Biochimica et Biophysica Acta, 986 (1989) 33–40
Elsevier

BBAMEM 74626

Adsorption of Al^{3+} to phosphatidylcholine vesicles

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(Received 31 July 1989)

Key words: Aluminum adsorption; Stern model; Aluminum toxicity

Aluminum toxicity to soil and aquatic organisms is widespread, but the mechanisms of toxicity are unknown. To understand these mechanisms, it is important to know how aluminum reacts with cell surfaces. In this report, we studied adsorption of Al^{3+} to liposomes composed of phosphatidylcholine, the most abundant phospholipid in plasma membranes of eukaryotic cells. Our equilibrium dialysis and electrophoresis experiments both showed that Al^{3+} has a 560-fold higher affinity for the phosphatidylcholine surface than Ca^{2+}. Unlike previous reports for adsorption of divalent metals, adsorption of Al^{3+} to phosphatidylcholine was predicted only approximately by the Stern model. Adsorption of AlF^{2+} and AlF_2^+ to the surface was not detectable at the activities we used. From our data, we calculate that Al^{3+} at 5×10^{-6} activity could neutralize the surface charge on plant cell plasma membranes and cause a surface potential shift from -30 to $+11$ mV. This is consistent with non-specific Al^{3+} inhibition of cation uptake by root cells. Al^{3+} adsorption to phosphatidylcholine may also play a role in aluminum uptake into cytoplasm by endocytosis.

Introduction

Aluminum (Al) is toxic to a variety of cells, but the mechanisms of toxicity are unknown. Among plants, a major step in the toxic reaction appears to be adsorption of Al to the root surface which may be the direct cause of a lesion or which may be a necessary first step prior to uptake into root cytoplasm [1]. In either case, Al adsorption diminishes divalent cation binding to the root surface [1] and vice versa [2].

Binding sites on the membrane surface are likely to be either carboxylate or phosphate groups because Al forms electrostatic bonds preferentially with oxygen donor ligands [3]. In the absence of a chelate effect, Al^{3+} has a greater affinity for phosphate oxyanions than it does for carboxyl oxyanions [3]. Important phosphate ligands on the plasma membrane surface of eukaryotic cells are anionic phosphodiesters associated with phospholipids. The neutral, zwitterionic phospholipids phosphatidylcholine (PC) and phosphatidylethanolamine (PE) together constitute about 80% of the phospholipid in soya bean root plasma membranes [4], and NMR analysis indicates that PC is preferentially sequestered in the outer leaflet of erythrocyte and platelet plasma membranes [5]. Thus, for practical reasons, it is important to define the nature of Al bonding to PC on plasma membrane surfaces.

Most evidence indicates that metals bind to the phosphate group in PC with stability constants ranked as trivalent metals > divalent metals > monovalent metals [6]. It is sometimes found, however, that metals complexed to halides [7] or hydroxyl ions [8] adsorb more readily to surfaces, presumably because the free energy of metal solvation is reduced at lower valency [8]. Al^{3+} is readily hydrolysed above pH 5 and it is known to form stable, cationic complexes with F^- and SO_4^{2-}. Thus it is important to determine if Al^{3+} is preferentially adsorbed to PC surfaces or if monovalent or divalent Al-complexes are preferred.

One of our objectives in this study was to examine the chemistry of Al adsorption to phosphatidylcholine. Specific questions we addressed were: (a) what is the affinity of Al^{3+} for PC surfaces, and how does it compare with the affinity of Ca^{2+}?; (b) is Al^{3+} preferentially bound to the PC surface or do monovalent and divalent Al-halides contribute measurably?; and (c) can the Stern model of the diffuse double layer predict Al^{3+} adsorption to the PC surface? McLaughlin et al. [9] have shown that one form of the model can predict binding of divalent cations to PC as bulk solution concentration of the cations is varied.

Our other objective was to determine if Al^{3+} bonding to PC could help to explain antagonisms between Al and other cations in plant nutrition. The observations we considered are: (a) 5 μM Al^{3+} significantly inhibits

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root growth, but a variety of cations (notably Ca^{2+}) can overcome the inhibition at millimolar concentrations via a non-specific reaction at the root surface [2]; and (b) binding of Al^{3+} to root surfaces significantly inhibits adsorption and uptake of Ca^{2+} and K^+ by barley plants, while increasing Cl^- adsorption [1].

We conclude that Al^{3+} alone among monomeric Al ions binds strongly to PC surfaces, and that the Stern model underestimates adsorption for the conditions we used. This reaction is consistent with Al^{3+} significantly changing plasma membrane surface potential and thus decreasing cation adsorption and uptake by plant roots. Adsorption of Al^{3+} to PC may also play a key role in Al absorption into cytoplasm by pinocytosis.

