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The surface charge density of plant cell membranes (σ): an attempt to resolve conflicting values for intrinsic σ

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Abstract

The electrical potentials at membrane surfaces (ψ_0) strongly influence the physiological responses to ions. Ion activities at membrane surfaces may be computed from ψ_0 , and physiological responses to ions are better interpreted with surface activities than with bulk-phase activities. ψ_0 influences the gating of ion channels and the driving force for ion fluxes across membranes. ψ_0 may be computed with electrostatic models incorporating the intrinsic surface charge density of the membrane (σ_0), the ion composition of the bathing medium, and ion binding to the membrane. Some of the parameter values needed for the models are well established: the equilibrium constants for ion binding were confirmed for several ions using multiple approaches, and a method is proposed for the computation of other binding constants. σ_0 is less well established, although it has been estimated by several methods, including computation from the near-surface electrical potentials [zeta (ζ) potentials] measured by electrophoreses. Computation from ζ potentials yields values in the range -2 mC m^{-2} to -8 mC m^{-2} , but other methods yield values in the range -15 mC m^{-2} to -40 mC m^{-2} . A systematic discrepancy between measured and computed ζ potentials was noted. The preponderance of evidence supports the suitability of $\sigma_0 = -30 \text{ mC m}^{-2}$. A proposed, fully parametrized Gouy–Chapman–Stern model appears to be suitable for the interpretation of many plant responses to the ionic environment.

Key words: Charge density, membrane, plant, surface potential, ζ potential.

Introduction

Membrane surface potentials, arising from membrane surface charges, profoundly affect plant function, especially interactions between roots and ions in the rooting medium. Knowledge of the plasma membrane (PM) surface electrical potential (ψ_0) enables the computation of ion activities at the PM surface. The Nernst equation, $\{I^Z\}_0 = \{I^Z\} \exp[-Z_1 F \psi_0 / (RT)]$, relates the activity of ion I with charge Z at the PM surface to the activity of the ion in the bulk-phase medium (see Methods). (The subscript 0 in $\{I^Z\}_0$ and ψ_0 denotes quantities at zero distance from the PM surface; the absence of a subscript in $\{I^Z\}$ indicates activity in the bulk-phase medium.) To illustrate, a value

of -59.2 mV for ψ_0 will enrich (relative to the bulk-phase medium) ion activities at the PM surface 10-, 100-, and 1000-fold for monovalent, divalent, and trivalent cations, respectively. Anions will be depleted in a reciprocal fashion. Root responses to ions (*e.g.* elongation, intoxication, alleviation of intoxication, transport across PMs, and PM enzyme activity) often correlate poorly with $\{I^Z\}$ and often correlate well with $\{I^Z\}_0$ (Gibrat *et al.*, 1985; McLaughlin, 1989; Zhang *et al.*, 2001; Yermiyahu and Kinraide, 2005; Kinraide, 2006; Wang *et al.*, 2008). In addition to the enrichment or depletion of ions at membrane surfaces, ψ_0 influences the surface-to-surface

potential difference across the membranes. This potential difference influences ion channel gating and the driving force for ion fluxes across membranes (Hille, 2001). Intracellular functions, such as electron transport in mitochondria (Møller *et al.* 1984a) and chloroplasts (Conjeaud and Mathis, 1986), are also responsive to ψ_0 .

In this article we shall argue that measured or computed values of ψ_0 are proportional to actual values but may be different from the actual values themselves. In many instances, proportional values for ψ_0 are sufficient for the interpretation of results such as the alleviation of the toxicity of toxic cations (Al^{3+} , Cu^{2+} , and H^+) or the enhancement of the toxicity of toxic anions (SeO_4^{2-} and H_2AsO_4^-) by treatments that reduce PM surface negativity (e.g. increases in the ionic strength or decreases in the pH of bathing media) (Gimmler *et al.*, 1991; Yermiyahu and Kinraide, 2005; Kinraide, 2006; Wang *et al.*, 2008). However, results have been reported in which detailed interpretations require more precise knowledge of actual values for ψ_0 . An example of this is presented in Wang *et al.* (2008) in which the extrinsic sensitivity to $\{\text{Cu}^{2+}\}$ (sensitivity to bulk-phase $\{\text{Cu}^{2+}\}$) is reduced by reductions in ψ_0 negativity, as expected, but the intrinsic sensitivity to Cu^{2+} (sensitivity to $\{\text{Cu}^{2+}\}_0$) is increased. This apparent increase in intrinsic sensitivity could be genuine (our view) or it could be the consequence of erroneous values for ψ_0 derived from erroneous values for surface charge density (σ) all leading to a miscalculation of $\{\text{Cu}^{2+}\}_0$.

Computation of PM surface activities (e.g. $\{\text{Cu}^{2+}\}_0$) requires knowledge of ψ_0 , and computation of ψ_0 requires knowledge of PM σ . σ is the contingent surface charge density, that is, σ in a bathing medium where ions may be bound to the membrane surface, thereby altering σ from the condition where no ions are bound. In the absence of ion binding, σ is designated the intrinsic surface charge density, denoted σ_0 , and σ_0 must be known in order to compute σ .

