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SURFACE POTENTIAL OF PHOSPHATIDYLSERINE MONOLAYERS

II. DIVALENT AND MONOVALENT ION BINDING

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Ion binding constants for phosphatidylserine membranes have been derived from the variation of the surface potential of phosphatidylserine monolayers with divalent cation concentrations in the presence of various monovalent salts in the aqueous subphase. The observed surface potential data for the monolayers, analyzed by use of the Gouy-Chapman diffuse potential theory, together with a simple binding reaction formula, yield, for Ca^{2+}, Mg^{2+}, Na^+ and $(Me)_4N^+$ binding constant values of $30\,M^{-1}$, $10\,M^{-1}$, $0.6\,M^{-1}$ and $0.05\,M^{-1}$, respectively. The effect of pH on surface potential of phosphatidylserine monolayers was found to be dependent upon ionic species other than H^+ in the subphase solution. The distinction between apparent and intrinsic dissociation constants of H^+ for biomolecules was made in terms of ion binding due to other ions at the same site as for H^+ in biomolecules.

Introduction

In a previous study [1], we attempted to determine the association constants for binding of various divalent ions to phosphatidylserine membrane surfaces by measuring the surface potential of the monomolecular film. However, in the calculation of the binding constants, we assumed that monovalent ions did not bind to the phosphatidylserine polar groups. Consequently, the values for binding constants of divalent cations thus derived were too small.

Since then, it has been shown that monovalent ions do bind to model membranes of phosphatidylserine [2–5]. Therefore, the previously obtained binding constants of divalent ions [1] should be reevaluated. Although the monovalent ions bind to phosphatidylserine membranes more weakly than do divalent ions, the effects of monovalent ions are important in the interpretation of such phenomena as membrane aggregation and fusion of phospholipid systems in different ionic environments.

In this work we describe surface potential measurements for phosphatidylserine monolayers and the dependence of this potential on the concentration of divalent ions in the presence of different monovalent ionic salts at various ionic strengths. The data are analyzed to obtain the binding constants of divalent as well as monovalent ions. We also interpret the difference in phosphatidylserine monolayer pH-surface potential ($\Delta\Delta V$) curves obtained for the two subphase salt solutions (NaCl and tetramethylammonium chloride, $(Me)_4NCl$) in terms of the different binding constants obtained from the former experiments for Na^+ and tetramethylammonium ion ($(Me)_4N^+$).

Finally, we discuss the determination of pK_a values of biomolecules in a salt solution medium and the importance of taking into account the effect of other cation binding on the evaluation of the dissociation constant of H^+ for various biomolecules.

Materials and Methods

Materials. Phosphatidylserine was extracted from beef brain and purified according to the method of Rouser et al. [6] with minor modifications. Ultrapure NaCl (Brinkman Instruments); histidine (D-αamino-β-imidazole propionic acid); Tes (N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid) both from Sigma; $(Me)_4NCl$ (tetramethylammonium chloride, purum, Fluka); and other analytical grade reagents (Alfa Chemicals) were used in the experments. NaCl was roasted at about 600°C for 1 h to eliminate contaminants of organic materials. Purification of tetramethylammonium chloride was done in the following two ways: (i) $(Me)_4NCl$ was dissolved in distilled water (3 M) and washed with redistilled hexane (Fluka) several times in order to remove soluble contaminants in $(Me)_4N$ solution to the hydrocarbon (hexane) phase. Then the washed $(Me)_4N$ solution was transferred into a teflon trough and the surface of the solution was scraped by a teflon bar several times in order to remove possible surfactants. (ii) The other purification method used recrystallization of $(Me)_4N$ salt from ethanol and acetone solution. By treating $(Me)_4N$ salt in these ways, a reasonably stable (time-independent) surface potential of a phosphatidylserine monolayer was observed when this salt was used in the subphase solution. $(Me)_4N$ salts purified by the above two methods gave identical results, within the experimental error. Water was triply distilled, including treatment by alkaline $KMnO_4$.

