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# $Na^+$ and $H^+$ Dependent $Mn^{2+}$ Binding to Phosphatidylserine Vesicles as a Test of the Gouy-Chapman-Stern Theory

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**Summary.** $Mn^{2+}$ binding to phosphatidylserine (PS) vesicles was measured by EPR as a function of $[Na^+]$ and pH. At nearly physiological monovalent salt concentration the apparent $Mn^{2+}$ affinity ($K_a$) increased monitonically over the pH range 5.7–8.35, with $K_a$ roughly $\alpha [H^+]^{-1}$ above pH 7.3. It was found, moreover, that $K_a$ fell off more rapidly with added NaCl at pH 6.1 than at pH 7.87. Qualitatively, these results are consistent with two types of $Mn^{2+}$-PS binding: (i) simple adsorption and (ii) adsorption with the release of an amino proton from PS. The existence of $Mn^{2+}$-induced $H^+$ displacement from PS was verified through titration measurements, employing a pH electrode.

When $H^+$ displacement is taken into account, the variation in $K_a$ with $[Na^+]$ observed at pH 6.1 is found to be in reasonably good agreement with that expected from the Gouy-Chapman-Stern theory of ionic binding to charged surfaces.

The electrostatic interactions between aqueous cations and anionic phospholipid head groups are relevant to many aspects of membrane structure and function. The simplest framework for treating these interactions consists of the Gouy-Chapman diffuse double layer theory supplemented by appropriate "Stern equations" to account for specific ion adsorption to the bilayer [5]. This approach is attractive both from the standpoint of relative ease of calculation and of the qualitative insight it provides.

McLaughlin and coworkers, in particular, have been highly successful in using this theory to explain the results of conductance, ion binding, and $\zeta$-potential measurements on model phospholipid membranes [2, 6, 7]. Given the drastic simplifications inherent in the Gouy-Chapman postulates (point ions in the aqueous phase, uniform planar surface charge distribution, no change in dielectric constant near the surface) this agreement is a little puzzling. Future theoretical work may nevertheless eventually show the agreement to be not entirely fortuitous. Meanwhile additional empirical tests may either extend our confidence in this approach or reveal discrepancies pointing to a fuller description of the interactions on a molecular level.

Some apparent deviations from the theory have arisen with respect to past measurements of $Mn^{2+}$ binding to anionic phospholipid vesicles [10, 11]. It can be deduced from the Gouy-Chapman and Stern equations that if the surface charge density $\sigma$ is reasonably large and negative, and if the divalent cation concentration is low compared to concentrations of monovalent cation and phospholipid binding sites, then the apparent affinity for Mn-phospholipid complexation should vary as [10]:

$$K_a \simeq K_o \sigma^4 / [M^+]^2 \tag{1}$$

where $[M^+]$ is the bulk concentration of monovalent cation and $K_o$ is the "intrinsic association constant". Under these conditions, therefore, a log-log plot of $K_a$ *vs.* $[M^+]$ should have a constant slope $s = -2.0$. While excellent agreement was obtained with respect to cardiolipin ($s \approx -1.99$), measurements on phosphatidylserine (PS) vesicles yielded a linear plot having a slope of only about $-1.49$ [10]. Furthermore when the surface charge density of anionic phospholipid vesicles was made less negative either by adding phosphatidylcholine (PC) [10] or by making the vesicles more fluid [11], the observed $K_a$ did not fall off as predicted by Eq. (1).

Two assumptions were made in arriving at Eq. (1) which now appear to be questionable. First, it was assumed that, apart from the diffuse double layer association (screening), monovalent cation binding to the vesicles was negligible. However, recent evidence [2, 3, 8] indicates that $Na^+$, the monovalent cation

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present in most of the $Mn^{2+}$ binding studies, is specifically bound (complexed) by the anionic phospholipids which were employed in the vesicles.

