxicants. Thus wheat root elongation was highly correlated with the computed activity of several ionic toxicants at root PM surfaces, but elongation was often

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Abbreviations: ψ_0, electrical potential at the plasma membrane surface; σ, charge density on the plasma membrane surface; pH_{Eq} or $[I^Z]_{Eq}$, equilibrium pH or concentration of ion I with charge Z in the bulk-phase medium after sorption reactions have been completed; $\{I^Z\}_0$ or $\{I^Z\}_\infty$, activity of ion I with charge Z at the plasma membrane surface or in the bulk-phase medium, respectively; $[I^Z]_0$ or $[I^Z]_\infty$, concentration of free ion I with charge Z at the plasma membrane surface or in the bulk-phase medium, respectively; K_I, binding constant for ion I to the plasma membrane ligand P^-; PM, plasma membrane; $[I^Z]_T$, total concentration of ion I with charge Z in the reaction mixture whether I^Z is sorbed to vesicles or free in the bulk-phase medium; P_T, total molar concentration of the plasma membrane ligand, P, in the vesicle suspension; *RMSE*, root mean square error.

poorly correlated with toxicant activities in the external rooting medium (Kinraide et al., 1992; Kinraide, 1994). Some toxicants appear to act by displacing Ca^{2+} from PM surfaces. Thus melon root elongation, in high concentrations of NaCl, correlated strongly with the computed activity of Ca^{2+} at the PM surfaces and with the amount of Ca^{2+} bound to the PM (Yermiyahu et al., 1997).

Al rhizotoxicity is a worldwide problem in acidic soils (Foy, 1984), but the mechanisms of toxicity are unknown. The plasma membrane (PM) may be a site of Al injury, and certainly the PM plays a role in toxicity if Al must accumulate intracellularly prior to toxic effects. Al interacts with the PM in ways that may play a role in toxicity. Al reduces membrane fluidity (Vierstra & Haug, 1978; Deleers, Servais & Wülfert, 1986; Chen, Sucoff & Stadelmann, 1991; Zel et al., 1993) and alters the permeability to water and several solutes (Zhao, Sucoff & Stadelmann, 1987; Cakmak & Horst, 1991*a;* Chen et al., 1991). Al inhibits the uptake of cations by roots (Rengel & Robinson, 1990; Huang et al., 1992; Nichol et al., 1993), cells (Rengel & Elliott, 1992), and PM vesicles (Huang et al., 1996) and appears to be a channel blocker for Ca^{2+} (Ding, Badot & Pickard, 1993; Piñeros & Tester, 1995) and K^+ (Schroeder, 1988; Gassmann & Schroeder, 1994). Transmembrane electrical potential differences usually are not affected much (Kinraide, 1988; Miyasaka et al., 1989), but sometimes the potential difference is increased (Kinraide, 1988, 1993; Reid, Tester & Smith, 1995) or, less often, decreased (Olivetti, Cumming & Etherton, 1995; and references in Kinraide, 1993). Lipid peroxidation is synergistically enhanced by Al and Fe (Cakmak & Horst, 1991*b;* Yamamoto et al., 1996).

Low levels of Al reduce or reverse the surface negativity of biological and artificial membranes. This signifies binding and electrostatic attraction of Al to the membrane surface. Akeson, Munns and Burau (1989) studied sorption of Al to uncharged liposomes composed of zwitterionic phosphatidylcholine using equilibrium dialysis and electrophoresis and concluded that the vesicle surface had a 560-fold higher affinity for Al^{3+} than Ca^{2+}. The electrophoresis measurements of Wilkinson et al. (1993) indicate a high affinity of fish gill cells for Al^{3+}. Using Tb phosphorescence, Caldwell (1989) concluded that Al binds to wheat-root PM protein and that Al induces changes in protein conformation. Though the role of the PM in Al rhizotoxicity remains uncertain, there appears to be diverse interactions between Al and the PM. Clearly, the sorption of Al to the PM is of interest to investigators of Al rhizotoxicity.

Membrane surface electrical potentials (ψ_0, expressed in mV), surface charge densities (σ, expressed in Cm^{-2} or $Å^2$ per charge [$16.02/(Cm^{-2}) = Å^2$ per charge]) and ion sorption have been incorporated into a Gouy-Chapman-Stern model (McLaughlin, 1977; Nir, Newton & Papahajopoulos, 1978; Barber, 1980; Lau, McLaughlin & McLaughlin, 1981; Kinraide, 1994; Rytwo, Banin & Nir, 1996*a;* Rytwo, Nir & Margulies, 1996*b*). We define ion sorption as the combination of tight binding to the membrane (leading to alterations of σ and ψ_0) and the electrostatic attraction of ions into the diffuse layer near the membrane surface (leading to alteration of ψ_0 only). The Gouy-Chapman portion of the model relates σ, ψ_0, and the concentrations of ions in the external solution. The Stern modification takes into account the effect of ion binding on σ. Use of the Gouy-Chapman-Stern model requires the specification of binding mechanisms and binding constants for each ion.

Information regarding surface properties (σ and binding constants) of root PM is limited. Estimates of intrinsic σ, using 9-aminoacridine fluorescence, have been reported a few times. The intrinsic σ is the value that would occur in the absence of tightly bound ions. Møller, Lundborg and Bérczi (1984) obtained values of 552 $Å^2$ per charge for oat root PM and 801 to 1001 for wheat root PM, bracketing the value of 890 for wheat root PM reported by Bérczi et al. (1984). Körner et al. (1985) reported 843 $Å^2$ per charge for barley roots, and Oka et al. (1988) reported 411 for *Vigna mungo* roots. Using Ca^{2+} sorption, Yermiyahu et al. (1994) estimated an intrinsic σ for melon root PM vesicles of 370 $Å^2$ per charge.

Binding constants for various cations have been reported for phospholipid vesicles (Nir et al., 1978; Lau et al., 1981; Ohki & Kurland, 1981; Bentz et al., 1988; Akeson, Munns & Burau, 1989; Tocanne & Teissie, 1990), but few studies have concerned the binding of cations, especially trivalent cations, to biological membranes (Abe & Takeda, 1988; Obi et al., 1989*a*,*b;* Wilkinson et al., 1993; Kinraide, 1994; Yermiyahu et al., 1994).

Aluminum rhizotoxicity is generally attributed to Al^{3+}, but this species hydrolyzes in solution so that the hydroxoaluminum species, such as $Al(OH)^{2+}$ and $Al(OH)_2^+$, exceed the trivalent species at pH > 5 (Kinraide, 1991). The tendency of Al^{3+} to undergo hydrolysis, and polynucleation, would complicate the sorption studies described in this paper because the differential sorption of one Al species over another would change both the concentration and the ratio of ions, including H^+, in the bulk medium. This would cause a reequilibration among species in the medium and at the PM surface. To avoid some of these problems, we studied initially the sorption of La^{3+} (itself a rhizotoxic ion [Kinraide, 1994]) as a surrogate for Al^{3+}. The advantage of La^{3+} is that the first hydrolysis constant is only $10^{-9.13}$ (Baes & Mesmer, 1986), so hydrolysis is minimal in neutral and acidic media. The result of the initial investigation was a model for La^{3+} sorption that includes bind-

ing constants for La^{3+}, Ca^{2+}, H^+ and K^+. The model was then adapted to the more difficult problem of Al^{3+} sorption.

Materials and Methods

Plant Material

Caryopses of wheat (*Triticum aestivum* L cv. Scout 66) were surface sterilized with 1% NaOCl for 5 min and thoroughly rinsed in distilled water. The seeds were then placed between sheets of germination paper that were kept saturated with 1 mM $CaCl_2$ and held on glass plates placed in a slanted position in a nearly closed container in an incubator at 25°C. After 4 d the primary roots were 8 to 12 cm long.