Methods and procedures. The surface potential was measured by use of an Americium air electrode (approx. 3 mm above the air/water interface) and a calomel pH reference electrode [1]. The latter electrode was grounded and the ionizing air electrode was connected to the input of an electrometer (Keithley 610C). The output potential of the electrometer, which was related to 'surface potential', was monitored by a strip chart recorder. The entire apparatus was shielded by a Faraday cage. The pH of the subphase solution was monitored continuously by a pH meter (Corning) through a thin combination pH electrode (Futura, Beckman) which was placed in the subphase solution.

A lipid monolayer was formed on the aqueous phase in a glass dish (9 cm in diameter) by spreading an aliquot of the hexane-lipid solution from a microsyringe (Hamilton). The subphase solution was either 0.1 M NaCl or 0.1 M $(Me)_4N$ containing 1 mM histidine/Tes, pH 7.4. Each experimental solution also contained 1 μM of EDTA to remove polyvalent cationic contaminants possibly present in trace amounts in the system. The concentration of phospholipid was determined by phosphate analysis [7]. All the monolayers used in the experiment had the same area per molecule of 65 $Å^2$ (0.65 nm^2). This value was also determined to be correct within 2 $Å^2$ by surface pressure (π) measurements, the results of which corresponded to those determined for similar systems from a π vs. surface area curve [8].

Before the addition of salt, the aqueous subphase was stirred well and it was ascertained that the monolayer surface potential was not altered by further stirring of the subphase solution. Then a concentrated salt solution was injected into the subphase of the monolayer from a microsyringe. The subphase solution was again stirred until a stable surface potential was obtained. The standard mean error for the observed surface potentials were within ±1 mV for the case of NaCl, and within ±2 mV for the $(Me)_4N$ salt. All experiments were done at room temperature of 24°C ± 2°C. Each data point represents the average of five or more experiments.

Results and Discussion

Fig. 1 shows the surface potential changes ($\Delta\Delta V$) of phosphatidylserine monolayers with respect to Ca^{2+} concentrations in the presence of either 0.1 M NaCl or 0.1 M tetramethylammonium chloride as a subphase solution. The magnitudes of the surface potential changes with the change of divalent ion concentrations were much greater in the case of tetramethylammonium chloride than with NaCl as a subphase solution. This feature qualitatively indicates the large difference between the binding of the two cations to the phosphatidylserine membrane.

Experimental data similar to the above, except that Mg^{2+} was used instead of Ca^{2+}, are shown in Fig. 2. The dependence of surface potential on Mg^{2+} concentration was qualitatively the same as for Ca^{2+}. However, the magnitude of the surface potential change was smaller in the case of Mg^{2+} than for Ca^{2+} over the range of divalent ion concentrations examined.

From these experimental data and the ion adsorption theory (see Appendix), the binding constants of divalent and monovalent ions were calculated. The best fit values of the association constants K (see Appendix) of the above four ions ($(Me)_4N^+$, Na^+,