Also implicit in the derivation was the assumption that the uncomplexed anionic phospholipid molecules each carried a net charge of $-1$/head group at the pH used in these studies ($\approx 7.3$) and that, moreover, no changes in protonation of the lipids took place as a result of $Mn^{2+}$ complexation. Evidence is presented below, however, which clearly indicates that protons are displaced when divalent cations bind to PS.

Presented here along with the evidence for $H^+$ displacement are further comparisons between the results of Mn binding experiments and predictions based on the Gouy-Chapman-Stern theory. It is found that, although some difficulties remain, the agreement is considerably improved when $Na^+$ binding and $H^+$ displacement are taken into account.

## Materials and Methods

Bovine PS (sodium salt) was purchased from Grand Island Biological Co. Lipid purity was verified by thin layer chromatography.

PS vesicles were prepared with the aid of a bath type sonicator as described previously [10, 11]. (Electron microscopy of a negatively stained preparation showed that nearly all were small unilamellar structures.)

For experiments in which Mn binding was to be assayed as a function of $[Na^+]$, PS was first sonicated in a buffer containing: (*a*) 60 mM NaCl, 30 mM Na-MES (pH 6.1) or (*b*) 75 mM NaCl, 30 mM Na-HEPES (pH 7.87). In preparing each sample, 114 µl of an appropriate NaCl solution was mixed with 266 µl of the vesicle stock suspension. Samples were then equilibrated at 37 °C for 1 hr and then at room temperature for at least 30 min. (Longer incubations produced no detectable changes in results.) Sequentially, $MnCl_2$ solution (19 µl) was added to each sample, and the binding was determined by EPR.

Similarly, where Mn binding to PS was to be measured as a function of varying pH, vesicles were sonicated in a medium containing 140 mM NaCl, 6 mM HEPES, and 2 mM MES adjusted to pH 7.5 with NaOH. The pH of 500-µl aliquots of the vesicle suspension were adjusted up or down with 10-µl volumes of trizma base or HCl. Samples were incubated as above, first at 37 °C and then at room temperature, $MnCl_2$ was added to each sample in a small volume of suspension medium, the pH was measured, and Mn binding was assayed by EPR. After its EPR spectrum had been recorded, the pH of the sample was rechecked; no appreciable pH shifts were detected.

EPR measurements of Mn binding were carried out on a Varian E-9 spectrometer equipped with a dual cavity, as described previously [10]. To minimize thermal differences between samples: (a) room temperature $N_2$ was blown through the cavity; (b) a 5-min equilibration was allowed after the flat cell had been inserted and after power and modulation had been set before a spectrum was taken. Measurements were completed within 15 min after Mn addition. Controls showed that no appreciable changes in binding due to Mn penetration occurred during this period.

Proton displacement from PS induced by divalent cation binding was studied in unbuffered vesicle suspensions. Vesicles were prepared as usual except that the sonication process was interrupted one or more times to adjust pH to within $\pm 0.1$ pH units of the desired value. A 1-ml aliquot of the final suspension was transferred to a cut-down 12-mm diameter polypropylene test tube containing a micro stir bar. A semi-micro pH electrode (Markson E-2885 or Beckman 39030) was placed into the solution, which was kept stirring except for short time intervals when readings were being taken. Appropriate small adjustments in pH were made and the sample was equilibrated until the expanded reading on the pH meter (Corning Model 10) was stable for several minutes. Divalent cations were added in small volumes from solutions nearly identical in pH and NaCl concentrations as the vesicle suspensions. As in the EPR experiments, the molar ratio of divalent cation to lipid was low ($<1:25$). Proton displacement was inferred from the amount of NaOH required to reverse the pH changes produced by manganese or calcium chloride additions.