Isolation of Plasma Membrane Vesicles

Right-side-out PM vesicles were isolated from whole roots according to Larsson, Widell and Sommarin (1988) with slight modification. The final wash solution included 0.25 M sucrose and 5 mM KCl adjusted to pH 7.2 with 1 M KOH. The vesicles were kept at 2°C as a pellet overnight and then were washed again with final solution and resuspended to achieve a protein concentration of 500 or 1000 μg mL^{-1}. Protein concentration was determined according to Bradford (1979) using BSA as a protein standard. Specific activity of the vanadate-sensitive ATPase was 17.4 ± 0.1 (mean ± SE, $n = 3$) μmol P_i released (h mg protein)$^{-1}$ in the presence of 1 μg Triton X-100 (μg protein)$^{-1}$ assayed according to Brauer et al. (1989). The pH of the vesicle suspension was 6.0 ± 0.2. In each sorption experiment some of the final wash solution was adjusted with HCl to the same pH as the vesicle suspension. This solution was used, as described later, for a vesicle-free control.

Estimation of Intrinsic σ

The intrinsic σ of the PM vesicles was determined by the 9-aminoacridine fluorescence method used by Chow and Barber (1980) and Møller et al. (1984). Fluorescence emission spectra, from 445 to 480 nm, of 20 μM 9-aminoacridine were determined in a solution of 0.25 M sucrose, 50 μM EDTA and 2 mM HEPES (titrated to pH 7 with KOH) with a Perkin-Elmer LS-5B fluorometer (Norwalk, CT) using an excitation wavelength of 340 ± 3 nm. PM vesicles were added to the solution to a protein concentration of 50 μg mL^{-1}, and after 5 min of incubation an emission spectrum was obtained.

Thereafter, the mixture was progressively supplemented with the chloride salt of a monovalent cation (KCl or tetramethylammonium · Cl) or a divalent cation (methyl viologen · Cl_2 or hexamethonium · Cl_2). After each increase in salt concentration, the fluorescence (F) increased. Finally, the mixture was supplemented with 20 mM $MgCl_2$ to yield F_{max}. Because methyl viologen reduced slightly the fluorescence of 9-aminoacidine in the absence of vesicles, corrections were made for readings in the presence of vesicles.

The ratio F/F_{max} relates to ψ_0 (Chow & Barber, 1980), and equal values of F/F_{max} are taken to indicate equal values of ψ_0, irrespective of the salt milieu. If none of the solutes bind tightly to the membrane surface, then σ values are constant. Consequently, σ and ψ_0 in one salt system must equal σ and ψ_0 in another salt system, provided F/F_{max} is the same in each. Since the Grahame equation (*see* Eq. 4) relates σ, ψ_0 and the salt concentrations, trial substitutions of σ into the equation can be done until a pair of values for σ and ψ_0 are found that is the same for each salt system.

Sorption Studies

The initial sorption experiments dealt with La^{3+}, not Al. Sorption experiments were performed according to Yermiyahu et al. (1994) with some modifications. Unless otherwise specified, 100 μL of $LaCl_3$ or $CaCl_2$ solution, adjusted to the desired pH, were added to a 350-μL solution containing 0.321 M sucrose adjusted to the same pH. The sorption reaction was started with the addition of 50 μL of PM vesicle suspension or 50 μL of the final wash solution adjusted to the same pH (about 6) as the vesicle suspension (for without-vesicle control solutions). The reaction solution contained 25 ± 2 μg protein for the La^{3+} experiments or 50 ± 2 μg protein for the Ca^{2+} experiments. The final volume of the reaction solution was 500 μL and contained 0.25 M sucrose, 0.5 mM KCl, various concentrations of La^{3+}, Ca^{2+} and H^+, and 0, 50 or 100 μg vesicle protein per mL. Triplicate measurements were made at 25°C, and each experiment was repeated three times.

The sorption reactions were run for 30 min, but measurements, not reported here, indicated that the sorption reaction was complete after a few minutes. After the pH of the suspensions was measured, the suspensions were transferred to Microfilterfuge tubes (0.2 μm; Rainin) and centrifuged for 15 min at 8,000 × g. [La^{3+}] was determined in the equilibrium solution (the filtrate) using inductively coupled argon plasma emission spectrometry (Jobin Yvon Emission Instruments S.A. model JY 46 P) capable of detecting <0.02 μM La at 408.67 nm (Winge et al., 1985). The amount of La^{3+} or H^+ sorbed to the vesicles was calculated from the difference between corresponding with-vesicle and without-vesicle solutions.

Sorption of Al was also measured as reductions in concentration in media to which a concentrated suspension of vesicles (or a vesicle-free solution for control) was added. As before, the solutions were intentionally not buffered, nor did they contain any solute, other than the inevitable OH^-, likely to complex Al^{3+}. Al in the filtrate was assayed by inductively coupled argon plasma emission spectrometry (Jobin Yvon Emission Instruments S.A., model JY 46 P) capable of detecting <0.2 μM Al at 167.02 nm (Uehiro, Morita & Fuwa, 1984).

The measured depletions of cations logically reflects sorption to vesicle membranes rather than uptake into the bulk solution of the vesicle interior. The vesicle volume is <1% of the volume of the reaction mixture, so passive uptake into the bulk solution of the vesicle interior would reduce the ion concentration of the filtrate by <1% unless there were a transmembrane electrical potential difference. That could occur only if there were an energy source for the establishment of the potential difference, which there is not. The possibility of ion sorption to the inner surface of the vesicle membranes is not excluded, but that possibility is not particularly problematical for our study. The modest differences in global electrostatic properties between the inner and outer surfaces of the PM are not likely to affect our model parameters greatly (Møller, Lundborg & Bérczi, 1984; Bérczi et al., 1984). (*See* Yermiyahu et al., 1994 for further discussion.)

Sorption Model

The PM is modeled as though it contained a singly charged ligand denoted P^-. This ligand has a characteristic surface density, which must be estimated and assigned a value in units of mol m^{-2}. In addition, the total molar concentration of P (P_T) in the vesicle suspension must be estimated. This value is needed only for a closed system, that is, a system where the concentration of vesicles is high enough to cause

a reduction in the concentration of ions in the bulk solutions, a condition required for our measurements.

P^- can bind ion I^Z, where Z is the charge of the ion, to form the complex PI^{Z-1}, expressed in mol m^{-2}. The binding of ions changes the actual σ, which depends upon the surface density of free ligand (P^-), but not the intrinsic σ, which depends upon the surface density of total ligand whether free or complexed. The reaction

$$P^- + I^Z \rightleftharpoons PI^{Z-1} \quad (1)$$

has a characteristic binding coefficient, K_I, expressed in M^{-1} and given by

$$K_I = [PI^{Z-1}]/([P^-][I^Z]_0) \quad (2)$$

where $[I^Z]_0$ is the concentration of free I^Z at the surface of the membrane. This concentration is computed by the Boltzmann equation

$$[I^Z]_0 = [I^Z]_{Eq}\exp(-Z_iF\psi_0/RT) \quad (3)$$

where $[I^Z]_{Eq}$ is the concentration of the solute in the bulk solution, and the other symbols are defined as in Eq. 4.