In this article we shall attempt to assess the ability to measure or compute σ_0 , ψ_0 , and membrane surface ion activities at the PM and other cell membranes. The computation of ψ_0 requires an appropriate electrostatic model, and the model in most common use is the Gouy–Chapman–Stern (GCS) model (Barber, 1980; Kinraide, 1994; Tatulian, 1999; Yermiyahu and Kinraide, 2005). This model incorporates σ_0 , the ion composition of the bathing medium, and ion binding to the membrane. In our view, σ_0 is the parameter simultaneously of greatest importance and greatest uncertainty, and in this study we marshal the evidence for the suitability of $\sigma_0 = -30 \text{ mC m}^{-2}$ as the best general value while noting the occurrence of variability among membranes. This variability is small relative to the difference in estimates of σ_0 by electrophoreses $[-4.3 \pm 0.9 \text{ mC m}^{-2} \text{ (mean} \pm \text{SE)}]$ and all other methods $[-27.5 \pm 2.1 \text{ mC m}^{-2} \text{ (mean} \pm \text{SE)}]$. These values for σ_0 would lead to $\psi_0 = -18.8 \text{ mV}$ and $\psi_0 = -36.4 \text{ mV}$, respectively, for membranes bathed in 1 mM CaCl_2 at pH 6. $\{\text{Ca}^{2+}\}_0$ would be 3.4 mM and 13.3 mM, respectively. The datum tables presented later provide

further illustrations of the interactions among σ_0 , ψ_0 , and ion activities.

Methods

Reported values for σ_0 , as assessed by several methods, are presented in Table 1. Zeta (ζ) potential measurements taken from published reports and contributed data are presented in Table 2. The ζ potential is the near-surface potential of membrane vesicles or cell protoplasts measured by electrophoresis. ψ_0 is related to the ζ potential, which is the electrical potential at a distance s from the membrane surface; s is the location of the hydrodynamic plane of shear (the slipping plane). Thus ζ potential $= \psi_s$, and $\psi_s = \psi_0 \exp[-3.29s\mu^{1/2}]$ where μ is the ionic strength and 3.29 is a constant appropriate at 25 °C when s is expressed in nm and μ is expressed in M (Morel and Hering, 1993). The negative exponential ensures that $|\psi_s| < |\psi_0|$.

If ψ_0 is known or can be computed, then σ_0 may be computed by the GCS model, and vice versa (see below). For these computations the detailed ionic composition of the bathing medium must be known (detailed chemical speciation is required), and some quantitative parameters for the model, including equilibrium constants for ion binding to the membrane surface, must be known. Some parameter values for a model currently in use are presented in the second column of Table 3, and henceforth the GCS model using those parameter values will be referred to as the ‘standard model’.

For the GCS model, the PM was modelled as though it were composed of negatively charged (R^-) and neutral (P^0) sites to which ions (I^Z) may bind. Ions may bind according to the reactions $R^- + I^Z \leftrightarrow RI^{Z-1}$ and $P^0 + I^Z \leftrightarrow PI^Z$ for which the binding constants $K_{R,I} = \{RI^{Z-1}\}/\{R^-\}[I^Z]_0$ and $K_{P,I} = \{PI^Z\}/\{P^0\}[I^Z]_0$ are needed. $[R^-]$, $[P^0]$, $[RI^{Z-1}]$, and $[PI^Z]$ denote PM surface densities in mol m^{-2} , and $[I^Z]_0 = [I^Z] \exp[-Z_1 F \psi_0 / (RT)]$, a Boltzmann equation, denotes the concentration in M of an unbound ion at the PM surface. F , R , and T are the Faraday constant, the gas constant, and the temperature, respectively; $F/(RT) = 1/25.7$ at 25 °C for ψ_0 expressed in mV. The contingent surface charge density (σ) can be expressed as the sum of the products of the surface density of each species and the charge of each species all times F in order to express σ in units C m^{-2} :

$$\sigma = \{-[R^-] + \sum_{RI} (Z-1) [RI^{Z-1}] + \sum_{PI} Z [PI^Z]\} F \quad (1)$$

Equation 1 expresses the Stern portion of the GCS model. Stern reactions are strong interactions represented as binding of ions to PM sites as noted above. The computation of σ by Equation 1 requires both binding constants and ψ_0 (in order to obtain $[I^Z]_0$ by the Boltzmann equation above). The Gouy–Chapman portion of the model is expressed in this equation:

$$\sigma^2 = 2\epsilon_r \epsilon_0 RT \sum_I [I^Z] \{\exp[-Z_I F \psi_0 / (RT)] - 1\} \quad (2)$$

$2\epsilon_r \epsilon_0 RT = 0.00345$ at 25 °C for bulk-phase concentrations of I^Z expressed in M and σ^2 expressed in $(\text{C m}^{-2})^2$ (ϵ_r is the dielectric constant for water, ϵ_0 is the permittivity of a vacuum). Thus in any bathing medium there are two equations with two unknowns (ψ_0 and σ). To compute ψ_0 , trial values were assigned to it, and σ in Equations 1 and 2 were computed until the values for σ from the two equations converged. See Yermiyahu and Kinraide (2005) for more detailed descriptions and references.

The possibly problematical necessity of the simultaneous truth of both the Nernst and Boltzmann equations (the first incorporating activities and the second concentrations, $\{I^Z\}_0 = \{I^Z\} \exp[-Z_I F \psi_0 / (RT)]$ and $[I^Z]_0 = [I^Z] \exp[-Z_I F \psi_0 / (RT)]$, respectively) has been discussed, but not resolved (Kinraide, 1994; Rytwo, 2004). This matter will be discussed later in conjunction with a systematic discrepancy between measured and computed ζ potentials observed in the present study. A computer program for the GCS model may be obtained from the authors.