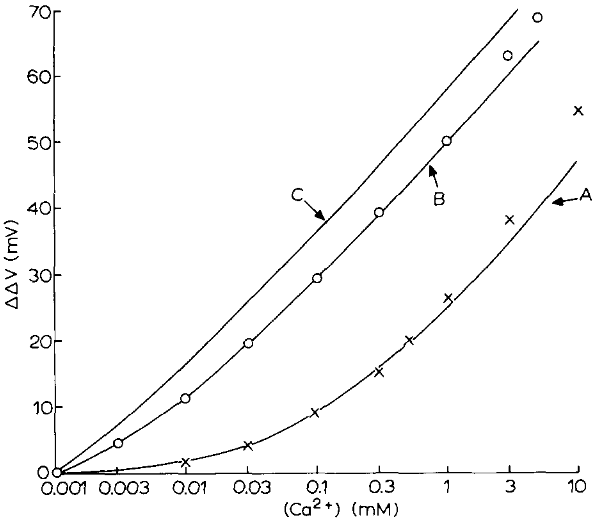

Fig. 1. Surface potential changes ($\Delta\Delta V$) of phosphatidylserine monolayer versus $CaCl_2$ concentrations in the subphase bulk solution. The monolayers having an area 65 Å^2 per molecule were formed at the air/water (0.1 M of either NaCl or $(Me)_4NCl$, 1 mM histidine/Tes, 1 μM EDTA, pH 7.4) interface. Bulk concentrations of Ca^{2+} were corrected for essentially quantitative formation of the Ca^{2+}-EDTA complex ($K_F \simeq 10^{10.59}$) by 1 μM EDTA present in solution. X, NaCl; o, $(Me)_4NCl$. The solid lines correspond to theoretical surface potential values. A: $K_1 = 0.6$ M^{-1} (monovalent ion binding constant) and $K_2 = 30$ M^{-1} (for divalent ion binding constant). B: $K_1 = 0.05$ M^{-1} (monovalent ion) and $K_2 = 30$ M^{-1} (divalent ion). C: $K_1 = 0$, $K_2 = 30$ M^{-1}. The initial surface charge density $\sigma^{int} = -e/65$ Å^2.

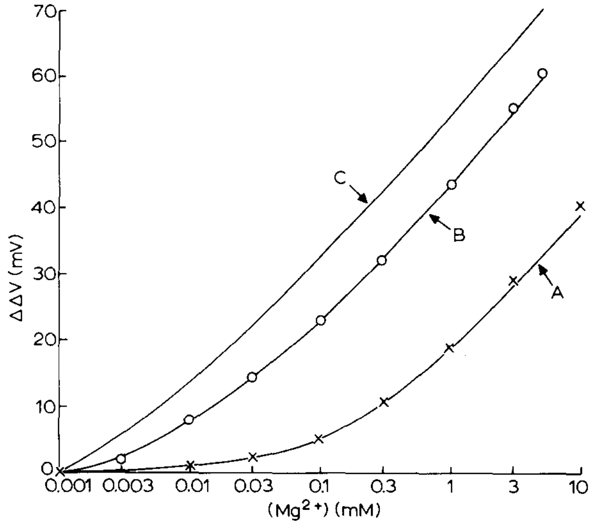

Fig. 2. Surface potential changes ($\Delta\Delta V$) versus $MgCl_2$ concentrations for phosphatidylserine monolayers at area 65 Å^2 per molecule formed at the air/water (0.1 M of either NaCl or TMA, 1 mM histidine/Tes, 1 μM EDTA, pH 7.4) interface. Bulk concentrations of Mg^{2+} were corrected for essentially quantitative formation of the Mg^{2+}-EDTA complex ($K_F \sim 10^{8.69}$) by 1 μM EDTA present in solution. X, NaCl; o, $(Me)_4NCl$. The solid lines are theoretically calculated surface potential values. A: $K_1 = 0.6$ M^{-1} (monovalent ion) and $K_2 = 10$ M^{-1} (divalent ions). B: $K_1 = 0.05$ M^{-1} (monovalent ion) and $K_2 = 10$ M^{-1} (divalent ions). C: $K_1 = 0$ M^{-1}, $K_2 = 10$ M^{-1}. The initial charge density $\sigma^{int} = -e/65$ Å^2.

Mg^{2+} and Ca^{2+}) to the present experimental data were 0.05 M^{-1} for $(Me)_4N^+$; 0.6 M^{-1} for Na^+; 10 M^{-1} for Mg^{2+} and 30 M^{-1} for Ca^{2+}.

The above values agree fairly well with those obtained by other methods: namely, Nir et al. [2] obtained 0.8 M^{-1} for Na^+; 4 M^{-1} for Mg^{2+} and 35 M^{-1} for Ca^{2+} by analysis of an equilibrium dialysis study of the adsorption of Ca^{2+} and Mg^{2+} to phosphatidylserine vesicles. Kurland et al. [3] reported 0.4–1.2 M^{-1} for Na^+ intrinsic binding constants to phosphatidylserine vesicles, interpreted from a ^{23}Na-NMR study. Eisenberg et al. [4] have obtained 0.6 M^{-1} for Na^+ and no binding for $(Me)_4N^+$, from electrophoretic mobility measurements of multilamellar phosphatidylserine vesicles in salt solution. A disagreement in the association constants for $(Me)_4N^+$ derived in this work and that previously reported [4] may be due either to an essential difference between the two measurement methods or to the differences arising from the fact that monolayer membranes were studied here, whereas multilamellar vesicles were used in the electrophoretic study [4].

