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Effect of surface ionization of dimyristoylphosphatidic acid vesicle membranes on the main phase-transition enthalpy and temperature

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The main phase transition of phospholipid bilayers is a property expressed by the order-disorder conformational change of the lipid tails. Nevertheless, with ionizable phospholipids, changes in the surface charge have large effects on the membrane properties. The free energy of a charged phospholipid membrane depends on the degree of ionization, area per phospholipid molecule, and the temperature. Here, the effect of surface electrostatic charges on the temperature and the enthalpy of the main phase transition of dimyristoylphosphatidic acid vesicle membranes is analyzed. A simple equation is presented that describes the relationship among the surface charge density, the phase-transition temperature, the surface area ratio between solid and liquid membranes, and the excess enthalpy. The theory indicated that the pH-induced shift in the excess enthalpy is attributable to the change in the surface area ratio between the solid and liquid membranes.

Introduction

In elucidating local anesthetic actions, the interaction between cationic anesthetics and anionic phospholipid membranes constitutes a major part because the standard local anesthetics are aromatic amines and exist about 90% in the cationic form at physiological pH, and cell membranes usually carry negative surface charges. As a part of our effort to elucidate local anesthetic interactions with lipid membranes, this control study analyzed the effects of pH and ionic strength on the main phase transition of acidic phospholipid membranes to gain basic parameters for the interaction.

The main phase-transition temperature of acidic phospholipid vesicles decreases with increasing pH, apparently due to the change in ionization and the consequent increase in the electrostatic repulsion between negatively charged hydrophilic moiety [1–4]. The concentration of the monovalent cation in the solution also affects the phase transition temperature [2,3]. Isothermal phase transition was demonstrated by the change in the electrostatic environment [2]. Also, the enthalpy change for the phase transition is affected by the solution pH [5].

There are several theories for the electrostatic effects on the phase transition of charged membranes [1,2,6–11]. Träuble and Eibl [1] and Träuble et al. [2] analyzed the pH-induced change in the transition temperature by calculating the electrostatic free energy according to the Gouy-Chapman theory. For membranes with high surface poten-

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tial, they found that the transition temperature bears a linear relationship with the degree of ionization of the phospholipid.

Jähnig [6] contended that the above theory did not include the effect of the ionization-induced change in the surface area, and separated the ionization-induced shift of the transition temperature into two parts: (1) charge-induced shift caused by the difference in the surface electrostatic free energy between the solid-gel and liquid-crystalline states, and (2) area-induced shift, caused by the change in the surface area between in each phase. He concluded that these two shifts were of comparable magnitude.

Apparently, the changes in the surface area per lipid molecule, caused by ionization of the polar head group, cannot be ignored in analyzing the phase transition of charged phospholipids [11–13]. We present a theory here that describes the effect of surface ionization on the temperature and the enthalpy changes in the phase transition of ionizable lipid membranes. The effects of pH and ionic strength on the main phase transition of dimyristoylphosphatidic acid were measured by differential scanning calorimetry and by optical methods, and the results were compared with the theory.

Experimental procedures

Synthetic dimyristoylphosphatidic acid, Coumarin 311 (7-dimethylamino-4-methylcoumarin), and DPH (1,6-diphenyl-1,3,5-hexatriene) were obtained from Sigma. All other chemicals were reagent grade. Water was triply distilled, once from alkaline potassium permanganate solution, and stored in Teflon bottles to avoid contamination by divalent cations.

The main phase-transition of the dimyristoylphosphatidic acid vesicle membranes was evaluated by differential scanning microcalorimetry and three optical methods. The optical methods measured a sudden change at the main phase-transition in the (1) liposome turbidity or (2) the fluorescence yield of Coumarin 311 adsorbed to the membrane or (3) the fluorescence anisotropy of DPH incorporated into the membrane core. The optical methods had an advantage over the calorimetry method because dilute phospholipid suspension could be used, typically 0.2 mM, making the pH measurement accurate. Our calorimetry method required higher phospholipid concentrations, typically above 20 mM.