## Results

### *Theoretical Background*

The free and bound fractions of $Mn^{2+}$ present in a suspension of negatively charged phospholipid vesicles can be quantitated with EPR. If the total $[Mn] \ll$ the concentration of phospholipid binding sites, the apparent affinity for Mn complexation can be calculated from the relationship [11]:

$$K_a = \beta [Mn]_{bound} / [Mn]_{free} [P] \tag{2}$$

where [P] is the total concentration of anionic phospholipid head groups and $\beta$ is a normalization factor. In the case where Mn is added to performed small vesicles, $\beta$ can be conveniently set equal to 1.5 [11].

The $[Mn^{2+}]$ at the surface of the vesicles, and hence the magnitude of $K_a$, is modulated by the surface potential $\psi_o$. This dependence is usually assumed to be the form:

$$K_a = K_o \exp(-2e\psi_o/kT) \tag{3}$$

where $K_o$ is the "intrinsic" binding constant, the affinity in the limit of zero surface potential.

If the level of divalent cations is sufficiently low, their influence on surface potential can be neglected. The value of $\psi_o$ can then be estimated from the Gouy equation [1]:

$$\sinh e\psi_o/2kT = \sigma/(8NC\varepsilon\varepsilon_o kT)^{\frac{1}{2}} \tag{4}$$

where $\sigma$ is the surface charge density, $N$ is Avogadro's number, $\varepsilon$ is the dielectric constant of water, and $C$ is the bulk concentration of monovalent salt. For $\sigma$ large and negative, Eqs. (3) and (4) readily combine to give Eq. (1).

Possible specific adsorption of monovalent cation to the vesicles has not been considered to this point. If the cation, say $Na^+$, does complex to the surface, two changes are required. First, assuming the same stoichiometry for $Na^+$ and $Mn^{2+}$ binding, the number of available $Mn^{2+}$ binding sites will be reduced by a factor, $y = 1 + K'_o[Na^+] \exp(-e\psi_o/kT)$,

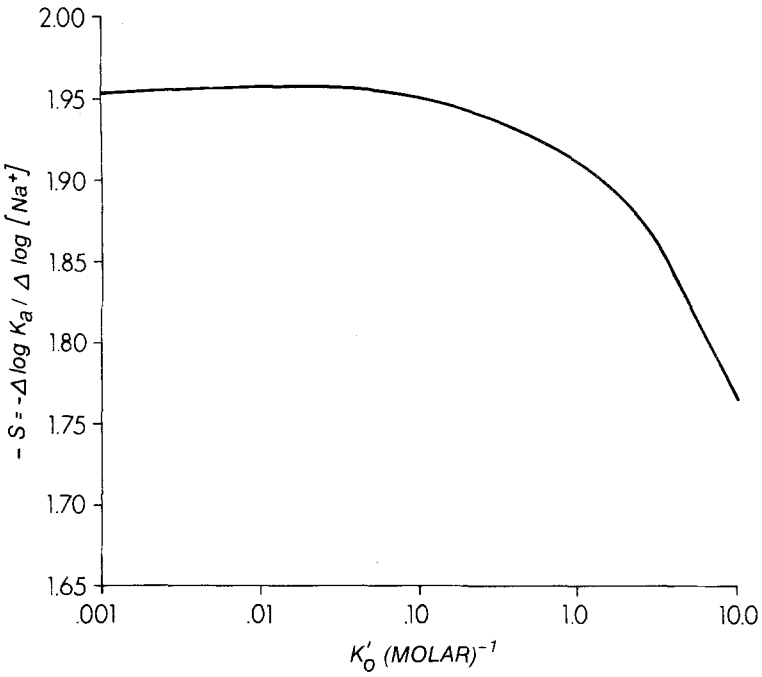


**Fig. 1.** Theoretical variation in apparent divalent cation affinity for anionic phospholipid vesicles with $[Na^+]$ and intrinsic $Na^+$-phospholipid binding affinity. The Gouy-Chapman-Stern theory, modified for $Na^+$ binding as described in the text, was used to calculate the relative $K_a$ at 100 and 200 mM $[Na^+]$, as a function of $K'_o$. The surface charge density, uncorrected for ion binding, was taken to be $\sigma = -e/75$ Å$^2$. The ordinate represents the calculated average slope, $s = \log(K_{a,100\text{ mM}}/K_{a,200\text{ mM}})/\log 2$

where $K'_o$ is the intrinsic affinity of the lipid for $Na^+$ and $[Na^+]$ is the bulk concentration of sodium. Likewise in calculating $\psi_o$, the surface charge density in the absence of $Na^+$ adsorption must be replaced by $\sigma/y$ in Eq. (4). Both these corrections will tend to lower the apparent $K_a$, as defined by Eq. (2).