A membrane surface charge establishes an electrical potential at the membrane surface (ψ_0) and in a diffuse layer near the membrane (ψ_x at distance x from the surface). The potential in the bulk medium, away from the membrane surface, is taken to be zero ($\psi_\infty = 0$). ψ_0 and σ are related to the ionic composition of the bulk medium according to the Grahame equation, which incorporates Gouy-Chapman theory (McLaughlin, 1977; Barber, 1980),

$$\sigma^2 = 2\epsilon_r\epsilon_0 RT\Sigma_i C_{i\infty}(\exp[-Z_iF\psi_0/RT] - 1) \quad (4)$$

where σ is expressed in units C m^{-2}; $2\epsilon_r\epsilon_0 RT = 0.00345$ at 25°C for concentrations expressed in M (ϵ_r is the dielectric constant for water, ϵ_0 is the permittivity of a vacuum, R is the gas constant, and T is temperature); $C_{i\infty}$ is the concentration of the ith ion at infinite distance from the membrane (also $[I^Z]_{Eq}$ or $[I^Z]_\infty$ in our notation); F is the Faraday constant; and $-Z_iF\psi_0/RT = -Z_i\psi_0/25.7$ at 25°C for ψ_0 expressed in mV.

The equations described thus far allow the computation of bound and free ions at the membrane surface, but do not allow the computation of the total amounts of ions sorbed to the membranes. That requires the additional computation of ions attracted into the diffuse layer above the concentration of ions in the bulk phase. For that, it is necessary to compute $[I^Z]$ at every distance (x) from the membrane surface ($[I^Z]_x$) under the influence of the electrical potential generated by the surface charges. Then, $[I^Z]_x - [I^Z]_\infty$ must be integrated from $x = 0$ to $x = \infty$ in order to obtain the sorbed ions in the diffuse layer. The methods of computation have been developed and summarized over a period of years (Nir et al., 1978, 1994; Nir, 1984; Rytwo et al., 1996*a*,*b*), and the computer program has been documented in the dissertation of Rytwo (1994).

The great advantage of being able to compute diffuse-layer accumulation of ions is that sorption experiments, using small volumes in which ion sorption can be measured as reductions in ion concentrations in the equilibrium solution, can be used to compute the value of sorption model parameters.

Al^{3+} Hydrolysis

Hexaaquaaluminum ($Al(H_2O)_6^{3+}$) undergoes reactions with OH^- that can be summarized as

$$Al(H_2O)_6^{3+} + nOH^- \rightleftharpoons Al(H_2O)_{6-n}(OH)_n^{3-n} + nH_2O \quad (5)$$

or, for convenience,

$$Al^{3+} + nH_2O \rightleftharpoons Al(OH)_n^{3-n} + nH^+ \quad (6)$$

where $n = 1$ to 4. The equilibrium constant (hydrolysis constant) for each reaction is defined as

$$K_n = \{Al(OH)_n^{3-n}\}\{H^+\}^n/\{Al^{3+}\} \quad (7)$$

where braces denote chemical activity. For the hydrolysis reactions used in our model we adopted the values compiled by Nordstrom and May (1989): $K_1 = 5.00$, $K_2 = 10.1$, $K_3 = 16.8$, $K_4 = 22.7$. To prevent the formation of polynuclear Al complexes we prepared Al solutions within hours of use, never added bases to Al solutions, and avoided solutes, including buffers, likely to react with Al^{3+} (*see* Bertsch & Parker, 1996).

The model membrane ligand, P^-, was assumed to bind the Al species according to the reaction

$$P^- + Al(OH)_n^{3-n} \rightleftharpoons PAl(OH)_n^{2-n} \quad (8)$$

with binding constants

$$K_{A13-n} = [PAl(OH)_n^{2-n}]/([P^-][Al(OH)_n^{3-n}]_0) \quad (9)$$

where $[Al(OH)_n^{3-n}]_0$ is the concentration of the Al species at the membrane surface. Binding constants for the species $AlOH^{2+}$ and $Al(OH)_2^+$ (the only important hydroxo-aluminum species in our system) were assigned values of $K_{A12+} = K_{Ca} = 30\ M^{-1}$ and $K_{Al+} = K_K = 1\ M^{-1}$.

Changes in $[H^+]$ in the vesicle suspension can occur because of H^+ sorption (or desorption) and because of the hydrolysis reaction (Eq. 6). If $K_{A13+} \gg K_{A12+} > K_{Al+}$ (which is the case), then the addition of vesicles to an Al solution will cause a preferential sorption of less hydroxylated species leading to the reversal of the hydrolysis reaction (Eq. 6). This decrease in $[H^+]$ and in hydroxoaluminum species could lead to an overestimate of the sorption of H^+ and the hydroxoaluminum species. Our model takes all of these reactions into account and partitions the changes in solute concentrations into sorption reactions and hydrolysis reactions.

Results

Sorption of La^{3+} to PM Vesicles

The amount of La^{3+} sorbed to PM vesicles as a function of the total $[La^{3+}]$ ($[La^{3+}]_T$) at five treatment pHs are presented in Fig. 1*A*. The amount of sorbed La^{3+} increased with increasing $[La^{3+}]_T$ and with increasing treatment pH. The term ''treatment pH'' refers to the pH of the 450-μL solution to which 50 μL of vesicle suspension or vesicle-free solution (for control) is added. The treatment pH ranged from 3.7 to 6.4, while the pH of the 50-μL added solution was 6.0 ± 0.2. Figure 1*B* presents the amount of La^{3+} sorbed as a function of $[La^{3+}]_{Eq}$, i.e., the concentration in the filtrate after the 30-min equilibration. Sorbed La^{3+} was computed from the difference between $[La^{3+}]_{Eq}$ in the with-vesicle suspension and $[La^{3+}]_{Eq}$ in the without-vesicle control.

Changes in the pH_{Eq} as a function of $[La^{3+}]_T$ are presented in Fig. 2*A*. pH_{Eq} decreased with increasing

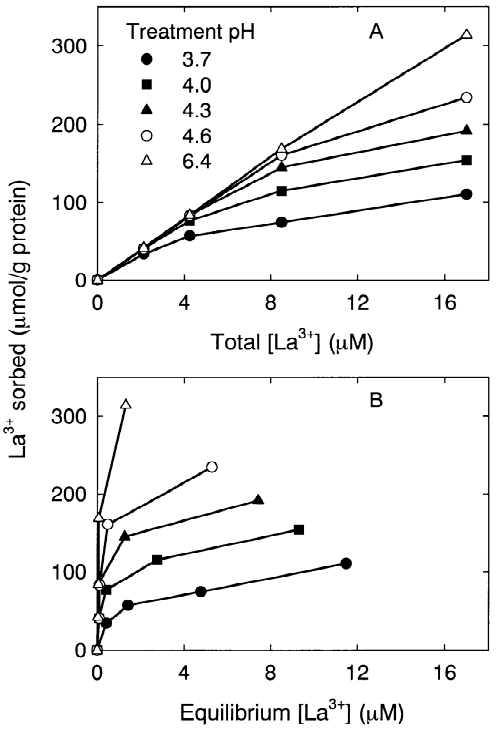

Fig. 1. La^{3+} sorbed to plasma membrane vesicles as a function of total $[La^{3+}]$ in the reaction mixture (*A*) and equilibrium $[La^{3+}]$ in the bulk-phase medium (*B*).

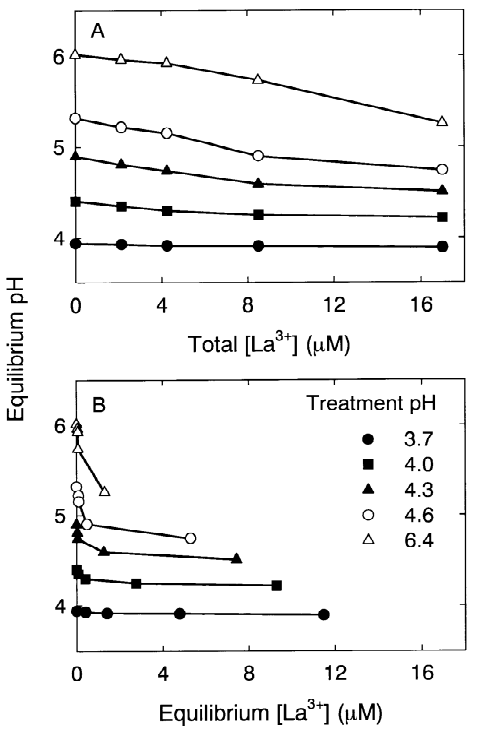

Fig. 2. Equilibrium pH of the vesicle suspension as a function of total $[La^{3+}]$ in the reaction mixture (*A*) and equilibrium $[La^{3+}]$ in the bulk-phase medium (*B*).