Dimyristoylphosphatidic acid was dissolved in a chloroform/methanol mixture (9:1, v/v), and the solvent was removed in a rotary evaporator under the flow of nitrogen gas. An appropriate amount of suspending solution was added into the evaporator flask and agitated by a vortex mixer for about 5 min or until homogeneous suspension was obtained. The dimyristoylphosphatidic acid concentration was 0.2 mM. The suspending solution consisted of several concentrations of NaCl (50, 100, and 200 mM) with 0.05 mM EDTA, and at various pH values. The pH was adjusted with 1 M HCl or NaOH, using a Radiometer Ion 85 Analyzer and a combination glass electrode (Copenhagen, Denmark), while keeping constant sodium ion concentrations. The obtained multilamellar suspension was sonicated in a cuphorn of a Branson Sonifier Model 185 (Danbury, CT) at above the phase-transition temperature for 15 min.

A Perkin-Elmer Model 554 UV-visible spectrophotometer (Norwalk, CT) was used to measure the light absorbance of the liposome at 400 nm. The temperature of the sample was measured with a thermistor probe inserted into the cuvette slightly above the lightpath and monitored by a Digitec thermometer Model 5810 (Dayton, OH) with 0.01 C° resolution. The cuvette temperature was scanned at a rate of 0.5 C°/min by a programmable Perkin-Elmer digital temperature controller and an electronic heat exchanger. The absorbance change at 400 nm wavelength and the temperature of the sample were recorded on a Heath/Schlumberger *X-Y* recorder Model SR-207 (Benton Harbor, MI). During the temperature scan, the sample solution in the cuvette was continuously mixed with a direct drive Spectro-Stir (Oreland, PA). The phase transition was determined by the sudden change in the absorbance. The transition temperature obtained by this optical method agreed with that obtained with the differential scanning calorimetry as previously reported [14,15].

Coumarin 311 was added to the aqueous phase of the vesicle suspension. The mole ratio of Coumarin to the phospholipid was less than

$1 \cdot 10^{-2}$. The phase-transition temperature was not affected by the presence of the fluorophore at this low mole ratio.

The fluorescence of Coumarin 311 was monitored by a Perkin-Elmer model MPF-44B fluorescence spectrophotometer. The cuvette temperature was controlled by flowing water from a Neslab model EX-300 water bath equipped with an Endocal-350 external cooler and an ETP-3 temperature programmer (Neslab, Portsmouth, NH). The aqueous solution of Coumarin 311 in the absence of the phospholipid showed a maximum excitation wavelength at 370 nm and a maximum emission wavelength at 466 nm. The emission intensity decreased with increasing temperature, but the wavelength of the maximum emission was not affected. At the transition temperature, the emission peak shifted to a shorter wavelength, and the fluorescence intensity suddenly increased. The magnitude of the blue shift was less than 5 nm and was inadequate to be used as a signal for the phase transition. Hence, the sudden increase in the fluorescence yield was used to detect the phase transition. To minimize interference from the temperature effect upon the fluorescence yield, 420 nm was chosen for the emission wavelength.

Diphenylhexatriene (DPH) was dissolved in chloroform and added to the chloroform-methanol solution of the phospholipid at a mole ratio $1 \cdot 10^{-2}$ before the solvent removal. The vesicles were prepared as described above after removal of the solvent. The fluorescence anisotropy of the vesicle suspension was measured by the MPF-44B fluorescence spectrophotometer with a polarizer attachment. The excitation wavelength was 365 nm and the emission wavelength was 425 nm. The parallel and perpendicular emission intensities were measured and the anisotropy was estimated according to the methods described by Shinitzky [16]. These three optical methods showed the same temperature for the main phase transition. Therefore, the turbidity method was mainly used to detect the transition temperature, because the procedure is relatively simple and is free from the alleged impurity effect of probe molecules.