Taking into account these modifications, computer calculations were carried out as to the variation in $K_a$ with varying $[Na^+]$, $\sigma$, and $K'_o$.

The surface charged density of anionic phospholipid vesicles has not been measured. Watts *et al.* [12] have estimated, however, that dimyristoyl phosphatidylcholine head groups in the outer leaflet of a small unilamellar vesicle in the liquid crystalline state occupy $\approx 75$ Å$^2$, slightly higher than values ($\approx 60$ Å$^2$) usually obtained in multilayer systems. Thus for vesicles composed of acidic lipids such as PS, it appears reasonable to set $\sigma \simeq -e/75$ Å$^2$, in the physiological pH region, for temperatures above the phase transition. Based on this approximation, the ratio of Mn affinities at 100 and 200 mM NaCl were calculated as a function of $K'_o$. The results are shown in Fig. 1.

For $K'_o \lesssim 1$ M$^{-1}$, the calculated slope $s \equiv \Delta \log K_a / \Delta \log [Na^+]$ is very close to $-2$, as expected from the limiting relationship defined by Eq. (1). As $K'_o$ is increased further, the magnitude of $s$ falls off gradually to a value of $\approx 1.77$ at $K'_o = 10$ M$^{-1}$. Three independent studies of $Na^+$-PS binding have all yielded values of $K'_o$ in the range 0.6–1.2 M$^{-1}$ [2, 3, 8]. It would appear therefore that specific $Na^+$ binding *cannot* account for much of the discrepancy between the slope ($s \simeq -2$) predicted from the Gouy-Chapman-Stern theory and the slope ($s \simeq -1.49$) previously derived from measurements of Mn binding to PS [10]. Further computer calculations indicate, moreover, that the above assertion is valid for any reasonable assumed value of $\sigma$ (area/charge $\lesssim 100$ Å$^2$).

### *Variation in $Mn^{2+}$ Binding to PS with pH*

$Mn^{2+}$ binding to PS vesicles was monitored as a function of pH as described in *Materials and Methods*. As shown in Fig. 2, the apparent affinity of PS for $Mn^{2+}$ exhibited a biphasic dependence over the range pH 5.7–8.35. $K_a$ increased relatively slowly as pH was raised from 5.7 to $\approx 7.3$, but the rate of increase was much more rapid above pH 7.3. As indicated by the dashed curve, the measured $K_a$ at high pH was found to be nearly proportional to $[H^+]^{-1}$.

$H^+$ titrations of PS bilayers in media containing no divalent cations but comparable levels of NaCl have indicated that PS carried a net charge of $-1$ over the entire pH range in Fig. 2 [1, 9]. The results here do not conflict with those findings but suggest that protons are ejected from the bilayer concomitant with $Mn^{2+}$ complexation. (Presumably these protons are dissociated from the amino group of PS.)