In the case of Ca^{2+}, the theoretical values of surface potentials derived from the best fit binding constants do not agree well with the experimental data at high bulk Ca^{2+} concentrations, above 1 mM (Fig. 1). It should be noted that even at these high bulk Ca^{2+} concentrations (1 mM or greater), the Ca^{2+} concentration at the monolayer-solution interface is enhanced by a factor of 100 or more due to the Boltzmann term, $\exp(-2e\phi(0)/kT)$ (Appendix). Accordingly, the difference between experimental and theoretical points at these Ca^{2+} concentrations may reflect failure to take non-ideality into account in the theory, i.e. may reflect the use of unit activity coefficients. Alternatives to this explanation for the deviations could invoke more complicated schemes for the binding of Ca^{2+} to phosphatidylserine than the simple 1 : 2 stoichiometry and independent binding site model used here, as has been suggested previously [1,8,9] to explain anomalous effects at higher Ca^{2+} concentrations. One such binding scheme might invoke a 1 : 1 stoichiometry, with displacement of a proton by a bound Ca^{2+} (in order that Ca^{2+} binding would still yield a net decrease of negative surface charge and increase in surface potential) in addition to the 1 : 2 stoichiometry. From Fig. 2 it is evident that the difference between the calculated and experimental values was less for high concentrations of Mg^{2+} (greater than 1 mM) than for Ca^{2+}.

Fig. 3 shows the increase in surface potential, $\Delta\Delta V \equiv \Delta V(\mathrm{pH}) - \Delta V(7.4)$, as the pH is varied by addition of HCl; 0.1 M NaCl or 0.1 M tetramethylammonium chloride were the subphase solutions. The solutions taken at physiological pH (7.4), the reference pH, were adjusted to this pH value by use of 0.5 mM histidine/Tes buffer. The results for the NaCl sub-phase correspond to those previously reported [10] for this case. The $\Delta\Delta V$ vs. pH curve for the $(Me)_4N^+$ subphase is shifted about 0.7 pH units to higher pH, compared to that for NaCl. This shift can be explained by the following argument. Since Na^+ binds to the phosphatidylserine membrane more strongly than does $(Me)_4N^+$, then the surface potential at the phophatidylserine membrane would be more positive for the NaCl case than for $(Me)_4N^+$ if the concentration of the two cations in bulk solution is the same and if, as is the case at pH 7.4, the binding of H^+ is negligibly small. Accordingly, at a given H^+ concentration in bulk solution (i.e., at a given pH for bulk solution), the H^+ concentration at the phosphatidylserine-water interface will be higher for the case of $(Me)_4N^+$ than for Na^+, as the calculated values given in Table I show. When the surface H^+ concentration increases to,a sufficiently high value (of the order of K_a for the acidic group of phosphatidylserine) a greater fraction of phosphatidylserine negatively charged surface sites will be neutralized by bound H^+ for $(Me)_4N^+$ bulk solution than for Na^+, as