The excess enthalpy of the phase transition was measured by a Perkin-Elmer Differential Scanning Microcalorimeter DSC-2 (Norwalk, CT) with a sample sealed in an aluminum pan. The sample preparation for differential scanning calorimetry was the same as for the optical methods, except that the concentration of dimyristoylphosphatidic acid was increased to 20 or 30 mM. The heating rate was 2.5 C°/min. The temperature was calibrated by measuring the melting points of ice (273.2 K) and indium (429.8 K).

Results

Fig. 1 illustrates the pH dependence of the phase-transition temperature of dimyristoylphosphatidic acid vesicles at 50, 100 and 200 mM NaCl. The curve for 100 mM NaCl was characterized by two downfalls; below pH 4.5 and between pH 7.5 and 10. The transition temperature at pH 10 (303.9 K) was about 19°C lower than that at pH 7.5 (323.0 K). In the rest of the pH range, the phase-transition temperature was fairly constant (324.1 K between pH 5 and 7, and 300.8 K above pH 11).

In the pH range between 7.5 to 10, the transition temperature was higher in lower NaCl concentrations at the given pH. However, this tendency was reversed above pH 11. Between pH 5 and 7, the transition temperature did not change

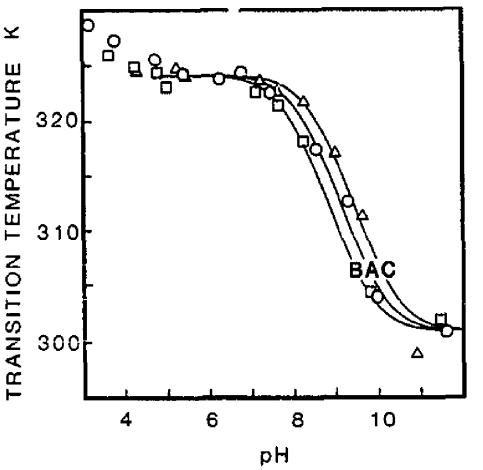

Fig. 1. The pH dependence of the phase-transition temperature of dimyristoylphosphatidic acid vesicle membranes and the effect of NaCl concentration. NaCl: 50 mM (△), 100 mM (○), and 200 mM (□). The curves are computer-generated according to Eqns. 23 and 26. The values are: $pK_{a2}^{int} = 6.5$ and $f = 60$ Å^2. A, 100 mM; B, 200 mM; and C, 50 mM NaCl.

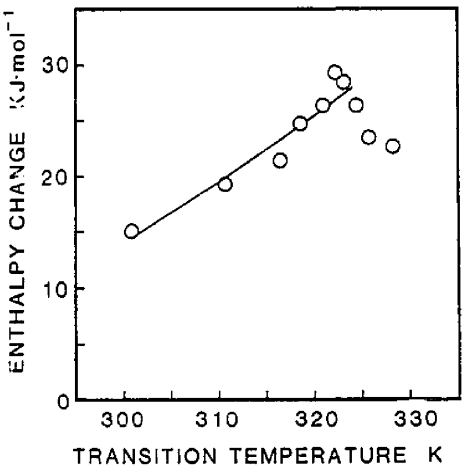

Fig. 2. The relationship between the enthalpy change and the transition temperature of dimyristoylphosphatidic acid membranes in 100 mM NaCl. The curves are computer-generated according to Eqns. 24 and 26, and $f_A/f_B = 0.523 + 0.145\alpha$.

significantly with the change in the NaCl concentrations.

Fig. 2 shows the differential scanning calorimetry data on the relation between the transition temperature and the enthalpy change. The NaCl concentration was fixed at 100 mM. When the transition temperature was lower than 323 K, the enthalpy change increased with increasing transition temperature. In contrast, above 323 K, the enthalpy change decreased with the increase in the transition temperature. The excess enthalpy had a maximum value of 7.0 kcal · mol^{-1} (29 kJ · mol^{-1}) at 322.3 K, and a minimum value of 3.5 kcal · mol^{-1} (15 kJ · mol^{-1}) at 300 K.