Table 1. Computed intrinsic surface charge densities (σ_0) of plant membranes

Source and method of estimation	σ_0 ($-\text{mC m}^{-2}$)	Material
Ca adsorption		
Yermiyahu <i>et al.</i> (1994)	43	Melon root PM vesicles
Electrophysiological (patch-clamp) study		
Pottosin and Martínez-Estévez (2003)	40	Beet taproot vacuole patches
Ionic strength effects upon electron transfer		
Conjeaud and Mathis (1986)	30	Spinach leaf thylakoids
Modelled from tissue cation content		
Bérczi <i>et al.</i> (1984)	18	Wheat root microsomes
Fluorescent dye quenching ^a		
Chow and Barber (1980)	14–36	Pea leaf thylakoids
Gibrat <i>et al.</i> (1983)	27	Horse bean microsomes (8-ANS)
Møller <i>et al.</i> (1984a)	33	Jerusalem artichoke mitochondria
Møller <i>et al.</i> (1984b)	16–20	Wheat root PM vesicles
	29	Oat root PM vesicles
Bérczi <i>et al.</i> (1984)	20	Wheat root PM vesicles
Gibrat <i>et al.</i> (1985)	5.7	Corn root PM vesicles (8-ANS)
Körner <i>et al.</i> (1985)	19	Barley root PM vesicles
Oka <i>et al.</i> (1988)	39	<i>Vigna mungo</i> root protoplasts
Bérczi and Møller (1993)	22–39	Wheat root PM vesicles
Yermiyahu <i>et al.</i> (1997a)	30	Scout wheat root PM vesicles
Yermiyahu <i>et al.</i> (1997b)	37	Scout wheat root PM vesicles
Yermiyahu <i>et al.</i> (1997b)	30	Atlas wheat root PM vesicles
Dahlin (2003)	24–34	Wheat thylakoids
Electrophoresis (ζ potentials)		
Gibrat <i>et al.</i> (1985)	7.3	Corn root PM vesicles
Obi <i>et al.</i> (1989a)	4.1	Barley mesophyll protoplasts
Obi <i>et al.</i> (1989c)	3.9	Barley mesophyll protoplasts
Obi <i>et al.</i> (1990)	2.3	Barley mesophyll protoplasts
Obi <i>et al.</i> (1989b)	2.9	<i>Rauwolfia serpentina</i> protoplasts
Murata <i>et al.</i> (2000)	3	Tobacco cell protoplasts
Filek <i>et al.</i> (2002)	2.4 (3.2) ^b	Wheat callus protoplasts
This study (Table 4, column G)	1.6–10.4	

^a The dye was 9-aminoacridine (9-AA) except where 8-anilino-1-naphthalene sulphonate (8-ANS) was used as noted in the Material column.

^b Recomputed to accommodate H^+ binding.

Results

Discrepancies in reported values for σ_0

Fluorescent dye quenching methods of σ_0 assessment, and several other methods, produce values ranging, usually, from -15 mC m^{-2} to -40 mC m^{-2} (Table 1), but they include one exceptional value: -5.7 mC m^{-2} (Gibrat *et al.*, 1985). Measurements with 9-aminoacridine (9-AA) have undergone several improvements and reassessments (Bérczi and Møller, 1993; Brauer *et al.*, 2000), and fair agreement between σ_0 computed by the 9-AA fluorescence method and σ_0 computed on the basis of liposome composition (where σ_0 is known) has been claimed (Brauer *et al.*, 2000). Estimates from ζ potential measurements yield dramatically different results. For them, the estimates of σ_0 range, usually, from -2 mC m^{-2} to -8 mC m^{-2} , and the averages of all values from the two sets differ by 6.4-fold [-4.3 ± 0.9 versus -27.5 ± 2.1 (means $\pm$ SEs)].

Within-study variation among σ_0 values

The 6.4-fold bimodal distribution of σ_0 values was large compared with the generally <2-fold variation among σ_0 values for different membranes within a study or for membranes of different type, sidedness, or experimental treatment. Membranes vary according to the plant genotype, the tissue, and the organelles from which the membranes were derived. In addition, treatments such as ionic, water, and temperature stress may affect the membranes. Except for the extreme value of -5.7 mC m^{-2} , the values assessed by fluorescent dye quenching vary by 2.8-fold in Table 1. In the study by Møller *et al.* (1984b) the inside and outside surfaces of PM vesicles were compared. The σ_0 for inside surfaces was 56% more negative than for outside surfaces. Yermiyahu *et al.* (1997a) compared Scout and Atlas cultivars of wheat (*Triticum aestivum* L.) and found Scout PM vesicles to be 26% more negative than Atlas. This difference was consistent with greater Scout sensitivity to cationic toxicants and greater Atlas sensitivity to anionic toxicants (Yermiyahu *et al.*, 1997a). The difference between dicotyledons and monocotyledons in Table 1 is statistically insignificant. Finally, weak red radiation produced a 31% reduction in negativity in wheat thylakoids relative to greenhouse controls (Dahlin, 2003).

Estimation of GCS model parameters from ζ potentials

Among the σ_0 estimates presented in Table 1 are several from electrophoresis studies. Table 1 also presents estimates (-1.6 mC m^{-2} to -10.4 mC m^{-2}) from the present study based upon the ζ potentials in Table 2. To achieve these estimates, values for ψ_0 were computed by a GCS model using parameter values selected to optimize the correspondence between the ζ potentials and ψ_0 . For these estimates, we assumed initially that ζ potential $\approx \psi_0$ as explained below. Optimization entailed the incorporation of starting values for the parameters into an iterative computer program to compute ψ_0 . Then one of the