The data in Fig. 2 are most easily accounted for by postulating two separate binding reactions:

$$Mn^{2+} + PS^- \rightarrow (Mn\text{-}PS)^+ \quad (5a)$$

$$Mn^{2+} + PS^- \rightarrow (Mn\text{-}PS)^o + H^+ \quad (5b)$$

having respective association constants:

$$K_{1a} = [(Mn\text{-}PS)^+]/[Mn^{2+}][PS^-] \quad (6a)$$

$$K_{2a} = [(Mn\text{-}PS)^o][H^+]/[Mn^{2+}][PS^-]. \quad (6b)$$

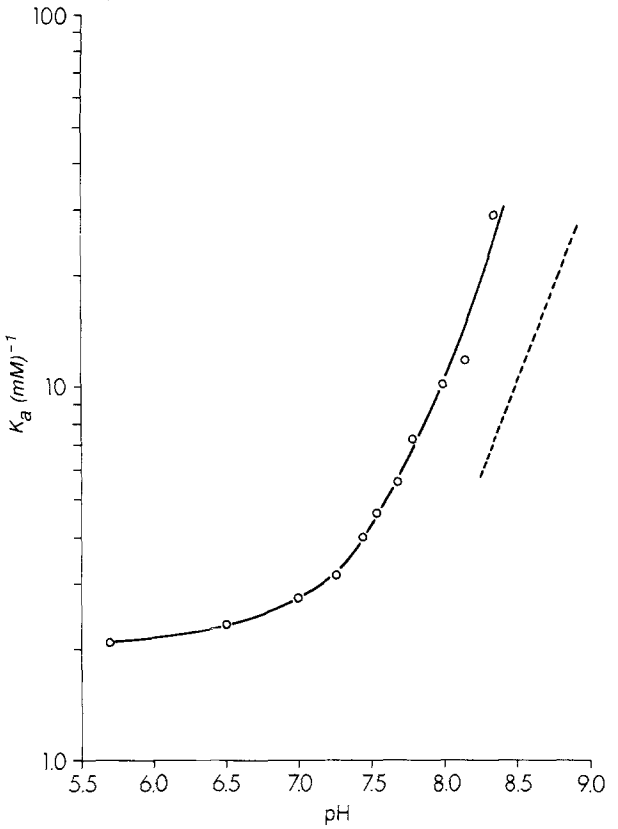


**Fig. 2.** Variation in $K_a$ with pH. $Mn^{2+}$ binding to PS vesicles, as a function of varying pH, was determined by EPR as described in *Materials and Methods*. Conditions were: 1.87 mg/ml PS, 140 mM NaCl, 3 mM MES, 6 mM HEPES, $5.57 \times 10^{-5}$ M $MnCl_2$. The slope of the dashed curve indicates the dependence for $K_a$ proportional to $[H^+]^{-1}$

EPR determinations yield a value for:

$$K_a = K_{1a} + K_{2a}/[H^+]. \quad (7)$$

The first term on the right of Eq. (7) dominates at low pH, the second at high pH. Under the conditions employed in Fig. 2 the two terms appeared to be comparable near pH 7.3.

### *Dependence of $K_a$ on $[Na^+]$*

If Mn binding to PS involves liberation of protons, then the theoretical relationship between $K_a$ and $[Na^+]$ discussed earlier must be modified. Consider, for purposes of discussion, the scheme defined by Eqs. (5)–(7). It follows from Fig. 1 that if $K'_o \lesssim 1.5\ M^{-1}$ a plot of $\log K_{1a}$ *vs.* $\log[Na^+]$ ought to have a slope $s_1 = -1.93 \pm 0.03$. On the other hand, at a fixed pH, the corresponding slope $s_2 = \Delta \log K_{2a}/\Delta \log[Na^+]$ is expected to have some other value (smaller in magnitude), also dependent on $K'_o$ ($s_2 \to s_1/2$ as $K'_o \to 0$). EPR enables one to measure the apparent affinity, $K_a = K_{1a} + K_{2a}/[H^+]$, as a function of $[Na^+]$. Over any $[Na^+]$ range, the slope $s = \Delta \log K_a/\Delta \log [Na^+]$ will lie between $s_1$ and $s_2$, inclusive. Furthermore it is predicted that the magnitude of $s$ should increase with pH as reaction (5b) becomes more important relative to (5a).