Theory

Effect of electrostatic interaction on the main phase-transition temperature of charged phospholipid membranes

For charged phospholipid bilayers, the free energy (G) at a given pH depends on the degree of ionization (α), the area per lipid molecule, f, and the absolute temperature T, and is composed of a non-electrostatic term, $G^*(f,T)$, and an electrostatic term, $G^{el}(f,\alpha,T)$.

$$G(f,\alpha,T) = G^*(f,T) + G^{el}(f,\alpha,T) \quad (1)$$

The area (f) is given by taking minima of the free energy, G

$$\left(\frac{\partial G}{\partial f}\right)_{\alpha T} = 0 \quad (2)$$

Consider the transition between solid-gel (A) and liquid-crystalline (B) states of a lipid membrane as a reversible two-state process, and the areas (f_A or f_B) for each state are given by Eqn. 2. At a constant pH, the difference (ΔG) in the molar free energy between these two states is written as

$$\Delta G = G(f_B,\alpha+\Delta\alpha,T) - G(f_A,\alpha,T) = \Delta G^* + \Delta G^{el} \quad (3)$$

with

$$\Delta G^* = G^*(f_B,T) - G^*(f_A,T) \quad (4)$$

$$\Delta G^{el} = G^{el}(f_B,\alpha+\Delta\alpha,T) - G^{el}(f_A,\alpha,T) \quad (5)$$

where $\Delta\alpha$ is the change in the degree of ionization caused by the change in the area per phospholipid molecule, and ΔG^* and ΔG^{el} are the differences in the non-electrostatic and the electrostatic free energies, respectively, for the phase transition. Also, ΔG is

$$\Delta G = \Delta H - T\Delta S \quad (6)$$

where ΔH and ΔS are the enthalpy and entropy changes between the solid-gel and liquid-crystalline states, respectively.

Consider the phase transition of a negatively charged phospholipid vesicle at two different pH values. At pH_1, the degree of ionization for the gel state (A) is α_1, and, because the difference in the molar free energy (ΔG_1) should be zero at the phase-transition temperature (T_{t1}), ΔG_1 is written as

$$\Delta G_1 = \Delta G_1^* + \Delta G_1^{el} = (T_{t1} - T)\Delta S_1 \quad (7)$$

Also, at pH_2, the difference in the molar free energy (ΔG_2) is written as

$$\Delta G_2 = \Delta G_2^* + \Delta G_2^{el} = (T_{t2} - T)\Delta S_2 \quad (8)$$

From Eqns. 4, 5, 7, and 8,

$$\begin{aligned}\Delta G_2 - \Delta G_1 &= (T_{t2} - T)\Delta S_2 - (T_{t1} - T)\Delta S_1 \\ &= [G^*(f_{B2}, T) - G^*(f_{B1}, T)] \\ &\quad - [G^*(f_{A2}, T) - G^*(f_{A1}, T)] \\ &\quad + [G^{el}(f_{B2}, \alpha_2 + \Delta\alpha_2, T) \\ &\quad - G^{el}(f_{B1}, \alpha_1 + \Delta\alpha_1, T)] \\ &\quad - [G^{el}(f_{A2}, \alpha_2, T) - G^{el}(f_{A1}, \alpha_1, T)] \end{aligned} \quad (9)$$

We expand the molar free energy in a Taylor series around each state at pH_1 at constant temperature T_{t2}, and assuming the first order approximation,