TABLE I

CALCULATED SURFACE CONCENTRATIONS OF MONOVALENT CATIONS AT THE PHOSPHATIDYLSERINE MONOLAYER-SOLUTION INTERFACE

pH	$[H^+]^s$ (M) [a]		$[M^+]^s$ (M) [b]		g_H [c]		g_{tot} [d]	
	Na [e]	$(Me)_4N$ [f]	Na [e]	$(Me)_4N$ [f]	Na [e]	$(Me)_4N$ [f]	Na[e]	$(Me)_4N$ [f]
8.0	$2.72 \cdot 10^{-7}$	$8.69 \cdot 10^{-7}$	2.79	9.00	$2.07 \cdot 10^{-4}$	$1.21 \cdot 10^{-3}$	0.620	0.304
7.0	$2.72 \cdot 10^{-6}$	$8.61 \cdot 10^{-6}$	2.79	8.91	$2.06 \cdot 10^{-3}$	$1.19 \cdot 10^{-2}$	0.621	0.310
6.0	$2.70 \cdot 10^{-5}$	$7.88 \cdot 10^{-5}$	2.76	8.14	$2.02 \cdot 10^{-2}$	$1.02 \cdot 10^{-1}$	0.627	0.355
5.5	$8.43 \cdot 10^{-5}$	$2.07 \cdot 10^{-4}$	2.73	6.80	$6.09 \cdot 10^{-2}$	$2.38 \cdot 10^{-1}$	0.639	0.426
5.0	$2.53 \cdot 10^{-4}$	$4.48 \cdot 10^{-4}$	2.61	4.47	$1.67 \cdot 10^{-1}$	$4.23 \cdot 10^{-2}$	0.672	0.539
4.5	$6.64 \cdot 10^{-4}$	$8.24 \cdot 10^{-4}$	2.15	2.66	$3.70 \cdot 10^{-1}$	$5.93 \cdot 10^{-1}$	0.721	0.639
4.0	$1.34 \cdot 10^{-3}$	$1.40 \cdot 10^{-3}$	1.37	1.43	$5.98 \cdot 10^{-1}$	$7.23 \cdot 10^{-1}$	0.778	0.742
3.5	$2.33 \cdot 10^{-3}$	$2.32 \cdot 10^{-3}$	0.746	0.746	$7.64 \cdot 10^{-1}$	$8.17 \cdot 10^{-1}$	0.836	0.824
3.0	$3.97 \cdot 10^{-3}$	$3.94 \cdot 10^{-3}$	0.401	0.397	$8.65 \cdot 10^{-1}$	$8.85 \cdot 10^{-1}$	0.981	0.888
2.5	$7.25 \cdot 10^{-3}$	$7.20 \cdot 10^{-3}$	0.230	0.229	$9.27 \cdot 10^{-1}$	$9.34 \cdot 10^{-1}$	0.936	0.936
2.0	$1.50 \cdot 10^{-2}$	$1.50 \cdot 10^{-2}$	0.151	0.150	$9.65 \cdot 10^{-1}$	$9.67 \cdot 10^{-1}$	0.968	0.968

[a] $[H^+]^s$, calculated hydrogen ion concentration (in M) at the membrane surface: $[H^+]^s = [H^+]_0 \exp(-e\phi(0)/kT)$, where $[H^+]_0$ is the bulk concentration of hydrogen ion.

[b] Calculated surface concentration (in M) of monovalent cation, $M^+ = Na^+$ or tetramethylammonium: $[M^+]^s = [H^+]_0 \exp(-e\phi(0)/kT)$, where $[M^+]_0$ is the bulk concentration of monovalent cation.

[c] Calculated fraction of negatively charged surface sites with bound H^+: $g_H = K_H[H^+]^s/(1 + K_1[M^+]^s + K_H[H^+]^s)$.

[d] Calculated fraction of negatively charged surface sites with either bound H^+ or bound monovalent cation, M^+; $g_{tot} = 1 - f$ (See Appendix).

[e] For assumed bulk NaCl concentration $[M^+]_0 = 0.100$ M.

[f] For assumed bulk tetramethylammonium chloride concentration $[M^+]_0 = 0.100$ M.

The association constants (K) of H^+, Na^+ and $(Me)_4N^+$ used for the above calculation were $2 \cdot 10^3\ M^{-1}$, $0.6\ M^{-1}$ and $0.05\ M^{-1}$, respectively.