Materials and Methods

Preparation of phospholipid vesicles (liposomes)

Vesicles were prepared by the method of Mayer et al. [10]. A 4-ml aliquot of 20 mg ml^{-1} egg phosphatidylcholine (Avanti Polar Lipids) was dried under a stream of nitrogen gas and then resuspended in 5.5 ml of a standard matrix which contained 0.12 M KNO_3 brought to pH 3.8 by the addition of 0.095 M HCl. This suspension was sonicated for about 15 s and alternately frozen in liquid nitrogen then thawed in lukewarm tap water. This step was repeated five times. The suspension was passed ten times through two 0.1 μm diameter pore-size Nuclepore filters at 200 psi. Under similar conditions, this procedure yielded unilamellar vesicles 0.1 μm in diameter with 50% of the phospholipid on the vesicle surface [10]. After filtration, the 20 mM phospholipid suspension was stored under argon at 5 °C for up to 4 days prior to use. Calculated phospholipid concentrations were tested periodically by colorimetric measurement of phosphate following acid digestion of the suspension [11].

Measurement of aluminum adsorption onto phosphatidylcholine surfaces by equilibrium dialysis

Aliquots from the PC stock were diluted ten-fold in solutions of the standard matrix that contained Al^{3+} at activities from 0 to 4×10^{-6}. Two-ml lots of each dilute suspension (1 mM surface PC) contained in Spectrapore dialysis membranes, were immersed in 200 ml of the matrix solution at the same Al^{3+} activities in a Teflon jar. They were sealed under argon and allowed to equilibrate for 4.5 h on a reciprocating shaker at room temperature. After equilibration, aliquots from inside and outside of the dialysis membranes were placed in 0.1 M HNO_3 and analyzed for Al content by graphite furnace atomic absorption spectroscopy [12]. The surface excess of Al, Γ_{Al}, was the difference between Al concentration inside and outside of the dialysis membranes. Equilibration times of up to 24 h did not change Al adsorption relative to 4.5 h controls.

Our experiments to measure the effects of Ca^{2+} and F^- on Al adsorption used the same protocol as above with the following modifications. In the Ca^{2+} competitive bonding experiments, Al^{3+} activity was maintained constant at 2×10^{-6}, and Ca^{2+} activity was varied between 0 and 5×10^{-3}. Ionic strength of these solutions was maintained constant by varying the addition of KNO_3. In the F^- experiments, total solution Al was maintained constant at 21 μM, and Al^{3+} activity was varied by additions of KF to yield final F^- activities between 0 and 3.5×10^{-5} measured by an ion specific electrode.

Measurement of the electrophoretic mobility of vesicles

The electrophoretic mobility of PC vesicles was measured by conventional procedures [9,13,14] using a Rank Brothers apparatus. To locate the stationary layer, we initially focused on a plane 0.293 μm from the wall of the electrophoresis cell (the theoretical stationary layer). We then adjusted the plane of focus to the true stationary layer using human erythrocytes as a standard for which the electrophoretic mobility, μ, is known to be 1.27 $\mu m\ s^{-1}$ per volt cm^{-1} in 0.067 M phosphate buffer at pH = 7.4 and 25 °C [15].

Large multilamellar vesicles were prepared by drying egg phosphatidylcholine (Avanti Polar Lipids) under a stream of nitrogen gas, redissolving the lipid in matrix and shaking glass beads in the suspension for 30 s [16]. Then, 0.4 ml of the lipid suspension was combined with 3.6 ml of matrix with or without Al^{3+} or Ca^{2+} to yield a final lipid concentration of 0.125 mg ml^{-1}. The matrix solutions we used were 0.12 M KNO_3 or 0.12 M KCl, at pH 3.8, generally without a pH buffer. We did not use a pH buffer because compounds that are useful at pH 3.8 are carboxylic acids, for example acetate which has a small but measurable affinity for Al^{3+}. To demonstrate that absence of buffer did not significantly affect our results, we repeated several assays in the presence of 10 mM sodium acetate/HNO_3. Aluminum stock solutions were 1 g l^{-1} in 1% HCl or 1% HNO_3. We were careful to add the Al^{3+} stock to pre-acidified assay solutions to prevent formation of Al-hydroxide polymers at higher pH.

Electrophoretic mobility of the PC vesicles was measured at 25 °C, 1.2 mA and 40 V. The value for a single assay is the composite of five measurements in each direction. All treatments were repeated at least three times using separate preparations. The zeta potential was calculated from the Helmholtz-Smoluchowski equation

$$\zeta = (4\pi\eta\mu)/\epsilon \qquad (1)$$

where ζ is the zeta potential in mV, η is the viscosity of water, ϵ is the dielectric constant of water and μ is the electrophoretic mobility in $\mu m \cdot s^{-1}$ per $V \cdot cm^{-1}$. Given

the common assumption that the viscosity and dielectric constant are the same in the diffuse layer as in bulk water [9], Eqn. 1 becomes $\zeta = 12.85\ \mu$. As an additional test of the accuracy of our data, we measured the electrophoretic mobility of egg phosphatidylcholine vesicles in 0.1 M NaCl, 0.01 M Tris/HCl (pH 7.5). In the presence of $CaCl_2$ ranging from 0 to 50 mM, mean zeta potentials calculated from our values of μ were consistently 1 mV higher than previously published zeta potentials for the same conditions [9], but the standard deviations overlapped.