$[La^{3+}]_T$ and increased with increasing treatment pH. The effect was stronger at the higher pH values, mainly because of the logarithmic nature of pH. Figure *2B* presents pH_{Eq} as a function of $[La^{3+}]_{Eq}$.

The amount of sorbed La^{3+} can be computed directly from the experimental data, but to evaluate K_I for La^{3+} (K_{La}) some other parameters are needed first. These include intrinsic σ, P_T, and K_I for H^+, K^+ and Ca^{2+} (K_K, K_H and K_{Ca}). Also needed are the initial total concentrations of cations, including Ca^{2+} and H^+ that may be unintentionally introduced with the 50 μL suspension of vesicles because some of these ions remained sorbed to the vesicles during their preparation.

Intrinsic σ

Intrinsic σ, estimated by the 9-aminoacridine fluorescence method from four internally replicated experiments using a combination of KCl and methyl viologen · Cl_2, was 540 ± 100 $Å^2$ per charge [(mean ± SE, $n = 4$). Experiments with tetramethylammonium · Cl and hexamethonium · Cl_2 yielded somewhat higher charge densities (lower values for $Å^2$ per charge). The values fall at the lower end of the range of reported values (when expressed as $Å^2$ per charge)] for σ for PM from grass root cells (*see* Introduction). The value of 540 $Å^2$ per charge was adopted for the model.

K_K and K_{Ca}

The binding coefficients for K^+ and Ca^{2+} were chosen from the literature. For K^+ we used a value of 1 M^{-1}, a value that Kinraide (1994) found to yield a good computational reproduction of the effects of KCl on zeta-potential measurements (Abe & Takeda, 1988; Obi et al., 1989*b*). For Ca^{2+} the range in the literature generally varies between 10 and 50 M^{-1} (Yermiyahu et al., 1994). We used 30 M^{-1} as an average, in part, because two investigations report that value for phosphatidylserine (Ohki & Kurland, 1981; Nir, 1984).

Computation of Some Additional Parameters

To evaluate additional parameters, other than K_{La}, a sorption experiment was performed where increasing amounts of Ca^{2+} were added at different treatment pH values. The pH_{Eq} was measured, and $[H^+]_{Eq}$ was calculated after computing the activity coefficients for H^+. The results appear in Fig. 3. The absence of a zero $[Ca^{2+}]$ occurs because a small amount of Ca^{2+} remained sorbed to the vesicles during their preparation. This

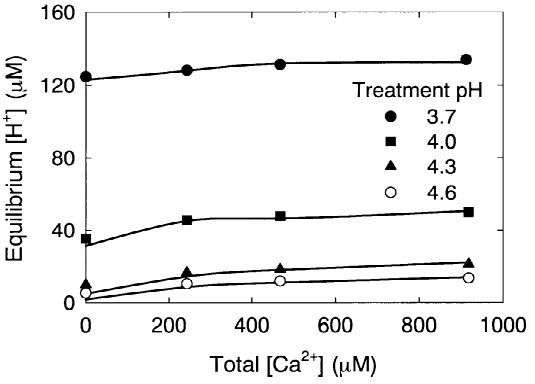

Fig. 3. Equilibrium pH of the vesicle suspension as a function of total $[Ca^{2+}]$ in the reaction mixture. The drawn curves were generated by the sorption model.

Ca^{2+} was measured directly in a concentrated suspension of vesicles, allowing a value of 18 μM (computed as a fraction of P_T) to be assigned for the present experiment.

While a rough estimate of P_T could be obtained from the protein analysis and previous work (Yermiyahu et al., 1994), a more precise value was estimated by systematically substituting trial values for P_T, K_H, and unintentionally added H^+ into the model until the closest correspondence between measured and computed values for $[H^+]_{Eq}$ was obtained. R^2 and *RMSE* were computed at each trial. The resulting values were 120 μM for P_T (=1.2 mmol [g protein]$^{-1}$; compare to 1.09 mmol [g protein]$^{-1}$ in Yermiyahu et al. [1994]), 45 μM for unintentionally added H^+, and 21,500 M^{-1} for K_H.. The drawn lines in Fig. 3 were produced by the sorption model using these values. The correlation between measured and calculated values for $[H^+]_{Eq}$ (μM) was very high (R^2 = 0.9995 and *RMSE* = 1.25).

K_{La}

To estimate K_{La}, two experiments were performed, each at two treatment pHs (4.6 and 5.4). For the first experiment, $[La^{3+}]_T$ ranged from 0 to 40 μM, and for the second experiment $[La^{3+}]_T$ ranged from 0 to 300 μM. (*See* Figs. 4 and 5) For the analysis of these data we incorporated most of the parameter values previously obtained, namely, intrinsic σ, K_{Ca}, K_H and K_K, and unintentionally added Ca^{2+}. P_T and unintentionally added H^+ had been determined previously also, but because the model is sensitive to these values and because they can change somewhat with the preparation, they were computed again. Using the same batch of vesicles used for the experiments represented in Figs. 4 and 5, a side experiment was done using five treatment pH values and no added La^{3+}. The results were 70 μM for P_T and 40 μM for unintentionally added H^+. 70 μM was not too distant from the expected value of 60 μM from the previous experiment. Recall that 25 μg protein per 500 μL was used here instead of 50 μg as before.

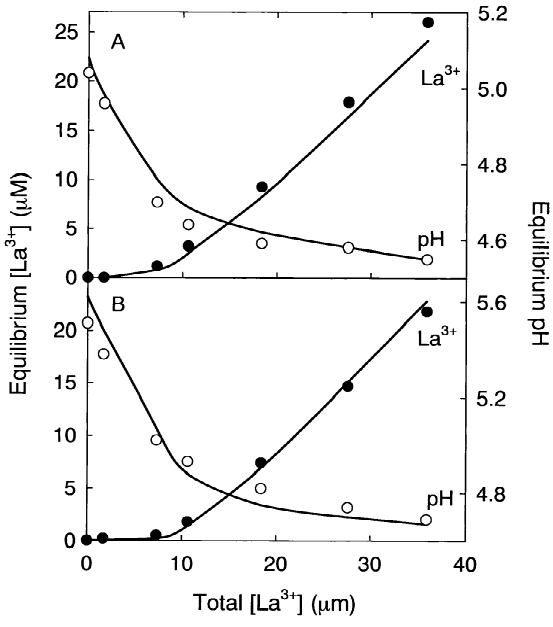

Fig. 4. Equilibrium $[La^{3+}]$ and equilibrium pH of the vesicle suspension as a function of total $[La^{3+}]$ (0 to 35.9 μM) and a treatment pH of 4.6 (*A*) and 5.4 (*B*). The drawn curves were generated by the sorption model.