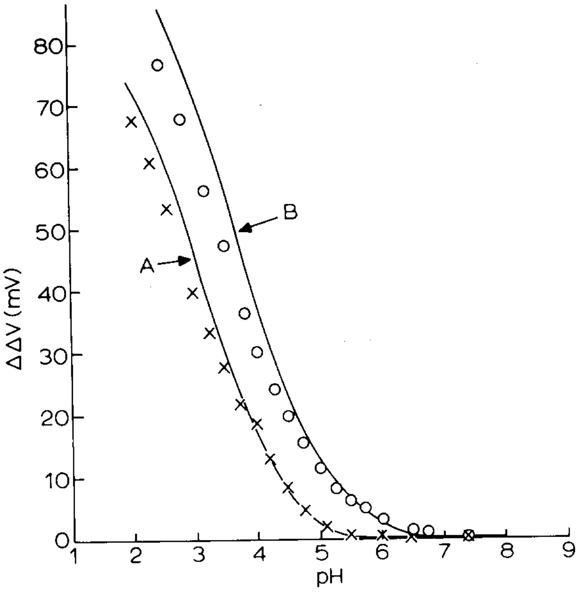

Fig. 3. Surface potential changes versus pH of phosphatidylserine monolayers at area 65 Å^2 per molecule formed at the air/water (0.1 M of either NaCl or $(Me)_4NCl$, 1.0 mM histidine/Tes, 1 mM EDTA, pH 7.4) interface. pH of subphase solution was altered by adding HCl in the subphase solution. X, NaCl; o, $(Me)_4NCl$. The solid lines are theoretically calculated surface potential-pH curves, where the curve A refers to the assumed conditions that the binding constant for the monovalent cations, M^+, is taken to be 0.6 M^{-1} and that for H^+ to be 2 000 M^{-1}, and the curve B refers to the assumed binding constant for M^+ taken to be 0.05 M^{-1}, respectively.

shown by the calculations given in Table I. Thus the surface potential will increase more rapidly with decreasing pH for the case of $(Me)_4N^+$ than for Na^+, corresponding to a shift to higher pH for the $\Delta\Delta V$ curve of $(Me)_4N^+$. The shift can be quantitatively accounted for by the use of the calculated surface potentials obtained from the ion binding constants to fit the above experiments (0.05 for $(Me)_4N^+$ and 0.6 for Na^+) and from an assumed intrinsic association constant, $K = 2 \cdot 10^3\ M^{-1}$ for H^+. The theoretical pH-surface potential curves are also shown in Fig. 3. The shift (0.8–0.9 pH unit) in the theoretically calculated curves is somewhat greater (0.2 pH unit) than that for the experimental ones.

Beside this slight disagreement between observed and calculated pH shifts, another point to be noted in Fig. 3 is that the shape of surface potential-pH relation is different in the two cases (experimental and theoretical); the experimental curve shows a more gradual increase in surface potential with lower pH than does the theoretical. This latter feature may be due to the fact that there are two acidic groups (phosphate and carboxyl groups, whose pK_a are close to each other) in a phosphatidylserine molecule in the pH range under consideration, whereas in our theoretical calculation, only one such acidic group was assumed. If there are two acidic groups, then we should take into account two different dissociation constants for H^+, and possibly two different binding constants for each monvalent cation in the calculation. These factors could result in a more gradual change of surface potential with respect to the pH change.

In order to simplify the analysis, similar experiments using phospholipids having a single dissociating group in the pH range considered, such as phosphatidic acid or phosphatidylglycerol should be carried out for comparison with the theoretical results. Conversely, if the intrinsic binding constant of H^+ to a molecule is known, then surface potential-pH measurements similar to those described above could be used to estimate binding constants of subphase ions.

Behavior similar to the shift in surface potential-pH curves for the cases of NaCl and $(Me)_4N$ subphase solutions has been observed for phosphatidylserine vesicle aggregation when the turbidity of a suspension was plotted against pH. In this case, the direction of the shift was reversed and the magnitude (~0.5 pH unit) of the shift was smaller than that for the surface potential-pH curves (Ohki, S., unpublished observation). This result may also be rationalized in terms of the different binding affinities of Na^+ and $(Me)_4N^+$ to a phosphatidylserine membrane surface, although the theoretical interpretation of vesicle membrane aggregation is much more complicated than that of the surface potential measurements.

It should be noted that the determination of pK_a values for biopolymers generally is for a situation such that these molecules are in a given ionic environment. Since there may be different binding affinities for different ions with a given biopolymer, the observed pK_a value must correspond to an 'apparent' dissociation constant, not the intrinsic dissociation constant. Such an 'apparent' dissociation constant of H^+ would therefore vary with differences in the ionic environment, such as ionic species and ionic strength. An example of this is shown in Fig. 4. Suppose that