Calculation of bulk phase solution activities

Bulk phase Al^{3+} and Ca^{2+} concentrations and activities for the various treatments were calculated using GEOCHEM version 1.2 [17]. In this program, computation involves a non-linear equation for each aquo ion, each complex and each ion pair in the system. This set of equations is solved simultaneously using an iterative numerical technique. The thermodynamic stability constants we used are those in the GEOCHEM version 1.2 database, which has been applied specifically to aluminum speciation by its authors [2].

We chose to use ion activities in our calculations rather than concentrations because electrolyte solutions in excess of 0.1 M are clearly non-ideal. Thus, use of concentrations to estimate surface binding constants would lead to substantial errors especially when comparing reactions of ions with different valences (e.g., Ca^{2+} vs. Al^{3+}). GEOCHEM 1.2 calculates individual ion activity coefficients, γ, using the Davies equation [17]. In 0.12 M electrolyte solutions, this gives $\gamma_1 = 0.77$ $l \cdot mol^{-1}$, $\gamma_2 = 0.36\ l \cdot mol^{-1}$ and $\gamma_3 = 0.10\ l \cdot mol^{-1}$ for mono-, di- and trivalent ions, respectively. Because activities are unitless, our calculated 1 : 1 thermodynamic stability constants (K_1) are also unitless. In this report, parentheses, ( ), denote activities and brackets, [ ], denote concentrations.

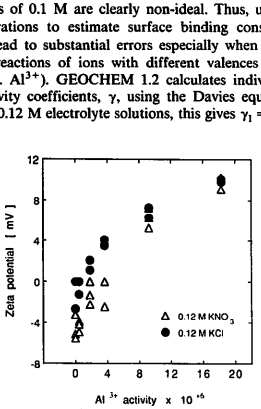

Fig. 1. The effect of Al^{3+} on the zeta potential of multilamellar phosphatidylcholine vesicles. Zeta potential was measured by microelectrophoresis at pH = 3.8 in 0.12 M KNO_3 or in 0.12 M KCl. Each point represents the mean of five runs in each direction at 25 °C, 1.2 mA and 40 V. Polynomial equations fit to the two data sets by the least squares method (not shown) were $Y = -0.6 + 1.1X - 0.03X^2$ (KCl matrix) and $Y = -4.4 + 1.5X - 0.04X^2$ (KNO_3 matrix) in which Y is zeta potential in mV and X is (Al^{3+}) multiplied by 10^6. R^2 was greater than 0.95 for both functions.

Results

The effect of Al^{3+} on the zeta potential of phosphatidylcholine vesicles

Figure 1 shows the effect of Al^{3+} on the zeta potential of PC vesicles. At zero added Al^{3+}, the zeta potential of vesicles in 0.12 M KCl was -0.9 ± 1.5 mV, in agreement with previous measurements on egg PC from the same source in 0.1 M NaCl [9]. This indicates that the vesicles did not contain negatively charged contaminants. In 0.12 M KNO_3 the zeta potential of the PC vesicles was -4.6 ± 1.2 mV, which suggests that NO_3^- binds to the PC choline head group as was proposed recently to explain a -3 mV potential on PC vesicles in 0.1 M $NaNO_3$ [13]. * Addition of Al^{3+} at activities as low as 1.8×10^{-6} caused a significant increase in ζ for PC vesicles in both KCl and KNO_3. Polynomial equations fit to the two data sets are $Y = -0.6 + 1.1X - 0.03X^2$ (KCl matrix) and $Y = -4.4 + 1.5X - 0.04X^2$ (KNO_3 matrix) in which Y is zeta potential in mV and X is (Al^{3+}) multiplied by 10^6. At low values of (Al^{3+}), the slope of the curve in the KNO_3 matrix is 1.5 and in the KCl matrix it is 1.1. This difference can be accounted for by the effect of negative surface potential on Al^{3+} activity at the membrane-solution interface in the KNO_3 matrix. In a subsequent section we will show that at zero surface potential the slopes of the curves are virtually identical.

Addition of 10 mM acetate buffer raised the zeta potential by 0.8 mV, but did not modify the effect of Al^{3+} in the KNO_3 solution.