$$\begin{aligned}(T_{t2} - T_{t1})\Delta S_1 &= \left[\left(\frac{\partial G^*(f_{B1}, T_{t2})}{\partial f}\right)_T \Delta f_B - \left(\frac{\partial G^*(f_{A1}, T_{t2})}{\partial f}\right)_T \Delta f_A\right] \\ &\quad + \left[\left(\frac{\partial G^{el}(f_{B1}, \alpha_1 + \Delta\alpha_1, T_{t2})}{\partial f}\right)_{\alpha,T} \Delta f_B \right. \\ &\quad \left. + \left(\frac{\partial G^{el}(f_{B1}, \alpha_1 + \Delta\alpha_1, T_{t2})}{\partial \alpha}\right)_{f,T} (\alpha_2 - \alpha_1 + \Delta\alpha_2 - \Delta\alpha_1)\right] \\ &\quad - \left[\left(\frac{\partial G^{el}(f_{A1}, \alpha_1, T_{t2})}{\partial f}\right)_{\alpha,T} \Delta f_A \right. \\ &\quad \left. + \left(\frac{\partial G^{el}(f_{A1}, \alpha_1, T_{t2})}{\partial \alpha}\right)_{f,T} (\alpha_2 - \alpha_1)\right] \end{aligned} \quad (10)$$

Here, Δf_A $(f_{A2} - f_{A1})$ and Δf_B $(f_{B2} - f_{B1})$ are the changes in the area per phospholipid molecule caused by the change in the degree of ionization.

By applying the condition given in Eqn. 2, we obtain

$$\begin{aligned}&(T_{t2} - T_{t1})\Delta S_1 \\ &= \left[\left(\frac{\partial G^{el}(f_{B1}, \alpha_1 + \Delta\alpha_1, T_{t2})}{\partial \alpha}\right)_{f,T} (\alpha_2 - \alpha_1 + \Delta\alpha_2 - \Delta\alpha_1)\right] \\ &\quad - \left[\left(\frac{\partial G^{el}(f_{A1}, \alpha_1, T_{t2})}{\partial \alpha}\right)_{f,T} (\alpha_2 - \alpha_1)\right] \end{aligned} \quad (11)$$

This equation can be further simplified by assuming that $\Delta\alpha_1$ and $\Delta\alpha_2$ are negligible. Thus, we have

$$\begin{aligned}&(T_{t2} - T_{t1})\Delta S_1 \\ &= \left[\frac{\partial G^{el}(f_{B1}, \alpha_1, T_{t2})}{\partial \alpha} - \frac{\partial G^{el}(f_{A1}, \alpha_1, T_{t2})}{\partial \alpha}\right](\alpha_2 - \alpha_1) \end{aligned} \quad (12)$$

The electrostatic molar free energy is written [2]

$$\begin{aligned}G^{el}(f, \alpha, T) &= -F\alpha\psi_0 \\ &\quad - \frac{4RT}{F} f N_A (2000\, \varepsilon\varepsilon_0 CRT)^{1/2} \times \left(\cosh\frac{F\psi_0}{2RT} - 1\right) \end{aligned} \quad (13)$$

where F is the Faraday constant, ψ_0 is the surface potential, ε is the relative permittivity of solution, ϵ_0 is the permittivity of a vacuum, N_A is the Avogadro number, C is the salt concentration given in units of molarity, and R is the gas constant. According to the Gouy-Chapman theory, surface potential of negatively charged membranes in a 1 : 1 electrolyte solution is written as

$$-\frac{e}{f}\alpha = (2000\, \varepsilon\varepsilon_0 CRT)^{1/2} 2\sinh\frac{F\psi_0}{2RT} \quad (14)$$

where e is the elementary charge, and $-e\alpha/f$ signify the negative surface charge density. When the negative surface potential is high enough, Eqn. 14 is arranged as

$$\psi_0 = -\frac{2RT}{F} \ln\frac{e\alpha}{(2000\, \varepsilon\varepsilon_0 CRT)^{1/2} f} \quad (15)$$

From Eqns. 13–15,

$$\begin{aligned}\left(\frac{\partial G^{el}(f, \alpha, T)}{\partial \alpha}\right)_{f,T} &= -F\psi_0 \\ &= 2RT \ln\frac{e\alpha}{(2000\, \varepsilon\varepsilon_0 CRT)^{1/2} f} \end{aligned} \quad (16)$$