Only K_{La} remains to be evaluated. Trial values for K_{La} were substituted into the model, and R^2 and *RMSE* were computed for each trial value using all the datum points presented in Figs. 4 and 5. The best fit was obtained with a value of K_{La} = 2200 M^{-1}. The drawn lines in Figs. 4 and 5 were produced by the sorption model using this value for K_{La}. As seen from the figure, the correlations between measured and calculated values were very high (R^2 = 0.9989 and *RMSE* = 2.85 for $[La^{3+}]_{Eq}$ [μM]; R^2 = 0.9715 and *RMSE* = 0.096 for pH_{Eq}).

Confirmation of Parameters

After computing K_{La} from the experiments presented in Figs. 4 and 5, the model was used to compute $[La^{3+}]_{Eq}$ and pH_{Eq} for the experiment presented in Figs. 1 and 2. These values, as well as the computed values for ψ_0, are presented in Table 1. Only the parameters P_T and unintentionally added H^+ were computed specifically for this experiment. Their values were 70 and 30 μM, respectively. Comparisons between measured and calculated values yielded R^2 = 0.9793 and *RMSE* = 0.94 for $[La^{3+}]_{Eq}$ [μM] and R^2 = 0.9849 and *RMSE* = 0.089 for

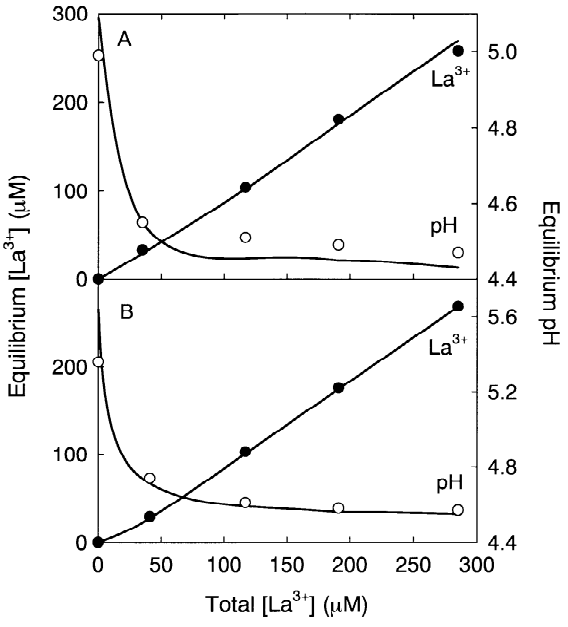

Fig. 5. Equilibrium $[La^{3+}]$ and equilibrium pH of the vesicle suspension as a function of total $[La^{3+}]$ (0 to 285 μM) and a treatment pH of 4.6 (*A*) and 5.4 (*B*). The drawn curves were generated by the sorption model.

pH_{Eq}). For the sorbed La^{3+}, the correlations were even higher. Note that the model computed successfully $[La^{3+}]_{Eq}$ and pH_{Eq} at treatment pH values both higher and lower than the treatment pH values used in the experiment where K_{La} was estimated.

Additional confirmation was obtained with a new experiment in which vesicles were suspended in a solution of 0.25 M sucrose and 2 mM HEPES titrated to pH 7.1 with KOH. Total $[K^+]$ = 1 mM and total $[Ca^{2+}]$ = 7.5 μM. 20 μM 9-aminoacridine was added and fluorescence measurements were taken after progressive additions of $LaCl_3$ as described for the estimation of intrinsic σ. Figure 6 indicates that $[La^{3+}]_T$ at a value somewhat higher than 100 μM caused the PM surfaces to be discharged to the same extent that they were discharged by 20 mM $MgCl_2$, presumably to a value close to 0 mV. The asymptotic nature of the curves near $F/F_{max} = 1$ means that an exact value cannot be read from the graph, but application of the model to the conditions of the present experiment predicts that $\psi_0 = 0$ when $[La^{3+}]_T$ = 248 μM.

Sorption of Al to PM Vesicles

The amount of Al sorbed to PM vesicles as a function of the total $[Al]_T$ at four levels of treatment pH are presented in Fig. 7*A*. The amount of sorbed Al increased with increasing $[Al]_T$ and with increasing treatment pH. The vesicles (or vesicle-free solution) were added in a 50-μL volume at pH 6.0 ± 0.2. Figure 7*B* presents the amount of Al sorbed as a function of $[Al]_{Eq}$. Sorbed Al was computed from the difference between $[Al]_{Eq}$ in the with-vesicle and without-vesicle reaction mixtures.

Changes in pH_{Eq} as a function of $[Al]_T$ and treatment pH are presented in Fig. 8*A*. pH_{Eq} decreased with increasing $[Al]_T$ and increased with increasing treatment pH. The Al-induced reduction of pH below the treatment pH reflects the displacement of some H^+ initially on the PM vesicles. Because of their logarithmic nature, pH changes were greater at the higher pH values. Fig. 8*B* presents pH_{Eq} as a function of $[Al]_{Eq}$.

Evaluation of K_{Al3+}

To evaluate K_{Al3+} for the sorption model, other parameters are needed. These include the following parameters already evaluated or assigned: intrinsic σ(540 $Å^2$ per charge), K_K (1 M^{-1}), K_H (21,500 M^{-1}), K_{Ca} (30 M^{-1}), K_{Al1+} (1 M^{-1}) and K_{Al2+} (30 M^{-1}). Also needed are the initial composition of the system including the P_T and the total concentrations of cations including Ca^{2+} and H^+ that may be unintentionally introduced with the vesicle suspension. Each of these values was estimated earlier, but the values for P_T and unintentionally added H^+ were also estimated for each Al experiment.

Once all the parameters other than K_{Al3+} were set, values for K_{Al3+} were substituted systematically into the model, and R^2 and *RMSE* were computed for each trial value using all datum points presented in both Figs. 7 and 8. The best fit for measured *vs.* model-computed values was obtained when K_{Al3+} = 20,000 M^{-1}. The drawn lines in Fig. 9 were produced by the sorption model using this value for K_{Al3+}. As seen from the figure, the correlation between measured and calculated values for $[Al]_T$ were very high (R^2 = 0.9964, and *RMSE* = 0.249) as was the correlation between measured and calculated values for pH_{Eq} (R^2 = 0.9966, and *RMSE* = 0.029).

Sorption in a Ternary System with Varying Al, Ca^{2+} and H^+

Vesicles were exposed to 24 different solutions composed of variable treatment concentrations of Al, Ca^{2+} and H^+. Because of hydrolysis, the number of treatment Al^{3+} concentrations was higher than the four levels of added $AlCl_3$. Various concentrations of $AlOH^{2+}$ and $Al(OH)_2^+$ were present as well. After the usual computation of P_T and the amount of unintentionally added H^+, the Al-sorption model was applied to the treatments without adjustment of parameters. Table 2 presents measured and computed values. R^2 = 0.8949 and *RMSE* =

Table 1. Measured and computed values from a sorption experiment with wheat-root plasma membrane vesicles