Al^{3+} has a high affinity for the phosphatidylcholine surface

Tests of the Stern model, and comparison of Al^{3+} adsorption to PC with adsorption of other metals, require a surface binding constant. McLaughlin et al. [18] assumed that the Langmuir expression is appropriate to describe binding of divalent metals to phosphatidylserine and PC. In our case this gives

$$\Gamma_{Al^{3+}} = K_1(\Gamma_0 - \Gamma_{Al^{3+}})(Al^{3+})_0 \qquad (2)$$

* The zeta potential of PC vesicles in 0.12 M $KClO_4$ at pH 3.8 was -25 mV. Thus, perchlorate would be expected to have a large effect on apparent binding constants to membrane surfaces and should be avoided for that reason. This contrasts with measurement of metal-ligand stability constants in solution where perchlorate salts are frequently the electrolytes of choice.

where $\Gamma_{Al^{3+}}$ is the excess of Al^{3+} on the PC surface in $mol \cdot m^{-2}$, $(Al^{3+})_0$ is the activity of Al^{3+} at the interface *, K_1 is a unitless constant and Γ_0 is the total number of phosphodiester binding sites on the PC surface assuming 1:1 stoichiometry between Al^{3+} and PC. For $\Gamma_0 \gg \Gamma_{Al^{3+}}$, rearrangement of Eqn. 2 yields

$$K_1 = \Gamma_{Al^{3+}}/\Gamma_0(Al^{3+})_0 \quad (3)$$

The least ambiguous estimate of K_1 is achieved when the potential at the PC surface is zero. This is true because at $\psi_0 = 0$ mV, surface Al^{3+} activity is equal to bulk phase Al^{3+} activity which can be accurately controlled. Fig. 1 shows that $\zeta = 0$ mV at 0 Al^{3+} activity (KCl matrix), and at 3.18×10^{-6} Al^{3+} activity (KNO_3 matrix). Assuming here that $\zeta = \psi_0$, the polynomial functions from Fig. 1 give $d\psi_0/d(Al^{3+})$ equal to 1.1 mV/1 μM and 1.2 mV/1 μM for the KCl and KNO_3 electrolytes, respectively. The surface charge density, σ, can be calculated from ψ_0 using the Gouy-Chapman equation [19]

$$\sigma = \left\{2\epsilon_r\epsilon_0 RT \sum C_{ib}[\exp(-z_i F\psi_0/RT) - 1]\right\}^{1/2} \quad (4)$$

where σ is in $C \cdot m^{-2}$, ϵ_r is the dielectric constant of water (80), ϵ_0 is static permittivity equal to 7.08×10^{-10} $J^{-1} \cdot C^{-2} \cdot m^{-1}$, C_{ib} and z_i are the bulk phase concentration and valence of the ith ion, ψ_0 is the surface potential in V and R and T have their usual meanings. By converting units, σ in $C \cdot m^{-2}$ becomes $\Gamma_{Al^{3+}}$ in mol Al^{3+} per m^2 PC. Thus, $d\psi_0/d(Al^{3+})$ becomes $d\Gamma_{Al^{3+}}/d(Al^{3+})$. Assuming that K_1 is a constant at low surface-bound Al^{3+}, it can be shown that $(d\Gamma_{Al^{3+}}/d(Al^{3+})) = (\Gamma_{Al^{3+}}/(Al^{3+}))$. Finally, this expression divided by $\Gamma_0 = 2.4 \times 10^{-6}$ mol per m^2 PC gives K_1 equal to 1278 in KCl, and K_1 equal to 1460 in KNO_3. The difference between the two constants is probably due to experimental error, although the lower value in 0.12 M KCl is consistent with formation of Al-Cl complexes which are not considered by GEOCHEM.

Recent studies of lipid bilayer surface potential [13,14,18,20] have assigned ψ_0 to the vesicle surface at 2 Å from the electrophoresis shear plane. Thus, ψ_0 is not equal to ζ but is somewhat greater. When we make the same assumption and calculate ψ_0 from ζ using diffuse double layer theory (Ref. 20, Eqn. 4), K_1 becomes 1610 in KCl and 1869 in KNO_3.

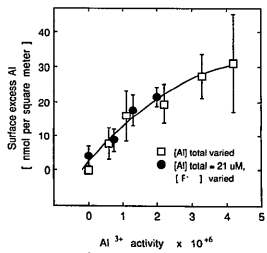

Fig. 2. The effect of Al^{3+} activity on surface excess Al adsorbed to PC vesicles. All assays were by graphite furnace atomic absorption spectroscopy following equilibrium dialysis at pH 3.8 in 0.12 M KNO_3. In one set of data, (Al^{3+}) was controlled by varying the total concentration of Al, [Al] total, in the reaction vessel; in the other set of data, [Al] total was constant at 21 μM, and (Al^{3+}) was controlled by varying F^- concentration. Each point represents the mean ±S.E. for at least six replicates.