From Eqns. 12 and 16, we obtain,

$$(T_{t2} - T_{t1})\Delta S_1 = \left(2RT_{t2} \ln\frac{f_{A1}}{f_{B1}}\right)(\alpha_2 - \alpha_1) \quad (17)$$

From Eqn. 17, the relationship between ΔH and f_A/f_B at two pH values is obtained.

$$\begin{aligned}\frac{1}{\Delta H_1}\left(\ln\frac{f_{A1}}{f_{B1}}\right)(\alpha_2 - \alpha_1) &= \frac{1}{\Delta H_2}\left(\ln\frac{f_{A2}}{f_{B2}}\right)(\alpha_2 - \alpha_1) \\ &= \frac{T_{t2} - T_{t1}}{2RT_{t1}T_{t2}} = \text{constant} \end{aligned} \quad (18)$$

Effect of ionic strength on the interfacial pH

According to the Boltzmann distribution law, the relation between the hydrogen ion concentration near the surface ($[H^+]_0$) of dimyristoylphosphatidic acid vesicle and that in the bulk solution ($[H^+]_\infty$) is written as

$$[H^+]_0 = [H^+]_\infty \exp\left(-\frac{F\psi_0}{RT}\right) \tag{19}$$

From Eqns. 15 and 19, and by the definition of pH, the relation between the interfacial pH (pH_0) and the bulk pH (pH_∞) is

$$pH_0 = pH_\infty - \log\frac{\alpha^2 e^2}{2000\ \varepsilon\varepsilon_0 CRTf^2} \tag{20}$$

The degree of ionization is expressed by the Henderson-Hasselbalch equation. Between $\alpha = 1$ and 2, this equation is approximately written as

$$\alpha = 1 + \frac{10^{(pH_0 - pK_{a2}^{int})}}{1 + 10^{(pH_0 - pK_{a2}^{int})}} \tag{21}$$

where pK_{a2}^{int} is the intrinsic pK_{a2}. From Eqn. 21,

$$pH_0 - pK_{a2}^{int} = \log\frac{(\alpha - 1)}{(2 - \alpha)} \tag{22}$$

From Eqns. 20 and 22, one obtains the relation between pH_∞ and α.

$$pH_\infty = pK_{a2}^{int} + \log\frac{e^2}{2000\ \varepsilon\varepsilon_0 RTf^2} + \log\frac{\alpha^2(\alpha - 1)}{(2 - \alpha)} - \log C \tag{23}$$

Discussion

From the pH titration of the phase-transition temperature of dimyristoylphosphatidic acid in Fig. 1, the degree of ionization changes from 0 to 1 below pH 4.5, and from 1 to 2 above pH 6.0. Between pH 5 and 7, the phospholipid should have one negative charge and above pH 11 two negative charges. The transition temperatures of the dimyristoylphosphatidic acid with singly and doubly charged states in 100 mM NaCl were 324.1 K and 300.8 K, respectively.

From Eqn. 18, we calculate

$$\frac{1}{\Delta H}\ln\frac{f_A}{f_B} = \frac{(T_{t2} - T_{t1})}{2RT_{t1}T_{t2}} = -1.44 \cdot 10^{-5}\ J^{-1} \cdot mol \tag{24}$$

When $(2RT_{t1}/\Delta S_1)(\alpha_2 - \alpha_1)\ln(f_{A1}/f_{B1}) \ll 1$, Eqn. 17 can be further simplified to

$$T_{t2} - T_{t1} = \frac{2RT_{t1}^2}{\Delta H_1}\ln\frac{f_{A1}}{f_{B1}}(\alpha_2 - \alpha_1) \tag{25}$$

Because the estimated value of $\ln(f_A/f_B)/\Delta H$ is $-1.44 \cdot 10^{-5}$, the error caused by this approximation is less than 1%. Eqn. 25 shows that the change in the phase-transition temperatures by pH is linearly related to the degree of ionization. When the degree of ionization was calculated by Eqn. 23, the best linearity was obtained at $pK_{a2}^{int} = 6.5$ with 100 mM NaCl, and the slope was -23.3 K, $r = -0.995$. Fig. 3 is the relationship between T_t and α, calculated from Eqn. 23, using $pK_{a2}^{int} = 6.5$.