$Mn^{2+}$ binding to the outside of PS vesicles was measured as a function of $[Na^+]$ at pH 6.1 and 7.87, respectively, i.e., at points located in the two asymptotic regions of $[H^+]$ defined by the curve in Fig. 2. Typical results are shown in Fig. 3. Plots of $\log K_a$ *vs.* $\log [Na^+]$ generally appeared to be nearly linear over the respective $[Na^+]$ ranges employed in Fig. 3, both for data obtained at pH 6.1 and at pH 7.87. When the data from a number of independent sets of measurements were subjected to least-squares analysis, the following mean slopes and standard deviations were obtained: $s = -1.59 \pm 0.14(6)$ at pH 6.1 and $s = -1.14 \pm 0.13(4)$ at pH 7.87. About half the observed variation in the slopes can be accounted for by uncertainties in EPR quantitation; the remainder is due to unknown causes, presumably relating to variations between batches of vesicles. By way of comparison, previous determinations carried out at pH 7.3, under conditions where $Mn^{2+}$ was present on both sides of the vesicles, yielded $s = -1.49 \pm 0.12$ [10].

The results in Fig. 3 further illustrate the enhancement of Mn-PS binding with increasing pH. In addition, they indicate that the slope $\Delta \log K_a/\Delta \log [Na^+]$ decreases substantially in going from pH 6.1 to 7.87. Consequently, the data are consistent with the general idea that two (or more) types of Mn-PS complex can be formed, each involving the release of differing numbers of protons. They further suggest that at least part of the discrepancy between the observed slope and that predicted by theory ($s \simeq -1.93$, taking into account $Na^+$ binding) may be attributable to $H^+$ displacement.

### *Measurements of $H^+$ Displacement*

Titration experiments (*see Materials and Methods*) were performed to further investigate the relationship between $H^+$ displacement and divalent cation binding to PS. The results are summarized in Table 1. At 100 mM NaCl, roughly one $H^+$ was released into the medium for each $Mn^{2+}$ ion added to a PS vesicle suspension at pH $\simeq$ 7.9, but only $\approx .097$ $H^+$ was released for each $Mn^{2+}$ added at pH $\simeq$ 6.1. Since the lipid concentration was chosen so as to ensure > 80% binding of $Mn^{2+}$, this confirms the inference drawn from Figs. 2 and 3 that $H^+$ displacement by $Mn^{2+}$ increases with pH.

At pH $\simeq$ 6.1, moreover, it was found that proton release increased to 0.16 $H^+/Mn^{2+}$ added when $[Na^+]$ was raised to 200 mM. This change is not unexpected since increased $Na^+$ should act to reduce