The adsorption constant for this reaction can also be predicted from our graphite furnace data. The slope of the curve in Fig. 2 is 3.9 nmol $Al \cdot m^{-2}$ per 1 μM Al^{3+} at $(Al^{3+}) = 3.3 \times 10^{-6}$ where the zeta potential in KNO_3 is approximately zero (see Fig. 1). The PC surface area was 421 m^2 per liter of solution within the dialysis membranes, assuming 70 $Å^2$ per lipid and assuming that only lipids in the outer bilayer leaflet reacted with Al^{3+} **. Using these values to solve for Eqn. 3 gives $K_1 = 1658$, or approximately 1.1-fold the value calculated from the zeta potential measurements in KNO_3. This result is noteworthy because it supports our assumption that Al adsorbs to PC as Al^{3+}. If Al on the PC surface were predominantly divalent due to hydrolysis, K_1 calculated from the graphite furnace data would be 1.5-fold higher than K_1 from the electrophoresis data; similarly assuming 1 charge per Al atom due to hydrolysis, K_1 (graphite furnace) would be 3-fold higher than K_1 from the electrophoresis experiment. This is supported by experimental evidence presented in the next section.

* In rigorous thermodynamic formulations, activities of electrolytes or ion activities that reduce to electrolyte activities are used. This is true because individual ion activities cannot be measured. Eqn. 3 is valid because the anion activities in the numerator and denominator cancel. Thus, $(Cl^-)^3/[(Al^{3+})(Cl^-)^3]$ reduces to $1/(Al^{3+})$.

** This is a reasonable assumption because the permeability coefficient of Al^{3+} is likely to be equal to or less than coefficients for divalent cations, i.e., 1×10^{-12} $cm \cdot s^{-1}$. Using Fick's Law and assuming 5×10^{-6} Al^{3+} activity in the external solution (the highest value we used) flux would be approximately 5×10^{-21} $mol \cdot cm^{-2} \cdot s^{-1}$. The surface area of a 0.1 μm diameter vesicle is 3.1×10^{-8} cm^2 and the trapped volume is 5.2×10^{-13} cm^3. Using these values it can be shown that at 4.5 h the activity of Al^{3+} inside the liposomes would be 5×10^{-9} or 1/1000th the external activity.

We conclude that the adsorption constant for Al^{3+} bonding to the PC surface is about 1500. As we will show later, this is at least 500-fold greater than K_1 for Ca^{2+} adsorption to PC.

Al^{3+} bonds to the PC surface; monovalent and divalent Al-F complexes do not

The data in Fig. 1 demonstrate that Al readily adsorbs to PC vesicles under conditions where Al^{3+} is the only significant species in solution. In most acidic surface waters, however, a variety of monovalent and divalent Al complexes exist. Would we expect these complexes to increase Al adsorption to PC as is true for cobalt complexes adsorbed to PC [7] and to a number of oxides [8]?

Fig. 2 shows Γ_{Al} when (Al^{3+}) was controlled either by varying [Al] total, or by adding F^- to a constant [Al] solution. The two procedures yield the same relationship between (Al^{3+}) and Γ_{Al} despite the presence of as much as 3.2×10^{-6} (AlF^{2+}) and 12×10^{-6} (AlF^{2+}) in the fluoride treatments. Indeed, least squares fits between (AlF^{2+}) and Γ_{Al}, or (AlF_2^+) and Γ_{Al} gave regression coefficients near zero. On this basis, we infer that Al^{3+} is the only one of these monomeric Al species that measurably adsorbs to PC surfaces.

Al^{3+} affinity for the PC surface is 560-fold higher than Ca^{2+} affinity

One of our major objectives in this study was to compare binding of Al^{3+} and Ca^{2+} to the PC surface. Figure 3 shows our data for the effect of Al^{3+} and Ca^{2+} on the zeta potential of PC vesicles in 0.12 M KNO_3. For comparison, we have included data from McLaughlin et al. [9] for Ca^{2+} adsorption to PC vesicles in 0.1 M NaCl. It is clear that Al^{3+} binding occurs at

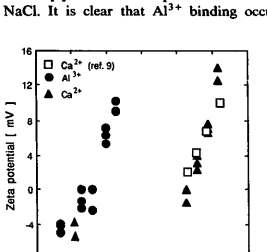

Fig. 3. The effects of Al^{3+} and Ca^{2+} on the zeta potential of multilamellar phosphatidylcholine vesicles. In our experiments, zeta potential was measured by microelectrophoresis at pH = 3.8 in 0.12 M KNO_3 as described in the text. The microelectrophoresis experiments of McLaughlin et al. [9] were conducted on phosphatidylcholine vesicles from the same source in 0.1M NaCl buffered at pH 7.0.