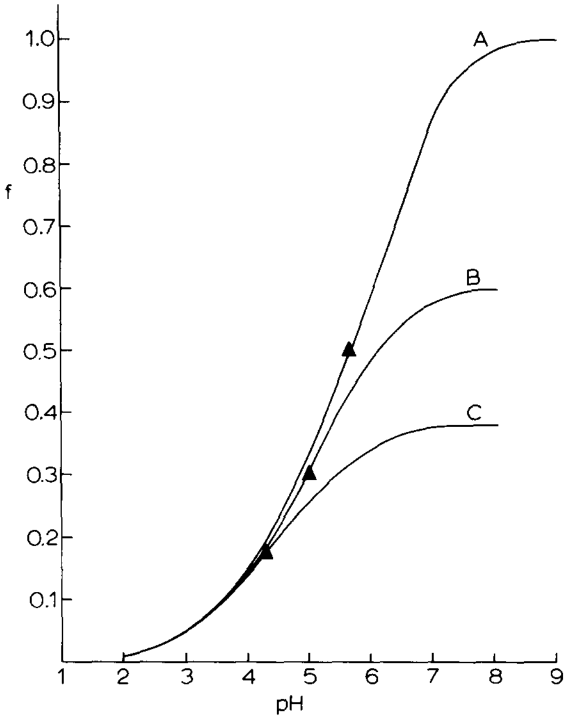

Fig. 4. Theoretical values of fraction of the charge density ($f = \sigma/\sigma^{\mathrm{int}}$) vs. pH of the subphase solution for a monolayer having initially one net negative charge site per 65 Å^2. The monolayer has been assumed to form on a 0.1 M monovalent salt solution and the cation binding constants have been taken as: (A) zero M^{-1}; (B) 0.08 M^{-1}; and (C) 0.6 M^{-1}, respectively. The binding constant for H$^+$ is assumed to be $1 \cdot 10^4$ M^{-1}.

an acidic phospholipid molecule has an acidic group with the H$^+$ association constant of 10 000 M^{-1}, and its monomolecular film, which has an area of 65 Å^2 per molecule, is formed on different 0.1 M monovalent salt solutions; the cations of the salts also have different binding constants to the phospholipid molecules. Then, the variation of surface potential (Gouy-Chapman double layer potential) of such a monolayer with respect to the pH of the subphase can be calculated, provided that the binding constants are known. Fig. 4 shows the fraction (f) of a charged group of a molecule of the monolayer (f is defined in the Appendix) in such cases. The curve A corresponds to the case where a monovalent cation does not bind to the lipid charged group, B, the case where a monovalent cation binds with the binding constant of 0.08 M^{-1} and C the case of a monovalent cation having the binding constant of 0.6 M^{-1}. The pH values corresponding to the mid-point of these curves are taken to be the apparent pK_a for H$^+$ (5.65 for A, 5 for B, and 4.3 for C) which are indicated with ▲ in Fig. 4. Although the given intrinsic p$K_a = 4$ is the same in each case, the apparent pK_a values obtained from a fraction of charge versus pH plot are different for different ionic conditions.

It is clear that the 'apparent' dissociation constant of H$^+$ depends on the binding affinities of other ions at the H$^+$ binding site and the ionic strength, as has been recognized for polyelectrolyte solutions [11]. The determination of the correct ion binding constants, including that for H$^+$ binding, is significant not only in the study of the mechanism of membrane adhesion and fusion, but also in studies of many other biochemical processes involving charged membranes.

Acknowledgements

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Appendix

We suppose that the surface potential change, $\Delta\Delta V$, measured by the above method, is due solely to the Gouy-Chapman double layer potential produced at the monolayer-electrolyte interface [1].

The formula for such potential is given by [12].

$$\sigma = \frac{1}{271}\left(\sum_i [i]_0\left(\exp\left(-\frac{eZ_i\phi(0)}{kT}\right) - 1\right)\right)^{1/2} \qquad (1)$$

where σ is the surface charge density (Å^2); $[i]_0$ the molar concentration of ith ionic species in the bulk phase; Z_i the valency of the ith ion; $\phi(0)$ the surface potential at the membrane interface, temperature 24°C.