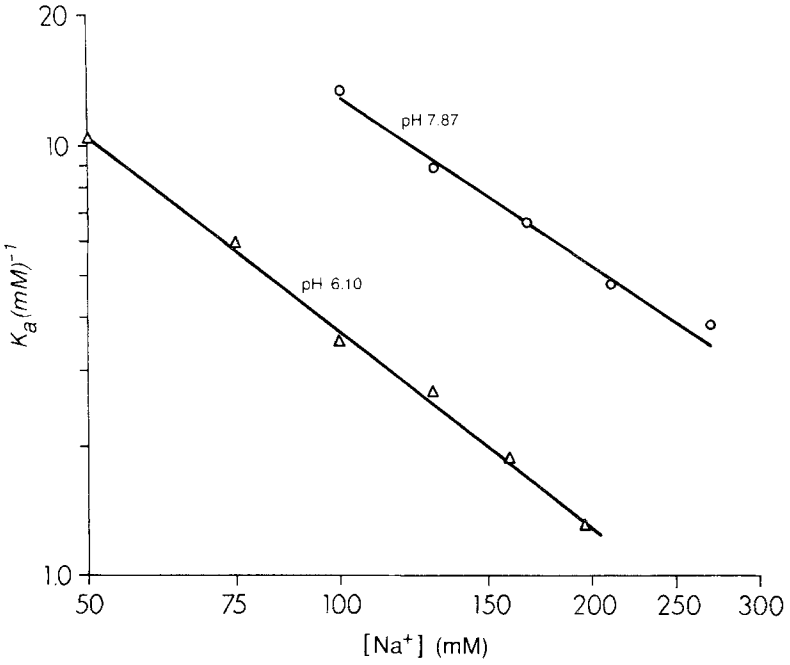


**Fig. 3.** Dependence of $K_a$ on $[Na^+]$. $Mn^{2+}$ binding to PS vesicles *vs.* varying $[Na^+]$ was determined by EPR as described in *Materials and Methods*. Conditions were: (○) 1.025 mg/ml PS, 20 mM HEPES (pH 7.87), $3.28 \times 10^{-5}$ M $MnCl_2$; (△) 2.18 mg/ml PS, 20 mM MES (pH 6.1), $6.65 \times 10^{-5}$ M $MnCl_2$

the surface potential which would, in turn, favor proton dissociation. No appreciable change of this sort was observed at elevated pH, however, consistent with the idea that reaction (5b) dominates at both sodium concentrations in this case.

Acidification of the medium was also found when low levels of $Ca^{2+}$ were added to PS vesicles at $pH \simeq 7.9$, but fewer protons were released than with a corresponding $Mn^{2+}$ addition. On the other hand, no detectable acidification occurred when $Ca^{2+}$ was added at pH 6.1 in presence of 0.1 M NaCl. This contrasts somewhat with results of Abramson *et al.* [1], who observed a significant release of protons when $Ca^{2+}$ was added to PS dispersions at $pH \simeq 6.1$. Those measurements, however, are not fully comparable with those reported here since they were performed in the absence of NaCl.

## Discussion

In media containing .05–0.5 M NaCl, but no divalent cations, the free carboxyl and amino moeities of PS membranes exhibit apparent pK's at $<3.5$ and $>10.0$, respectively [1, 4, 9]. Thus, PS would seem to have no titratable groups near physiological pH. It was nevertheless found (*see* Fig. 2) that, in NaCl solutions containing still very low levels of divalent cation, the apparent affinity of PS for $Mn^{2+}$ increased monitonically over the range pH 5.7–8.35, with a rapid increase above $pH \simeq 7.3$. Guided by the results in Fig. 2, and in particular by the observation that $K_a$ was nearly proportional to $[H^+]^{-1}$ at elevated pH, we proposed a scheme (*see* Eqs. (5a) and (5b)) in which two types of Mn binding can occur, one being simple $Mn^{2+}$ adsorption, the other involving release of an $H^+$ from the amino group of PS. The latter complex, in this view, grows stronger with increasing pH, predominating above $pH \simeq 7.3$. Such a scheme is also fully compatable with the titration data presented in Table 1, but other kinds of $Mn^{2+}$ complexes involving, e.g., release of protons from more than one PS head group cannot be ruled out. Alternative to $H^+$ displacement, formation of a ternary complex involving PS, $Mn^{2+}$ and $OH^-$ ions would produce a net acidification of the medium. Such a complex would also grow stronger with increasing pH as seen in Fig. 2. Although the experiments here do not distinguish between these possibilities, strong ternary complexes involving $Mn^{2+}$, $OH^-$, and either phosphoryl or carboxyl moieties appear to be rare, hence displacement of the amine $H^+$ seems more probable.

It was previously reported [10] that the variation in apparent $Mn^{2+}$-PS association constant with $[Na^+]$ observed at pH 7.3 was given by $s \approx -1.49$, while the Gouy-Chapman-Stern theory, in the limit of large negative $\sigma$, predicted $s = -2$. The results here indicate, however, a considerably narrower difference between experiment and theory.

First, as illustrated in Fig. 1, a calculation based on $\sigma = -e/75$ Å$^2$ (a reasonable surface charge density for anionic phospholipids in the liquid crystalline state [12]) yields a theoretical slope closer to $-1.95$. Evidence in favor of specific $Na^+$ binding to PS [2, 3, 8] forces another small revision in this estimate.