Table 2. ζ potentials of plant membranes

Material and source	Solution no.	pH	CaCl ₂ (mM)	MgCl ₂ (mM)	NaCl (mM)	KCl (mM)	LaCl ₃ (mM)	ζ potential (mV)
Tobacco leaf protoplasts (Nagata and Melchers, 1978)	1	5.8			6.7	10		-28
	2	5.8	1		6.7	10		-25
	3	5.8	10		6.7	10		-9
	4	5.8	100		6.7	10		0
Corn root PM vesicles (Gibrat <i>et al.</i> , 1985)	5	6.5			15			-24
	6	6.5			65			-14
	7	6.5		6	15			-8
	8	6.5		6	65			-6
Barley leaf protoplasts (Abe and Takeda, 1988)	9	6.7	0.1		0.5			-48
	10	6.7	0.1		6.5			-39
	11	6.7	0.1		0.5	6		-39
	12	6.7	0.1		0.5		0.1	-17
	13	6.7	0.1		0.5		0.3	0
	14	6.7	0.1		0.5		1	14
	15	3.6	0.1		6			-2
	16	3.6	0.1				1	23
Barley leaf protoplasts (Obi <i>et al.</i> , 1989a)	17	7.6			3			-29
	18	7.6			7.5			-20
	19	7.6			15			-13
	20	7.6			1	14		-13
	21	7.6			30			-10
	22	7.6	1		4.5			-10
	23	7.6		1	4.5			-11
	24	7.2			0.5		0.17	0
	25	6.5			5		1	3
<i>Rauwolfia serpentina</i> protoplasts (Obi <i>et al.</i> , 1989b)	26	7	0.02		6	1		-18
	27	6	0.02		6	1		-18
	28	5	0.02		6	1		-17
	29	4	0.02		6	1		-12
	30	3	0.02		6	1		3
Barley leaf protoplasts (Obi <i>et al.</i> , 1990)	31	7	0.02		6	1		-18
	32	6	0.02		6	1		-16
	33	5	0.02		6	1		-12
	34	4	0.02		6	1		1
	35	3	0.02		6	1		18
Tobacco leaf protoplasts (Zhang and Reid, contributed data; see Zhang <i>et al.</i> , 2001)	36	4	0.02		1			-3
	37	5	0.02		1			-20
	38	6	0.02		1			-32
	39	7	0.02		1			-37
	40	7	0.02	0.01	1			-33
	41	7	0.02	0.1	1			-28
	42	7	0.02	1	1			-17
	43	7	0.02	10	1			-7
Wheat root protoplasts (Zhang and Reid, contributed data; see Zhang <i>et al.</i> 2001)	44	6	0.02		1			-17
	45	6	0.02		1	1.6		-15
	46	6	0.02		1	8.1		-12
	47	6	0.02		1	48.6		-8
	48	6	0.02	0.54	1			-10
	49	6	0.02	4	1			-4
	50	6	0.02	13.9	1			-2

Table 2. Continued

Material and source	Solution no.	pH	CaCl ₂ (mM)	MgCl ₂ (mM)	NaCl (mM)	KCl (mM)	LaCl ₃ (mM)	ζ potential (mV)
Wheat root protoplasts (Wang <i>et al.</i> , 2008)	51	6	0.23	0.22	0.97	0.44		-27
	52	6	2.5	0.23	1.02	0.47		-15
	53	6	0.25	2.5	1.04	0.48		-15
	54	6	0.24	0.23	1.03	29.39		-18
	55	6	0.24	0.23	39.28	0.46		-22
	56	4.5	0.24	0.22	0.38	0.46		-17
	57	7	0.24	0.22	1.97	0.46		-29
	58	6	0.30	1.77	1.05	0.48		-18
	59	6	1.77	0.3	1.05	0.48		-17

Table 3. Parameter values for a Gouy–Chapman–Stern model for plant plasma membranes

Model parameters	Standard model	ζ potential-derived model	General binding strengths ^a
R_T μmol m ⁻²	0.3074	0.017–0.108	
P_T μmol m ⁻²	2.4	2.4 (897) ^b	
$K_{R,K}$ M ⁻¹	1	0	0.9
$K_{R,Na}$	1	0	1.0
$K_{R,Ca}$	30	30.5 (899)	27.5
$K_{R,Mg}$	30	31.4 (896)	29.0
$K_{R,Cu}$	400		697
$K_{R,La}$	2200	1950 (898)	2030
$K_{R,Al}$	20 000		14 800
$K_{R,H}$	21 500	19 400 (901)	
$K_{P,I}$	$K_{R,I}/180^c$	$K_{R,I}/1100^c$ (896)	

^a Based upon a scale linearly related to the logK values for binding to hard ligands (Kinraide, 2009). That scale may be converted to PM binding constants by the formula $K_{R,I}=10^{[3+1.75(\text{Hard Ligand Scale})]}$.

^b Results of a sensitivity analysis are presented in parentheses. The values are the sum of squares (SS) when the parameter value was reduced 10% and the other parameters remain as listed (SS=895 for all values as listed). See Table 4 for the sensitivity analysis of the R_T values.

^c These ratios refer to cations. Anion binding to R^- is taken to be zero, and $K_{P,I}=K_{P,K}$ for all monovalent anions, and $K_{P,I}=K_{P,Ca}$ for all divalent anions.

parameters was changed incrementally until the sum of squares [$SS=(\zeta \text{ potential}-\psi_0)^2$] was minimized. The program fixed that value and moved on to the next parameter. This process was repeated until all parameters were evaluated, then the size of the incremental changes was reduced and new rounds of evaluations occurred until the SS was minimized. The parameters so derived are presented in Tables 3 and 4, the latter presenting the computed ψ_0 for each of the 59 solutions and the computed σ_0 for each of the nine studies. For the regression of ζ potentials versus computed ψ_0 , $r^2=0.917$ (Fig. 1).