Treatment		Meas. $[La^{3+}]_{Eq}$	Calc. $[La^{3+}]_{Eq}$	Meas. pH_{Eq}	Calc. pH_{Eq}	Meas. $[H^+]_{Eq}$	Calc. $[H^+]_{Eq}$	Calc. ψ_0
$[La^{3+}]_T$	pH							
0	3.7	0	0	3.94	3.90	112.2	129.9	−36.2
2.125	3.7	0.42	1.29	3.93	3.91	114.9	126.8	−32.2
4.25	3.7	1.41	2.83	3.91	3.90	118.6	128.0	−29.1
8.5	3.7	4.78	6.26	3.91	3.91	120.2	127.9	−25.1
17.0	3.7	11.48	13.84	3.89	3.89	124.9	132.0	−20.2
0	4.0	0	0	4.40	4.34	38.5	47.0	−51.2
2.125	4.0	0.08	0.45	4.35	4.32	43.8	49.4	−43.5
4.25	4.0	0.41	1.38	4.30	4.29	49.1	52.9	−37.6
8.5	4.0	2.75	4.10	4.25	4.26	55.1	56.2	−30.6
17.0	4.0	9.30	11.03	4.22	4.24	58.9	59.8	−23.5
0	4.3	0	0	4.90	4.91	12.4	12.6	−69.9
2.125	4.3	0.03	0.05	4.81	4.83	15.2	15.2	−60.9
4.25	4.3	0.05	0.24	4.74	4.76	17.8	18.0	−52.3
8.5	4.3	1.26	1.69	4.59	4.64	25.0	23.2	−38.8
17.0	4.3	7.42	7.82	4.51	4.56	30.3	28.5	−27.1
0	4.6	0	0	5.32	5.50	4.7	3.3	−86.7
2.125	4.6	0.09	0.00	5.22	5.37	5.9	4.4	−78.5
4.25	4.6	0.10	0.02	5.15	5.26	6.9	5.7	−69.8
8.5	4.6	0.48	0.43	4.90	5.00	12.3	10.2	−50.1
17.0	4.6	5.29	5.49	4.74	4.80	17.7	16.4	−30.3
0	6.4	0	0	6.01	6.16	1.0	0.7	−105.3
2.125	6.4	0.04	0.00	5.96	6.00	1.1	1.0	−96.2
4.25	6.4	0.06	0.00	5.92	5.86	1.2	1.4	−88.0
8.5	6.4	0.07	0.04	5.73	5.55	1.8	2.9	−69.4
17.0	6.4	1.30	3.11	5.26	5.09	5.4	8.3	−35.2

Equilibrium values were measured after the sorption reaction. The measured pH change reflects both sorption and dilution upon the addition of the 50-μL vesicle mixture at pH 6.0 ± 0.2. Calculated values were obtained from the sorption model. Concentrations are expressed in μM and ψ_0 in mV.

0.570 for $[Al]_{Eq}$, and $R^2 = 0.9393$ and $RMSE = 0.067$ for pH_{Eq}.

Partitioning of Ions into Bound and Diffuse-layer Fractions

All cations, I^Z, will be attracted to a negatively charged surface according to the charge, Z. The higher the Z, the higher the attraction to the surface. The number of ions that bind to the surface, relative to the number that reside in the diffuse layer, will depend upon the binding constant, K_I. If K_I is low, most of the sorbed ions will be in the diffuse layer; if K_I is high, most of the sorbed ions will be bound. Table 3 presents some model-computed data to show the partitioning of some sorbed ions. Under the experimental conditions, more of the P^- charges are balanced by H^+ than by any other ion. The table shows also that nearly all of the sorbed K^+ is in the diffuse layer, that nearly all of the sorbed Al^{3+} and H^+ is bound, and that in a mixture of Al species, the overwhelming majority of sorbed Al will be in the trivalent state. It can be shown that the model is not very sensitive to K_{Al2+} and K_{Al+}, unless the values far exceed those that were assigned or unless the concentrations far exceed those

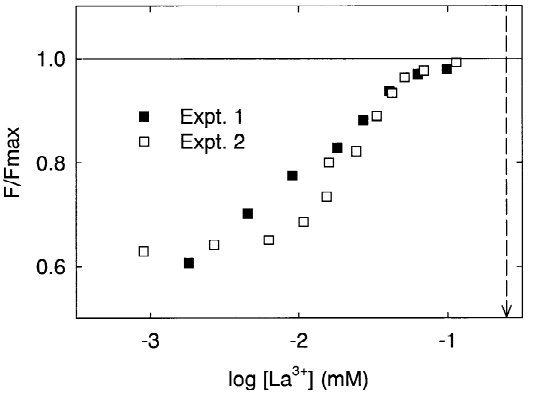

Fig. 6. The relative fluorescence (F/F_{max}) of 9-aminoacridine in a suspension of plasma membrane vesicles as a function of total $[La^{3+}]$. The arrow corresponds to 248 μM La^{3+}.

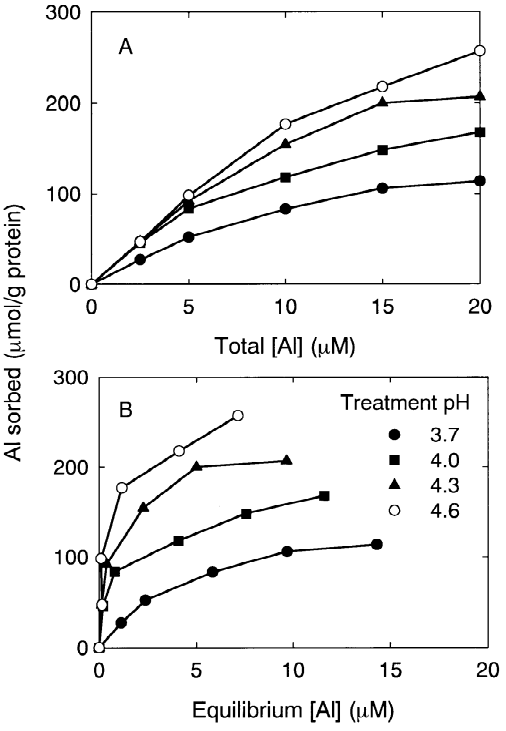

Fig. 7. Al sorbed to plasma membrane vesicles as a function of treatment pH and total [Al] in the reaction mixture (*A*) and equilibrium [Al] in the bulk-phase medium (*B*).

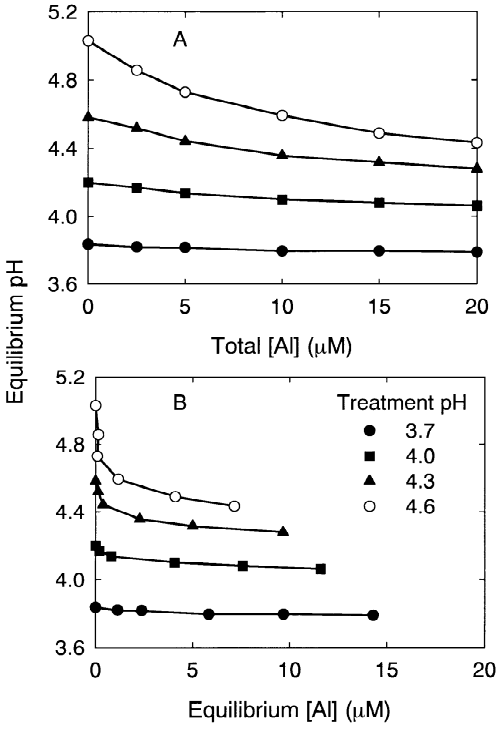

Fig. 8. Equilibrium pH of the vesicle suspension as a function of treatment pH and total [Al] in the reaction mixture (*A*) and equilibrium [Al] in the bulk-phase medium (*B*).

allowed by equilibrium with polynuclear or solid-phase Al species (*see* Bertsch & Parker, 1996). Note that the sum of sorbed cationic charges in Table 3 is slightly less than 100% of membrane-surface charges. This apparent deficiency of sorbed cations is balanced by *de*sorbed anions (*not shown*) so that the sum of charges in the whole system is zero.

Discussion

The plant root plasma membrane is composed of several classes of molecules, some of which are represented by several molecular species. These molecules may carry negative, or, less commonly, positive charges at one or more sites on the molecule. Each of these sites, and even uncharged sites, may be capable of binding various ions in various ligand-ion combinations. Thus it is a simplification to model the membrane as containing a single, negatively charged ligand, P^-, that binds all ions with a 1:1 stoichiometry. The success of the model in describing La^{3+}, Al^{3+} and H^+ sorption indicates the predominance of the type of reaction represented in Eq. 1 or indicates that a multiplicity of reaction types, in aggregate, can be represented by the equation.