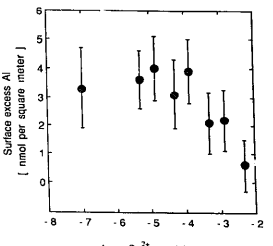

Fig. 4. The effect of increasing Ca^{2+} activity on adsorption of Al^{3+} to PC vesicles. (Al^{3+}) was fixed at 2.0×10^{-6}, and Ca^{2+} was varied by addition of $Ca(NO_3)_2$. Ionic strength was maintained constant at 0.12 M by the addition of KNO_3, and pH of the bulk phase was 3.8. Surface excess Al^{3+} was measured by graphite furnace atomic absorption spectroscopy following equilibrium dialysis. Each point represents the mean ±S.E. for six replicates.

activities several orders of magnitude lower than Ca^{2+} binding. To make a quantitative comparison between the two adsorption reactions, we calculated an adsorption constant, K, for Ca^{2+}-PC as described above. The result is $K_1 = 2.6$ ($\psi_0 = \zeta$) or $K_1 = 3.2$ (ψ_0 calculated from ζ assuming 2 Å between the shear plane and the vesicle surface). These values are nearly identical to the Ca^{2+}-PC stability constant measured by McLaughlin et al. [9] when the latter is corrected for ionic strength (i.e., $K_1 = 2.7$), and they show that Ca^{2+} affinity for the PC surface is about 560-fold less than Al^{3+} affinity.

To get a direct measure of competitive bonding of Al^{3+} and Ca^{2+} at the PC surface, we measured Al^{3+} adsorption at a fixed Al^{3+} activity in the presence of increasing levels of Ca^{2+}. The results in Fig. 4 indicate that the surface excess of Al^{3+} at 2.2×10^{-6} Al^{3+} activity is reduced to half in the presence of 1×10^{-3} Ca^{2+} activity.

The Stern model underestimates Al^{3+} adsorption to PC surfaces

Several studies conclude that a combination of Henry's Law, the Boltzmann distribution, and the Gouy-Chapman equation (a form of the Stern model) adequately describes monovalent and divalent cation adsorption to phospholipid surfaces [9,13,18]. Can the Stern model predict Al^{3+} adsorption to PC surfaces?

A conventional form of the Stern model [22,23] is

$$\Gamma_{ih} = \Gamma_0 K a_i \exp(-z_i F\psi_0/RT) \quad (5)$$

where Γ_{ih} is the surface excess of an ion in the inner Helmholtz plane (ions/m^2), a_i is the activity of ion i in bulk solution, and the other constants and variables are

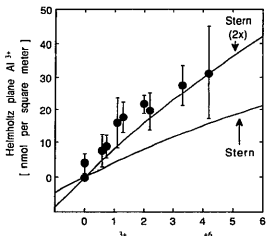

Fig. 5. Comparison between measured surface excess Al^{3+} in the inner Helmholtz plane, $\Gamma_{Al^{3+}_h}$ and $\Gamma_{Al^{3+}_h}$ predicted by the Stern model (Eqn. 5). In the lower curve, ψ_0 in the Stern model was set equal to ζ due to Al^{3+} adsorption to PC as shown in Fig. 1. The upper curve was arbitrarily set at twice the value predicted by the Stern model. Measured values of $\Gamma_{Al^{3+}_h}$ (means ±S.E.) are equal to Γ_{Al} in Fig. 2 corrected for minor changes in diffuse double layer Al^{3+} due to changes in ψ_0 (Ref. 20, Eqn. 4).

as defined earlier. In Eqn. 5, the stoichiometry of the adsorption reaction is factored out between Γ_0 and K. This is an important difference from the form of the model used by McLaughlin et al. [9], wherein reaction stoichiometry is a variable. Eqn. 5 assumes that $\Gamma_{ih} \ll \Gamma_0$ which is true for Al^{3+} adsorption to PC at the activities we used. At $\psi_0 = 0$, Eqn. 5 becomes Eqn. 3 which we used earlier to solve for K_1.

To estimate $\Gamma_{Al^{3+}_h}$ from the Stern model, we solved Eqn. 5 setting $K = K_1 = 1500$, a_i equal to (Al^{3+}) and ψ_0 equal to the zeta potentials due to Al^{3+} adsorption to PC in 0.12 M KNO_3 (Fig. 1). The results shown in Fig. 5 reveal that predicted $\Gamma_{Al^{3+}_h}$ was about one-half $\Gamma_{Al^{3+}_h}$ measured by equilibrium dialysis even when the latter was corrected for minor changes in diffuse double layer Al associated with changes in ψ_0 [19]. The fit of the Stern model to the data was not improved significantly when we solved Eqn. 5 for ψ_0 calculated 2 Å from the shear plane and for $K = K_1 = 1710$.