Equilibrium constants for ion binding

Among the parameter values derived by optimization are the equilibrium constants for ion binding to negative

binding sites (R^-) on the PM surface ($K_{R,K}$, $K_{R,Na}$, $K_{R,Ca}$, $K_{R,Mg}$, $K_{R,La}$, and $K_{R,H}$). A notable feature of these results is the close correspondence between the binding constants evaluated by two entirely different techniques—electrophoresis, as just described, and adsorption (second column in Table 3). For the latter, the binding constants for ions were computed on the basis of measured ion adsorption to PM vesicles of wheat root cells (Yermiyahu *et al.*, 1997b; Vulkan *et al.*, 2004). Figure 2A illustrates this close correspondence in a plot of rescaled values (cube roots) for which $r^2=0.998$. Estimation of equilibrium constants for ion binding to neutral binding sites (P^0) on the PM surface ($K_{P,K}$, $K_{P,Na}$, $K_{P,Ca}$, $K_{P,Mg}$, $K_{P,La}$, and $K_{P,H}$) will be described in the next section.

The column designated General binding strengths in Table 3 is derived from the relative strength of ion binding to hard ligands (*e.g.* ligands with F⁻ or O donors) as opposed to soft ligands (*e.g.* ligands with I⁻ or S donors). These relative binding strengths to 13 hard ligands were determined in a survey of 81 metal ions (Kinraide, 2009). The scale presented in that survey is related linearly to the logK values for binding to the hard ligands, and that scale may be converted to PM binding constants by the formula $K_{R,I}=10^{[3+1.75(\text{Hard Ligand Scale})]}$. Figure 2B illustrates the close correspondence between these estimates and binding constants evaluated by adsorption. The Hard Ligand Scale (Kinraide, 2009) allows the attribution of binding strengths to numerous additional ions, with some uncertainty.

Surface densities of negative binding sites (R^-) and neutral binding sites (P^0)

σ_0 may be stated in several ways ($-1 \text{ mC m}^{-2}=0.0104 \text{ μmol negative charges m}^{-2}=16 \text{ 000 Å}^2 \text{ per negative charge}=0.00624 \text{ e}^- \text{ nm}^{-2}$). We use R_T as an expression of σ_0 . It is the total number of negative binding sites (R^-) per m², and a value of $0.3074 \text{ μmol m}^{-2}$ has been assigned previously (Yermiyahu *et al.*, 1997b; and see Table 3). In the analysis of the ζ potential data (Table 2), values for R_T were assigned individually, by optimization, for each of the nine studies. Inspection of the table reveals apparent differences in σ_0 for the studies. For example, one would expect the

Fig. 1. Measured ζ potentials plotted against ψ_0 . ψ_0 is the plasma membrane surface potential computed with a Gouy–Chapman–Stern model with parameters optimized for best correspondence between ζ potentials and ψ_0 (third column Table 3).

Fig. 2. Comparison of equilibrium constants for ion binding to negative sites on plasma membranes assessed by different methods. Plots are of the cube roots of binding constants assessed by ion adsorption, electrophoresis, and relative binding strength to hard ligands. Refer to Table 3.

ζ potential for solution no. 2 to be similar to or slightly less negative than the ζ potential for solution no. 22. The opposite occurs, indicating that σ_0 may be more negative for the tobacco leaf protoplasts (Nagata and Melchers, 1978) than for the barley leaf protoplasts (Obi *et al.*, 1989a). A similar argument applies in the case of solutions 5 and 20.

P_T is the total number of neutral binding sites (P^0) per m^2 , and a single value of $P_T=2.4 \mu\text{mol m}^{-2}$ was assigned on the basis of the number of phospholipids per m^2 in a typical membrane (Akeson *et al.*, 1989; Kinraide *et al.*, 1998; and see Table 3). Separate equilibrium constants for ion binding to P^0 were not determined. Instead, it was assumed, as an approximation, that the binding strengths for the neutral sites are a constant fraction of the binding strengths for negative sites. In a previous study (Kinraide *et al.*, 1998), an optimized value of 1/180 was determined for cations; that is (binding strength to P^0)/(binding strength to R^-)= $K_{P,I}/K_{R,I}=1/180$. In the present analysis of ζ potentials (Table 2) the optimized value for $K_{P,I}/K_{R,I}$ was 1/1100. This value is greatly different from the previous 1/180, but the smaller value (1/1100) is a consequence of the less negative values for σ_0 estimated from ζ potentials (analysis not shown). A sensitivity analysis of the parameters (see below) also indicated the relative insensitivity to changes in $K_{P,I}/K_{R,I}$, which in any case is a small number (<0.01).

The very close agreement between previous (adsorption) and present (electrophoresis) estimates of ion binding strength at negative sites (R^-) has been noted (Table 3 and Fig. 2A), but the disparities between previous and present estimates of R_T in Table 3 (second and third columns) are typical of the disparities observed in Table 1. In Table 3 the value $R_T=0.3074 \mu\text{mol m}^{-2}$ (second column) was computed by the 9-AA fluorescent dye quenching method (Yermiyahu *et al.*, 1997b), and the values 0.017–0.108 $\mu\text{mol m}^{-2}$ (third column) were computed from electrophoresis data as just noted.

The parameter s (distance of the slipping plane from the PM surface)

In the initial evaluation of parameters, ζ potential was taken to equal ψ_0 ; that is, $s=0$. This assignment should not introduce a great error if $s \approx 0.2 \text{ nm}$, as is commonly assumed (McLaughlin, 1989). At the small μ of 1 mM, $\psi_0=-20.4 \text{ mV}$ when $\psi_s=-20 \text{ mV}$, and at the large μ of 100 mM, $\psi_0=-24.6 \text{ mV}$ when $\psi_s=-20 \text{ mV}$.