Other binding models have appeared in published Gouy-Chapman-Stern models. Kinraide (1994) assumed two ligands, one singly charged and one neutral, each with 1:1 binding stoichiometry. K_I for binding to the neutral site was arbitrarily set to one tenth the K_I for binding to the negative site except that K_H (neutral site) $= K_H/100$ (negative site). Nir and some of his associates (Nir et al., 1994) have considered two binding reactions for divalent cations. One reaction is similar to that in Eq. 1, and the other combines two negative reaction sites with the divalent cation to form a neutral complex. These two reactions, and others, can be denoted by the general reaction

$$(P^-)_n + I^Z \rightleftharpoons (P_nI)^{Z-n} \tag{10}$$

where $(P^-)_n$ is a cluster of n number of negative binding sites. If this cluster reacts as a unit, the binding constant for I^Z will be

$$K_I = [(P_nI)^{Z-n}]/([I^Z]_0[P^-]/n) \tag{11}$$

where $[P^-]$ is the surface concentration (in mol m^{-2}) of unoccupied binding sites and $[(P_nI)^{Z-n}]$ is the surface concentration of the specific bound-ion complex.

Our choice of binding mechanism is based upon the fact that Eq. 1 (equal to Eq. 10 when $n = 1$) is the simplest mechanism that provides an excellent simulation of our sorption data. This simplicity means that

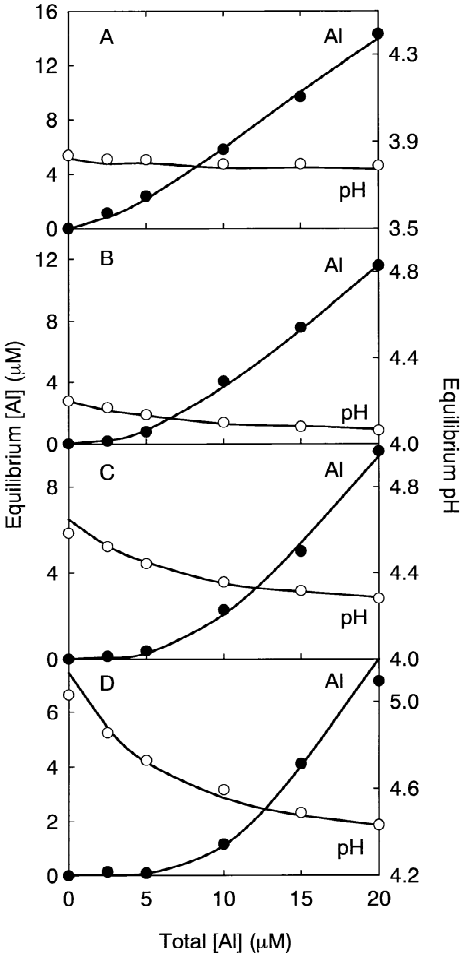

Fig. 9. Equilibrium [Al] and equilibrium pH of the vesicle suspension as a function of total [Al] and treatment pH values of 3.7 (*A*), 4.0 (*B*), 4.3 (*C*) and 4.6 (*D*). The drawn curves were generated by the sorption model. Note the changing scales for equilibrium [Al] and pH.

Table 2. Measured and computed values from a sorption experiment with three input variables

Treatment			Meas.	Calc.	Meas.	Calc.	Calc.
			$[Al]_{Eq}$	$[Al]_{Eq}$	pH_{Eq}	pH_{Eq}	ψ_0
$[Al]_T$	$[Ca^{2+}]_T$	pH					
0	16.5	4.0	0	0	4.59	4.50	−55.4
5	16.5	4.0	0.06	0.14	4.42	4.40	−40.5
10	16.5	4.0	1.39	0.92	4.28	4.31	−27.3
15	16.5	4.0	3.87	2.98	4.22	4.26	−18.3
0	266.5	4.0	0	0	4.48	4.40	−39.1
5	266.5	4.0	0.30	0.33	4.36	4.34	−30.6
10	266.5	4.0	1.46	1.40	4.26	4.28	−22.4
15	266.5	4.0	4.69	3.62	4.20	4.24	−15.7
0	2516	4.0	0	0	4.39	4.26	−19.5
5	2516	4.0	0.37	1.20	4.30	4.24	−15.7
10	2516	4.0	1.89	3.13	4.24	4.22	−12.1
15	2516	4.0	6.43	5.72	4.19	4.20	−9.2
0	16.5	4.3	0	0	5.12	5.22	−76.9
5	16.5	4.3	0.13	0.02	4.91	4.98	−61.9
10	16.5	4.3	0.83	0.14	4.71	4.77	−45.2
15	16.5	4.3	1.58	0.98	4.54	4.59	−29.0
0	266.5	4.3	0	0	4.86	4.82	−46.9
5	266.5	4.3	0	0.11	4.74	4.73	−40.0
10	266.5	4.3	0.83	0.47	4.59	4.65	−32.1
15	266.5	4.3	1.97	1.62	4.50	4.55	−23.3
0	2516	4.3	0	0	4.70	4.56	−22.4
5	2516	4.3	0.30	0.76	4.62	4.54	−19.0
10	2516	4.3	1.61	2.08	4.52	4.50	−15.4
15	2516	4.3	3.20	4.03	4.44	4.47	−12.2

Equilibrium values were measured after the sorption reaction. The measured pH change reflects both sorption and dilution upon the addition of the 50-μL vesicle mixture at pH 6.0 ± 0.2. Calculated values were obtained from the sorption model. Concentrations are expressed in μM and ψ_0 in mV.

fewer parameters need to be evaluated for the model than in the case of a two-ligand model (Kinraide, 1994) or a variable stoichiometry model (Nir et al., 1994). Other considerations include the fact that Eq. 1 allows charge reversal by divalent and trivalent cations, but not by monovalent cations. While there is some evidence that charge reversal may not occur upon the binding of ordinary metallic monovalent and divalent cations (Gibrat et al., 1985; Obi, 1989*b*), there is abundant evidence that La^{3+}, Al^{3+} and H^+ can cause the PM to become positively charged (Abe & Takeda, 1988; Akeson et al., 1989; Obi et al., 1989*a*,*b;* Wilkinson et al., 1993). Consequently, if a single binding mechanism is to be adopted, Eq. 1 seemed to be the best choice at pH values above those that induce charge reversal experimentally, and at Ca^{2+} concentrations below those that induce charge reversal according to our model (33.3 mM).

The determination of absolute, rather than relative, values for model parameters cannot be done by the adjustment of numerous parameters in a search for best fit. Initial constraints must be imposed. This was done by measurement in the case of intrinsic σ and by assignment in the case of K_K and K_{Ca}. Then, in a system composed only of vesicles, $CaCl_2$, HCl and KCl, other parameters, including P_T and K_H, were established by best fit. The resulting values for the suite of parameters (K_K, K_{Ca}, K_H and P_T) were consistent with values obtained from previous studies employing different methods (*see* the value for P_T obtained by Yermiyahu et al. [1994] using $^{45}Ca^{2+}$, and *see* cited literature for K_K and K_{Ca}). Each preceding set of parameters was then used to establish others (K_{La}, etc.). That the relative values for the parameters is good is indicated by the excellent fit between model and measurement, but the quality of the absolute values must be judged by additional criteria.