Discussion

We conclude that the surface binding constant for the Al^{3+}-PC complex is 560-fold greater than for the Ca^{2+}-PC complex. Unlike Co^{2+} binding to PC [7], halogenation does not increase Al^{3+} adsorption to PC. We further conclude that the Stern model underestimates Al^{3+} adsorption to PC in a KNO_3 solution when ψ_0 is calculated at the bilayer surface from ζ.

The activity of Al^{3+} was carefully controlled in these experiments. Frequently, in biological research, aluminum salts are added to neutral pH solutions and the metal is wrongly assumed to remain in the trivalent form. For example, Deleers et al. [24] added 30–200 μM Al salt to a pH 7.4 buffer and measured its effects on fusion of phosphatidylserine liposomes. Calculations using GEOCHEM suggest that, at 30 μM total Al, only about 1 pmol · l^{-1} exists as Al^{3+} and the remainder is present as hydroxy species and, at 20 μM Al, 85% would precipitate. Thus, the observed effect of Al on PS liposome fusion could be due to any of several Al hydrolysis products or to Al^{3+} at bulk solution concentrations 6 orders-of-magnitude lower than the authors assumed.

A theoretical framework with which to understand the relative affinities of Ca^{2+} and Al^{3+} for the PC surface was outlined by James and Healy [8]. They point out that any adsorption reaction is the sum of three free energy terms. Thus

$$\Delta G^0_{ads} = \Delta G^0_{coul} + \Delta G^0_{solv} + \Delta G^0_{chem} \quad (6)$$

where ΔG^0_{coul} is the change in coulombic energy due to adsorption, ΔG^0_{solv} is the change due to displacement of the hydration shell associated with the ion and the surface and ΔG^0_{chem} is any interaction term not included in ΔG^0_{coul} (e.g., image forces, hydrogen bonding, covalent bonding). Our data give no direct information about these free energy terms, however the following argument is reasonable. We assume that the attraction between each metal and the oxyanion is mainly coulombic. According to Coulomb's Law,

$$\Delta G^0_{coul} = z^+ z^- (e)^2/(4\pi\epsilon_0\epsilon_r r) \quad (7)$$

where z^+ and z^- are the charges on the cation and anion respectively, e is the electronic charge (1.6×10^{-10} C), r is the distance between charges in m, and ϵ_0 and ϵ_r are as defined earlier. In our calculations we assume the following radii: O^- (179 pm), Al^{3+} (50 pm), Ca^{2+} (99 pm) [25] and water (276 pm) [8]. Thus, ΔG^0_{coul} for the Al^{3+}–oxyanion bond is about -25 kJ · mol^{-1} (assuming all waters of hydration are removed) compared to -14 kJ · mol^{-1} for the Ca^{2+}–oxyanion bond. Addition of one water of solvation between the metal and the oxyanion reduces the bond energy to -11 kJ · mol^{-1} for Al^{3+} and to -7 kJ · mol^{-1} for Ca^{2+}. The point is that at any given hydration number, the smaller ionic radius and greater charge of Al^{3+} favor electrostatic bonding to the phosphodiester. In contrast, formation of the coulombic bond removes waters of hydration, therefore ΔG^0_{solv} is a positive term in Eqn. 6. For insulating solids (e.g., SiO_2) the solvation term is sufficiently important that larger ionic radius and lower charge may favor adsorption [8]. In our case, ΔG^0_{solv} appears to play a minor role because Ca^{2+} and AlF^{2+} bond much less strongly to the PC surface than does Al^{3+} (Figs. 3 and 4).

Our results indicate that the Stern model underestimates surface bound Al^{3+} when ψ_0 is set equal to ζ or when ψ_0 is calculated at the bilayer surface 2 Å from the shear plane. One explanation is that the anionic PC phosphodiester to which Al^{3+} probably adsorbs is 3 Å beneath the choline plane (Ref. 6, Fig. 12), so that bound Al^{3+} is several ionic radii from the bilayer surface. Aveyard and Haydon [21] show that in a uni-univalent electrolyte at 0.1 M the thickness of the diffuse layer is only 10 Å. It follows that a 3 Å underestimate of the distance to the adsorption plane could lead to a substantial underestimate of ψ_0. A second possible explanation is that discrete charge effects becomes important for Al^{3+} adsorption to PC. However, Winiski et al. [13] show that surface adsorption of most ions to phospholipid bilayers is best modeled by smeared charge theories (e.g., the Stern model), and that discrete theories are only accurate for highly charged solutes such as hexavalent melittin. Finally, in our calculations we assumed that Al^{3+} was the only ion bound in the Stern layer. In fact, NO_3^- can also bind to PC (Fig. 1) and this would partially neutralize the surface charge due to bound Al^{3+}. Thus, Eqn. 5 would underestimate surface bound Al^{3+} because it cannot account for enhancement of Al^{3+} adsorption by bound NO_3^-.