In order to evaluate s by the iterative optimization method, the model-computed value for ψ_0 was converted to the electrical potential at $s \text{ nm}$ from the membrane surface with the equation $\psi_s=\psi_0 \exp[-3.29s\mu^{1/2}]$. Nonsensically, s evaluated to -0.76 nm , indicating, impossibly, that the slipping plane was inside the vesicle and that the potential became more negative with increased distance from the membrane surface. The incorporation of $s=-0.76 \text{ nm}$ into the model caused minor changes in the other parameters: σ_0 became even less negative; SS declined from 895 to 839; and r^2 increased from 0.917 to 0.921. The apparently negative value of s may be related to a systematic error noted in the next section.

In further analyses, s was assigned positive values in order to force values for σ_0 into a range comparable with those computed by other methods (Table 1). When $s=2$ nm, σ_0 ranged from -2.2 mC m $^{-2}$ to -51.8 mC m $^{-2}$ (-17.0 average). However, SS $\{=[\zeta \text{ potential}-\psi_{(s=2)}]^2\}$ rose to 1557, and r^2 declined to 0.866. Thus ζ potential-derived values for σ_0 are compatible with other values if s is increased 10-fold the usually accepted value, but then the correspondence between measured and computed potentials declines.

The GCS model generates a systematic error relative to ζ potentials

The values for ψ_0 computed with optimized parameter values (third column in Table 3) differ systematically with measured ζ potentials: the differences (Table 4, column D) became more negative with increasing μ (Fig. 3A); that is, the model underestimates the depolarizing effectiveness of low μ media and overestimates the depolarizing effectiveness of high μ media as assessed by electrophoresis. To test the possibility that this effect of μ was the accidental consequence of random error or a consequence of very large μ , the data were trimmed. Solutions 12, 16, 29, 30, and 55 were eliminated because of relatively large residuals ($>|8|$), and solutions 4, 6, and 8 were eliminated because of large μ (>50 mM), then optimal parameters were recomputed. The error persisted with the trimmed data (Fig. 3B), and s continued to evaluate to negative values. When differences between ψ_0 computed by the 'standard model' and ζ potentials (Table 4, column F) were plotted against the square root of μ , a statistically significant relationship qualitatively similar to that in Fig. 3A was obtained also (not shown).

As mentioned earlier, the ζ potential= ψ_s , the potential at the slipping plane s nm from the membrane surface. Because of the dependence of ψ_s upon μ and s according to the equation $\psi_s=\psi_0\exp(-3.29s\mu^{1/2})$, we assessed the influence of s upon the μ -related error just noted. Adjustments of s from 0 nm to 2 nm failed to eliminate the error.

σ_0 inferred from toxicological studies

In toxicological studies one may compute σ_0 by making the (possibly false) assumption that the best value for σ_0 is the one that leads to the best correlation between the computed surface activity of the toxicant and the physiological response. Consider a study (Kinraide, 2006) evaluating wheat root elongation in response to variable pH and variable concentrations of CuSO $_4$, CaCl $_2$, and NaCl. Relative root length (RRL) declined as a function of $\{\text{Cu}^{2+}\}$, but the correlation ($r^2=0.565$) was poor (Fig. 3A in Kinraide, 2006). The correlation was much better ($r^2=0.823$) for RRL versus $\{\text{Cu}^{2+}\}_0$ (Fig. 3B in Kinraide, 2006) where $\{\text{Cu}^{2+}\}_0$ was computed by the 'standard model'. If $\{\text{Cu}^{2+}\}_0$ was computed after optimization of R_T , then $r^2=0.846$ (Table 5, experiment no. 8). This optimized value for R_T (1/2 the standard value) was still in the range of 9-AA-computed values. Furthermore, the elimination of the single greatest outlier increased r^2 to 0.856 for RRL

versus $\{\text{Cu}^{2+}\}_0$, and now the optimized value for R_T was equal to the standard value.

Table 5 and Fig. 4 present reanalyses of eight toxicity studies, including the one just mentioned. Only in the case of experiment 3 did σ_0 fall into the -2 mC m $^{-2}$ to -8 mC m $^{-2}$ range of ζ potential-derived values. On average, the optimized value for σ_0 was -46 mC m $^{-2}$. An attempt was made to avoid prejudice in the selection of studies. In fact, it was not possible to predict whether optimization would lead to greater or smaller values for σ_0 .

Discussion

The distribution of computed values for σ_0 is bimodal, with almost no overlap. Electrophoretic studies yield values in the range -2 mC m $^{-2}$ to -8 mC m $^{-2}$, and other studies yield values in the range -15 mC m $^{-2}$ to -40 mC m $^{-2}$. We know of no certain criterion by which to reject either of the two ranges, but the following arguments militate in favour of the more negative values: (i) the more negative values were obtained by five methods rather than just one (Table 1); (ii) values for σ_0 computed by the 9-AA fluorescence method correspond well to values computed on the basis of liposome composition (where σ_0 is known) (Brauer *et al.*, 2000); (iii) optimization of σ_0 in physiological studies generally led to more negative values (-46 mC m $^{-2}$ on average; Table 5); and (iv) a comparison of measured near-surface potentials (ζ potentials) and computed surface potentials (ψ_0) revealed a systematic error: a GCS model, whether with optimized parameter values or with 'standard model' parameter values, underestimated the depolarizing effectiveness of low μ media and overestimated the depolarizing effectiveness of high μ media. Furthermore, the evaluation of s from electrophoretic measurements yielded the nonsensical value of -0.76 nm.