A limited amount of published data allows some independent evaluation of the La^{3+} sorption model. In an electrophoresis study of barley leaf protoplasts, Obi et al. (1989*b*) demonstrated that additions of La^{3+} to NaCl

Table 3. Percentage of the negative sites on the membrane surface that are neutralized by sorbed cations

Treatment			Sorbed Al^{3+}	Sorbed H^+	Sorbed Ca^{2+}	Bound Ca^{2+}	Sorbed K^+	Bound K^+
$[Al]_T$	$[Ca^{2+}]_T$	pH						
0	16.5	4.0	0	85.8	3.6	1.0	7.7	0.0
5	16.5	4.0	13.3	77.8	1.7	0.4	4.8	0.0
10	16.5	4.0	24.8	69.8	0.8	0.2	2.8	0.0
15	16.5	4.0	32.8	63.7	0.4	0.1	1.7	0.0
0	266.5	4.0	0	78.3	16.3	6.2	2.8	0.0
5	266.5	4.0	12.7	72.8	10.1	3.7	2.1	0.0
10	266.5	4.0	23.5	67.1	6.1	2.2	1.4	0.0
15	266.5	4.0	31.0	62.7	3.8	1.4	0.9	0.0
0	2516	4.0	0	67.3	28.7	16.7	0.5	0.0
5	2516	4.0	10.4	64.1	22.2	13.2	0.4	0.0
10	2516	4.0	18.7	61.6	17.0	10.4	0.3	0.0
15	2516	4.0	25.3	59.3	13.3	8.6	0.2	0.0
0	16.5	4.3	0	70.2	12.7	6.9	14.0	0.2
5	16.5	4.3	13.6	67.7	6.3	2.9	9.5	0.1
10	16.5	4.3	26.9	62.3	2.5	1.0	5.8	0.1
15	16.5	4.3	38.2	55.8	0.9	0.3	3.1	0.0
0	266.5	4.3	0	62.0	31.6	17.1	3.6	0.1
5	266.5	4.3	13.3	59.9	21.1	10.6	2.9	0.1
10	266.5	4.3	26.0	56.3	13.2	6.1	2.2	0.0
15	266.5	4.3	36.5	52.6	7.5	3.3	1.5	0.0
0	2516	4.3	0	51.9	43.7	28.9	0.6	0.0
5	2516	4.3	11.6	49.9	34.6	22.9	0.5	0.0
10	2516	4.3	21.6	48.7	26.4	17.6	0.4	0.0
15	2516	4.3	29.9	46.7	20.6	14.1	0.3	0.0

Over 99% of the sorbed Al^{3+} and H^+ is bound to the membrane surface (*not shown*); between 30 and 70% of the sorbed Ca^{2+} is bound to the membrane; and over 90% of the sorbed K^+ resides in the diffuse layer. Treatment concentrations are expressed in μM; sorbed and bound values are expressed as %.

solutions of 14 to 15 mM at pH 7.2 caused the PM surface to progress from negative to positive as $[La^{3+}]$ exceeded 170 μM. Abe and Takeda (1988), in another electrophoresis study of barley leaf protoplasts, observed a similar cross over at 300 μM in a medium of 100 μM $CaCl_2$ and NaCl at 4 to 5 mM at pH 6.7. When we applied our model to their solute conditions we obtained a cross over ($\psi_0 = 0$) at $[La^{3+}] = 227$ μM in each case. Thus there are three independent studies against which to test our model. One of the studies was done by us (Fig. 6), but involved assumptions and techniques entirely different from those used in the sorption studies upon which the model was constructed. We believe that the results of the three studies are reasonbly well simulated by our sorption model.

The binding parameters of Al were established under conditions of variable $[AlCl_3]$ and pH, and the computed values for equilibrium [Al] and pH corresponded well to the measured values under those conditions. A subsequent experiment with variable pH, [Al] and $[Ca^{2+}]$ was also successfully simulated without the adjustment of any binding parameters. We know of no precedent for such a ternary system using membranes, either synthetic or biological, and only a few ternary systems have been studied using clays (Sposito et al., 1983; Nir et al., 1986; Barak, 1989; Rytwo, Banin & Nir, 1996).

The literature provides little opportunity for independent confirmation of our model with respect to Al. If we apply the model to the solute conditions ([NaCl] = 0.1 M, pH = 4.5) reported by Wilkinson et al. (1993), a predicted cross over from negative to positive surface potentials ($\psi_0 = 0$) will occur when $[AlCl_3]$ exceeds 27.6 μM. According to their electrophoretic measurements with fish gill cells, cross over occurred at 16 μM. A previous Gouy-Chapman-Stern model (Kinraide, 1994) predicted cross over at 5.1 μM $AlCl_3$ ([NaCl] = 0.1 M, pH = 4.5), but that model had no more to draw upon with regard to an Al^{3+} binding constant than a binding affinity ratio of 560 for Al^{3+}:Ca^{2+} for zwitterionic phosphatidylcholine liposomes (Akeson et al., 1989).

The successful simulation of measured Al sorption

increases the confidence with which a Gouy-Chapman-Stern model may be used in the analysis of Al rhizotoxicity. Growth experiments indicate high negative correlations between root elongation and the computed activity of free toxicant ions at the PM surface (Kinraide, 1994). The empirical equation

$$RL = c + d/\exp[(a\{Al^{3+}\}_0)^b] \quad (12)$$

in which *a, b, c* and *d* are constants, described the sigmoidal relationship between root length (*RL*) and the computed activity of Al^{3+} at the PM surface. Surface activities were computed by the Nernst equation

$$\{Al^{3+}\}_0 = \{Al^{3+}\}_\infty \exp(-ZF\psi_0/RT) \quad (13)$$

where $\{Al^{3+}\}_\infty$ is the activity in the external medium. Eq. 13 requires ψ_0, which is a quantity computed by the sorption model (Eq. 4).

Correlations between *RL* and $\{Al^{3+}\}_\infty$ were often low in experiments where negative correlations with $\{Al^{3+}\}_0$ were high (Kinraide, 1994). This higher negative correlation with $\{Al^{3+}\}_0$ than with $\{Al^{3+}\}_\infty$ is certainly a reasonable expectation. One might also expect a high negative correlation between root elongation and the amount of PM-bound, or apoplast-bound, toxicant. For Al intoxication, this expectation has been expressed repeatedly (*see* Horst, 1995). Nevertheless, negative correlations for root elongation vs. computed PM-bound Al (or La) can be very low in experiments where negative correlations for root elongation vs. $\{Al^{3+}\}_0$ (or $\{La^{3+}\}_0$ are high. This was observed when the present sorption models were applied to previously published data (Figs. 2 and 3 in Kinraide, 1994). The implication that rhizotoxicity is the consequence of surface activities of Al (or La) rather than PM-bound Al (or La) should be the subject of further inquiry.

Previous studies indicate that at constant $\{Al^{3+}\}_\infty$, increases in H^+, Ca^{2+}, Na^+, or other cations cause a decrease in Al toxicity (Kinraide, 1994). This was attributed to decreases in $\{Al^{3+}\}_0$ caused by a reduction in surface negativity. Nevertheless, Eq. 12 does not adequately express root responses to the combined effects of Al^{3+} and H^+, each of which is simultaneously toxic and ameliorative because each reduces the surface activity of the other (Kinraide, 1993). The following extension of Eq. 12 provides an adequate description of root responses to the combined effects of the ions, including growth stimulation by low $\{Al^{3+}\}_\infty$ at low pH (Kinraide, 1994).

$$RL = c + d/\exp[(a_1\{Al^{3+}\}_0)^{b1} + (a_2\{H^+\}_0)^{b2}] \quad (14)$$

These and other interactions among Al^{3+}, other ions, and the PM surface can now be assessed with the aid of a Gouy-Chapman-Stern model whose parameters (including the first published binding coefficients for La^{3+} and Al^{3+}) are derived from the measured sorption of ions to plasma membranes.

We thank Dr. David Bligh and Ms. Barbara White for developing the procedures for the assay of La and Al.

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