**Table 1.** $M^{2+}$ induced displacement of protons from PS vesicles

| $M^{2+}$ | pH | NaCl (mM) | $\Delta H^+/\Delta M^{2+}$ (mole displaced/ mole added) |
|---|---|---|---|
| $Mn^{2+}$ | 6.1 | 100 | 0.097 |
| | 6.1 | 200 | 0.16 |
| | 7.9 | 100 | 0.98 |
| | 7.9 | 200 | 1.04 |
| $Ca^{2+}$ | 6.1 | 100 | 0.00 |
| | 7.9 | 100 | 0.17 |

$H^+$ displacement from PS vesicles was determined as outlined in the Methods section. The titration procedure in each case was carried out over a range of $H^+$ encompassing $< \pm 0.1$ pH units of the respective nominal pH noted in the Table. Each tabulated value of $\Delta H^+/\Delta M^{2+}$ represents a mean derived from 3 independent sets of measurements on separate vesicle preparations. Reproducibility was generally within $\pm 15\%$ for the $Mn^{2+}$ data, and within $\pm 0.02$ for the $Ca^{2+}$ results.

PS concentrations were as follows: 5 mg/ml (pH 6.1); 2.0 mg/ml (pH 7.9, 100 mM NaCl); 2.5 mg/ml (pH 7.9, 200 mM NaCl).

If, for example, the intrinsic $Na^+$ affinity $K_o' \approx 0.6\ M^{-1}$, then the theoretical slope is, according to Fig. 1, $\simeq -1.92$. Higher assumed values for $K_o'$ would, as shown in Fig. 1, lead to still smaller estimates of $|s|$. More importantly, however, this analysis neglects changes in protonation and, consequently, only provides a theoretical estimate of $|s_1| = -\Delta \log K_{1a}/\Delta \log [Na^+]$ (*cf.* Eq (6a)).

If, however, $H^+$ release is important (Eq. (5b)), then the slope observed at a given pH will depend on the relative magnitudes of $K_{1a}$ and $K_{2a}$ (Eqs. (6a) and (6b)). Thus, with increasing pH, $|s|$ should decrease as $K_{2a}$ becomes larger while $K_{1a}$ remains constant. This prediction is consistent with the results in Fig. 3, which show a considerably steeper slope at pH 6.1 than at pH 7.87.

As seen from data in Table 1, appreciable $H^+$ dissociation accompanies $Mn^{2+}$ binding, even at pH 6.1. After suitable corrections have been made (based on EPR determinations of $K_a$) for the small $Mn^{2+}$ fraction not bound to the vesicles, the data indicate that, at this pH, simple adsorption (reaction (5a)) accounts for approximately 90 and 81% of the $Mn^{2+}$ binding at .1 M and .2 M $[Na^+]$, respectively. Noting that measurements of $K_a$ *vs.* $[Na^+]$ gave a mean value for $s$ of $-1.59$, it can then be estimated that $s_1 = \Delta \log K_{1a}/\Delta \log [Na^+] \simeq -1.74$; this is in fair agreement with the theoretical estimate, $s_1 \simeq -1.92$, arrived at above.

Additional computer calculations (results not shown) indicate that corrections for $Na^+$ binding and $H^+$ displacement might also explain most of the apparent discrepancy between the Gouy-Chapman-Stern theory and the previously observed slow fall-off in $Mn^{2+}$ binding with decreasing $\sigma$ [10, 11]. Further experimental work is needed, however, to test this point.

In conclusion, the findings here help to reconcile the observed characteristics of Mn binding to anionic phospholipid vesicles with predictions of the Gouy-Chapman-Stern theory. Although additional studies are necessary, it would appear that when proper attention is paid to all of the pertinent interactions, the theory may provide a useful framework for describing ionic interactions with membrane phospholipids.

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