It is uncertain whether these errors reflect a deficiency in the measurement of ζ potentials or in the electrostatic model for computing ψ_0 . The errors may relate to a possible problem in the GCS model previously discussed, but not resolved (Kinraide, 1994; Rytwo, 2004). The Nernst equation is accepted as the appropriate thermodynamic expression for the distribution of ions in an electric field [or at least for the equilibrium partition of ions between the bathing medium and the usually negative cell interior (Nobel, 2005)]. In contrast, the derivation of the Gouy–Chapman theory incorporates the Boltzmann equation as the thermodynamic expression for the distribution of ions at a charged membrane surface (Barber, 1980; Tatulian, 1999; and see Methods). For both equations to be correct the activity coefficients of the ions (γ_i) must remain constant at all distances from the charged surface. Only then can $\{I^Z\}_0/\{I^Z\}=\gamma_{i,0}[I^Z]_0/(\gamma_i[I^Z])=[I^Z]_0/[I^Z]=\exp[-Z_iF\psi_0/(RT)]$.

Should these errors call into question other parameter values computed from ζ potentials? Binding constants assessed from ζ potentials agree remarkably well with those assessed from other methods (Table 3 and Fig. 2). Furthermore, ζ potentials and computed potentials are usually

Table 4. Measured ζ potentials and computed ψ_0 of plant membranes (refer to Table 2)

Column A Solution no.	Column B Measured ζ potential, mV	Column C Computed ψ_0 (optimized parameters), mV	Column D Difference (B–C), mV	Column E Computed ψ_0 (standard parameters), mV	Column F Difference (B–E), mV	Column G Computed σ_0 , mC m ^{–2}
1	–28	–29.9	1.9	–55.1	27.1	–10.4 (911) ^a
2	–25	–20.8	–4.2	–31.5	6.5	
3	–9	–6.3	–2.7	–9.4	0.4	
4	0	3.9	–3.9	8.3	–8.3	
5	–24	–24.9	0.9	–64.0	40.0	–7.4 (911)
6	–14	–12.4	–1.6	–35.7	21.7	
7	–8	–7.9	–0.1	–14.7	6.7	
8	–6	–5.8	–0.2	–12.5	6.5	
9	–48	–54.0	6.0	–65.3	17.3	–9.2 (902)
10	–39	–37.8	–1.2	–58.4	19.4	
11	–39	–37.8	–1.2	–58.4	19.4	
12	–17	–6.7	–10.3	–6.0	–11.0	
13	0	1.5	–1.5	3.0	–3.0	
14	14	9.9	4.1	12.6	1.4	
15	–2	–1.2	–0.8	5.1	–7.1	
16	23	9.6	13.4	16.5	6.5	
17	–29	–27.0	–2.0	–100.0	71.0	–3.7 (905)
18	–20	–17.9	–2.1	–81.9	61.9	
19	–13	–12.9	–0.1	–66.2	53.2	
20	–13	–12.9	–0.1	–66.2	53.2	
21	–10	–9.2	–0.8	–51.2	41.2	
22	–10	–14.2	4.2	–35.6	25.6	
23	–11	–14.1	3.1	–35.6	24.6	
24	0	–1.9	1.9	–1.6	1.6	
25	3	7.6	–4.6	12.3	–9.3	–4.0 (905)
26	–18	–20.7	2.7	–70.9	52.9	
27	–18	–19.2	1.2	–66.3	48.3	
28	–17	–14.5	–2.5	–45.6	28.6	
29	–12	–3.4	–8.6	–10.5	–1.5	–3.3 (901)
30	3	12.0	–9.0	28.5	–25.5	
31	–18	–16.6	–1.4	–70.9	52.9	
32	–16	–16.0	0.0	–66.3	50.3	
33	–12	–12.2	–0.2	–45.6	33.6	
34	1	–2.5	3.5	–10.5	11.5	
35	18	12.1	5.9	28.5	–10.5	
36	–3	–4.6	1.6	–14.3	11.3	
37	–20	–23.6	3.6	–58.3	38.3	–3.0 (927)
38	–32	–33.0	1.0	–79.6	47.6	
39	–37	–34.2	–2.8	–83.0	46.0	
40	–33	–33.4	0.4	–78.5	45.5	
41	–28	–28.1	0.1	–62.5	34.5	
42	–17	–14.6	–2.4	–36.6	19.6	
43	–7	–2.8	–4.2	–10.1	3.1	
44	–17	–20.0	3.0	–79.6	62.6	
45	–15	–13.1	–1.9	–74.8	59.8	–1.6 (902)
46	–12	–7.2	–4.8	–63.3	51.3	
47	–8	–3.1	–4.9	–38.2	30.2	
48	–10	–11.7	1.7	–44.2	34.2	
49	–4	–3.9	–0.1	–20.0	16.0	
50	–2	–0.8	–1.2	–6.7	4.7	
51	–27	–31.9	4.9	–45.6	18.6	
52	–15	–15.4	0.4	–24.4	9.4	

Table 4. Continued

Column A Solution no.	Column B Measured ζ potential, mV	Column C Computed ψ_0 (optimized parameters), mV	Column D Difference (B–C), mV	Column E Computed ψ_0 (standard parameters), mV	Column F Difference (B–E), mV	Column G Computed σ_0 , mC m ⁻²
53	-15	-15.2	0.2	-24.3	9.3	
54	-18	-15.3	-2.7	-34.8	16.8	
55	-22	-13.7	-8.3	-32.7	10.7	
56	-17	-19.8	2.8	-29.9	12.9	
57	-29	-30.8	1.8	-45.4	16.4	
58	-18	-17.7	-0.3	-27.6	9.6	
59	-17	-17.8	0.8	-27.6	10.6	
			SS=895			
						SS=58 800

^a Results of a sensitivity analysis are presented in parentheses. The values are the sum of squares (SS) when the value of R_T for the indicated study was reduced 10% and the value of R_T for the other studies remain as listed. Compare these values with 895 at the bottom of column D.