Then, we assume that one divalent ion M^{2+} binds simultaneously to two phospholipids and one monovalent ion M$^+$ to one phospholipid A$^-$, independently, as in the following reactions:

$$M^+ + A^- \overset{K_1}{\rightleftharpoons} M^+A^- \qquad (2)$$

$$A^{2-} + M^{2+} \overset{K_2}{\rightleftharpoons} M^{2+}A^{2-}$$

where K_1 and K_2 are the association constants for the above reactions:

$$\begin{aligned} K_1 &= \frac{[\mathrm{M}^+\mathrm{A}^-]}{[\mathrm{M}^+]^s[\mathrm{A}^-]} \\ K_2 &= \frac{[\mathrm{M}^{2+}\mathrm{A}^{2-}]}{[\mathrm{A}^{2-}]\,[\mathrm{M}^{2+}]^s} \end{aligned} \tag{3}$$

and where it has been assumed that

$$[\mathrm{A}^{2-}] \equiv \frac{[\mathrm{A}^-]}{2}$$

and $[\mathrm{M}^+]^s$, $[\mathrm{M}^{2+}]^s$ are the surface concentrations of cations M^+ and M^{2+}, respectively ($[\mathrm{M}_i]^s = [\mathrm{M}_i]_0 \exp(-eZ_i\phi(0)/kT)$). Then, the surface charge density is

$$\begin{aligned} \sigma = \sigma^{\mathrm{int}}(1 &+ K_1 \cdot [\mathrm{M}^+]_0 \cdot \exp(-e\phi(0)/kT) \\ &+ K_2 \cdot [\mathrm{M}^{2+}]_0 \cdot \exp(-2e\phi(0)/kT))^{-1} \end{aligned} \tag{4}$$

where σ^{int} is the surface charge density with no ion binding.

With Eqns. 1 and 4, and for all ionic concentrations in the bulk phase known, and the initial surface charge density of the phosphotidylserine monolayer taken as $-e/65$ Å^2, the surface potential was calculated by a computer (CDC 4000 at SUNY/Buffalo). K_1 and K_2 were taken as variable parameters to give a best fit of the calculated surface potential to the experimental data.

For the case of two monovalent ions present in the subphase solution, the binding reaction equations are quite similar to the above, and the surface charge density is expressed by

$$\begin{aligned} \sigma = \sigma^{\mathrm{int}}(1 &+ K_1'[\mathrm{M}'^+]_0 \exp(-e\phi(0)/kT) \\ &+ K_1''[\mathrm{M}''^+]_0 \exp(-e\phi(0)/kT))^{-1} \end{aligned} \tag{5}$$

instead of Eqn. 4, where K_1' and K_1'' are the binding constants for one species ($'$) and the other species ($''$) of monovalent ions, respectively.

The fraction of the surface charge density, f, is expressed by

$$f = \frac{\sigma}{\sigma^{\mathrm{int}}}$$

References

1 Ohki, S. and Sauve, R. (1978) Biochim. Biophys. Acta 511, 377–387
2 Nir, S., Newton, C. and Papahadjopoulos, D. (1978) Bioelectrochem. Bioenerg. 5, 116–133
3 Kurland, R., Newton, C., Nir, S. and Papahadjopoulos, D. (1979) Biochim. Biophys. Acta 551, 137–147
4 Eisenberg, M., Brasalfi, T., Riccio, T. and McLaughlin, S. (1979) Biochemistry 18, 5213–5223
5 Puskin, J.S. (1977) J. Membrane Biol. 35, 39–55
6 Rouser, G., Bauman, A.J., Kirtchavsky, G., Haller, P. and O'Brien, J.S. (1961) J. Am. Oil. Chem. Soc. 38, 544–555
7 Fiske, C.H. and Subbarow, Y. (1925) J. Biol. Chem. 66, 375–400
8 Ohki, S. and Düzgünes, N. (1979) Biochim. Biophys. Acta 552, 438–449
9 Bangham, A. and Papahadjopoulos, D. (1966) Biochim. Biophys. Acta 126, 181–184
10 Papahadjopoulos, D. (1968) Biochim. Biophys. Acta 163, 240–254
11 Marinsky, J.A. (1976) Coord. Chem. Rev. 19, 125–172
12 Delahay, P. (1965) Double Layer and Electrode Kinetics, Interscience Publisher Inc., New York