Although the accuracy of the Stern model for describing Al^{3+} adsorption is questionable, our results are unambiguous and they may help to explain the observed antagonism between Al and Ca^{2+} in plant nutrition. Specifically we propose that Al reduces adsorption and uptake of cations (notably Ca^{2+}) by binding to membrane phospholipids as Al^{3+} and causing a positive increase in the membrane surface potential. As a corollary, Ca^{2+} and other divalent cations would be expected to reduce both uptake and surface adsorption of positively charged Al compounds when their concentrations were orders of magnitude higher than Al^{3+}. Again, this would not involve exchange of the divalent metal for Al^{3+} at a specific site; rather, the divalent metals would increase surface potential and thereby decrease the activity of Al^{3+} near the membrane surface.

For example, the zeta potential of tobacco protoplasts is about -30 mV in a 0.01 M KCl solution at pH = 5.8 [26]. To approximate the effect of 5×10^{-6} (Al^{3+}) on surface potential of the protoplasts under these conditions, we solved the Gouy-Chapman equation (Eqn. 4) and the Stern model (Eqn. 5) simultaneously. That is, the Gouy-Chapman equation was used to give ψ_0 for the initial surface charge density on the protoplast surface. This value of ψ_0 was then used to predict Al^{3+} bound to the surface based on the Stern model, which in turn gave a new value of ψ_0. The calculations were then repeated until changes in ψ_0 and surface bound metal became very small as shown graphically using a spread sheet program. In our calculations, we assumed that bound Al^{3+} was twice the quantity predicted by zeta potential incorporated into the Stern model (see Fig. 5), and that the binding constant for the Al^{3+}–PC bond was $K = K_1 = 1500$. * Our results show that bound Al^{3+} would be expected to neutralize the negative surface charge and generate a positive surface potential of 11 mV. Macdonald and Seelig (ref. 27, Fig. 10) calculated a similar effect of millimolar concentrations of Ca^{2+} on ψ_0 at the surface of mixed POPC : POPG bilayers in 0.1 M NaCl.

It follows that Al^{3+} would induce a significant decrease in adsorption of cations to the protoplast surface and thus a decrease in passive diffusion of cations across the membrane. This prediction is in qualitative agreement with results of Clarkson and Sanderson [1] who showed that 100 μM total solution Al reduced exchangeable Ca^{2+} by 80% on roots of intact barley plants. Furthermore, in the presence of 200 μM Al, plant absorption of Ca^{2+} and K^+ was reduced by 70 and 80%, respectively. They conclude that this is a non-specific reaction at the root surface dependent upon the polyvalency of Al rather than on its precise chemical nature. Our data show that one likely site for Al^{3+} binding is the PC phosphodiester and that the non-specific interaction may be due to an Al^{3+}-induced change in surface potential.

Our data are also consistent with a reported amelioration of Al^{3+} toxicity in wheat by monovalent and divalent cations [2]. Addition of 2 μM (Al^{3+}) to a 400 μM $CaCl_2$ medium at pH = 4.3 gave root growth of 33 mm over 2 days; the addition of 1 mM (Ca^{2+}) to the same Al^{3+} treatment resulted in 44 mm of growth, i.e., a 33% increase. Our results (Fig. 4) show that 1 mM (Ca^{2+}) reduces Al^{3+} adsorption to the PC surface by 50% in the presence of 2.2×10^{-6} (Al^{3+}). We note, however, that the rank among divalent metals in terms of affinity for PC surfaces (i.e., $Ca^{2+} - Mg^{2+} > Sr^{2+}$ [9]) is not the same as their ability to overcome Al^{3+} toxicity, which argues against the simple mechanism we have proposed.

Finally, our results suggest that adsorption of Al^{3+} to PC could contribute to Al uptake by endocytosis. Hübner and co-workers [28] have demonstrated that endocytosis occurs in higher plants. This mechanism may include both endocytosis of solutes (fluid phase pinocytosis) and absorption of compounds bound to the plasma membrane (adsorption pinocytosis) [29]. Adsorption pinocytosis accelerates uptake of many solutes by a factor of 100–1000 [29]. Our results show that Al^{3+} bound to surface PC of tobacco protoplasts could

* This is reasonable because Macdonald and Seelig [27] have shown that K_1 values for Ca^{2+} binding to pure POPC bilayers and to POPC:POPG bilayers are nearly identical. In their view, increased Ca^{2+} binding due to anionic POPG can be explained entirely by electrostatic terms in Gouy-Chapman theory.

be as high as 1×10^{-8} mol · m^{-2} when bulk phase Al^{3+} activity is 5×10^{-6}. Given an average diameter of 100 nm for the pinocytotic vesicles, adsorbed Al would be 6×10^{-22} mol per vesicle which is about ten times greater than fluid phase Al per vesicle under the same conditions.

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