Fig. 3. Residual values plotted against the square root of ionic strength (μ in mM). (A) Residuals from the first set of difference values in Table 4 (column D). (B) Residuals from a new analysis, using newly optimized parameters, with eight solutions removed because of large residuals or large μ .

proportional to one another and highly correlated within a study (compare values in column B with values in column C in Table 4). For the equation ζ potential = $a + b\psi_0$, r^2 ranged

from 0.708 to 0.998, b ranged from 0.630 to 1.24, and, for seven of nine studies from Table 4, a was not statistically different from zero ($a=0$ is, of course, a feature of proportionality). The statistics just cited are based upon ψ_0 computed with the optimized parameter values. Using ψ_0 computed from the 'standard model' (column E in Table 4), these statistics are obtained: r^2 ranged from 0.833 to 0.987, b ranged from 0.199 to 0.721, and a was not statistically different from zero for eight of the nine studies. Thus, the proportionality within studies was even stronger using potentials computed from the 'standard model'.

In conclusion, -30 mC m^{-2} appears to be the best available value for σ_0 for plant cell membranes. Within-study differences for σ_0 among membranes differing by membrane type, sidedness, or experimental treatment are small (<2-fold) compared with the 6.4-fold difference between ζ potential-derived values and values derived from all other methods. Other parameter values can be taken from the 'standard model' in Table 3, and additional binding constants can be computed as instructed there. The much smaller negative values for σ_0 computed from ζ potentials may be related to the preparation of vesicles or protoplasts or to some unexpected electrophoretic behaviour. Alternatively, these values may reflect some deficiency in theory, and perhaps the present study will inspire theoreticians to address the problem. For many applications the actual value of σ_0 over a wide range is relatively unimportant because of the probable proportionality between computed ψ_0 and actual ψ_0 . That is, physiological responses correlate better with computed surface activities of ions than with bulk-phase activities irrespective of assumed σ_0 over a wide range: note the relatively small effect upon r^2 of changes for R_T from standard to optimal in Table 5. Use of the 'standard model' has contributed to several novel insights into plant-ion interactions (Yermiyahu and Kinraide, 2005) that are not negated by the use of σ_0 values differing by several fold from the standard value. Nevertheless, some interpretations are dependent upon exact values, rather than proportional values,

Table 5. Plant responses to ion activities in the bathing medium ($\{I^Z\}$) or at the PM surface ($\{I^Z\}_0$)

Surface activities were computed with the ‘standard model’ ($R_T=0.3074\text{ }\mu\text{mol negative charges m}^{-2}$; see Table 3) or with the ‘standard model’ with an optimized value for R_T .

Experiment no.	Material ^a	Function ^b	r^2 for $\{I^Z\}$	r^2 for $\{I^Z\}_0$ ‘standard model’	r^2 for $\{I^Z\}_0$ optimized R_T	Optimum R_T
1	Wheat	RL versus Al^{3+}	0.454	0.947	0.947	R_T
2	Red clover	RL versus Al^{3+} , H^+	0.875	0.922	0.925	$R_T/3$
3	Lettuce	RL versus Al^{3+} , H^+	0.817	0.908	0.921	$R_T/7$
4	Turnip	RL versus Al^{3+} , H^+	0.865	0.933	0.948	$4R_T$
5	Wheat	Ca uptake versus Ca^{2+}	0.786	0.962	0.980	$R_T/2$
6	Wheat	Se uptake versus SeO_4^{2-}	0.710	0.893	0.916	$5R_T$
7	<i>Chlorella</i>	Pb, Zn uptake versus Pb^{2+} , Zn^{2+}	0.728	0.968	0.968	R_T
8	Wheat	RL versus Cu^{2+}	0.565	0.828	0.846	$R_T/2$
Column means			0.725	0.920	0.931	$1.56R_T$

^a Sources of data: Experiment 1, Kinraide and Parker (1987); Experiment 2, Kinraide and Parker (1990); Experiment 3, Kinraide and Parker (1990); Experiment 4, Kinraide and Parker (1990); Experiment 5, Huang *et al.* (1996); Experiment 6, Kinraide (2003); Experiment 7, Hassler *et al.* (2004); Experiment 8, Kinraide (2006).
^b Correlations were based upon equations relating a response, such as root length (RL), to one or two ions. See Fig. 4 for a graphical presentation of Experiment 6.

Fig. 4. Se uptake plotted against SeO_4^{2-} activity in the rooting medium or at the plasma membrane surface. $\{\text{SeO}_4^{2-}\}_0$ was computed using the ‘standard model’ value for σ_0 ($R_T=0.3074\text{ }\mu\text{mol m}^{-2}$) or with a value 5-fold greater. The values for r^2 in parentheses apply in the absence of the datum point denoted by the filled circle. Figure adapted from Kinraide (2003).

for computed ion activities at cell surfaces (Wang *et al.*, 2008). Finally, it is important to realize that all references to and measurements of σ in this study refer to global or

average values. Local values at ion channel openings, for example, may be different (Hille, 2001). Nevertheless, even global values for σ have contributed greatly to the interpretation of plant–ion interactions.

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