$$T_t = 324.1 - 23.3(\alpha - 1) \tag{26}$$

Because the apparent pK_{a2} (pK_{a2}^{app}) equals the bulk pH when the degree of ionization is 1.5, the differences between pK_{a2}^{int} and pK_{a2}^{app} ($\Delta pK_{a2} = pK_{a2}^{app} - pK_{a2}^{int}$) at 50, 100, and 200 mM NaCl

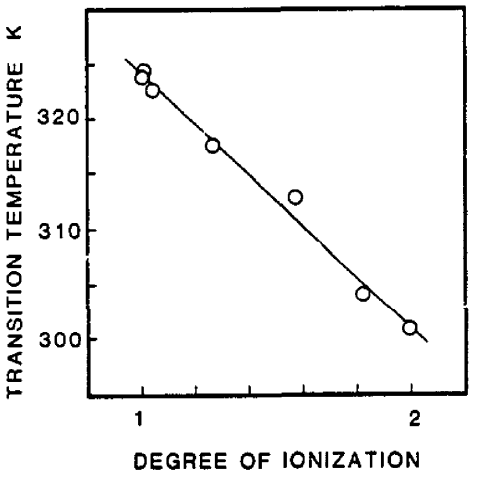

Fig. 3. The relationship between T_t and α calculated from Eqn. 23, using $pK_{a2}^{int} = 6.5$, $f = 60$ Å^2. The line is $T_t = 324.1 - 23.3(\alpha - 1)$, and $r = -0.995$.

are calculated to be 2.9, 2.6, and 2.3 units, respectively, according to Eqn. 23. The lines in Fig. 1 are drawn according to Eqns. 23 and 26. The numerical values used were $pK_{a2}^{int} = 6.5$, $T_{tl} = 324.1$ K and (the mean area per phospholipid) = 60 Å^2. The curve for 100 mM NaCl is in good agreement with the present experimental data. The deviation of the experimental values from the theoretical curves at pH values above 10 for 50 and 200 mM NaCl concentrations may be attributable to the counterion binding (Na^+) to the phospholipid surface.

Träuble and Eibl [1] and Träuble et al. [2] assumed that membrane ionization does not perturb the non-electrostatic free energy appreciably, and the area per phospholipid molecule for each phase was independent of ionization. They obtained the expression for ΔT_t ($= T_t - T_t^*$) as follows.

$$T_t - T_t^* = \frac{4RT}{F\Delta S^*} N_A (2000\ \varepsilon\varepsilon_0 CRT)^{1/2} \left(\cosh\frac{F\psi_0}{2RT} - 1\right) \Delta\bar{f} \tag{27}$$

where $\Delta\bar{f}$ is the change in the area per phospholipid molecule at the phase transition, and ΔS^* and T_t^* are the entropy change and the phase-transition temperature of uncharged phospholipids, respectively. This expression indicates that the change in the transition temperature is originated mainly from the second term in Eqn. 13 rather than from the first term. On the other hand, the present expression Eqn 17, derived from Eqns. 12 and 16, shows that the change is originated mainly from the first term in Eqn. 13. Fig. 4 shows the contribution of these two terms to the electrostatic molar free energy, calculated from Eqn. 13. The electrostatic free energy depends more on the first term than the second.

From Eqn. 27, the difference in the transition temperature at pH_1 and pH_2 ($\Delta T_{t(2-1)} = T_2 - T_1$) is written as

$$\Delta T_{t(2-1)} = -\frac{2RT}{\Delta S^*} \frac{\Delta\bar{f}}{\bar{f}} (\alpha_2 - \alpha_1) \tag{28}$$

at high surface potential. Here, $\bar{f}$ is the area per phospholipid molecule independent of the degree of ionization [1,2]. Because $-\Delta\bar{f}/\bar{f}$ is nearly equal

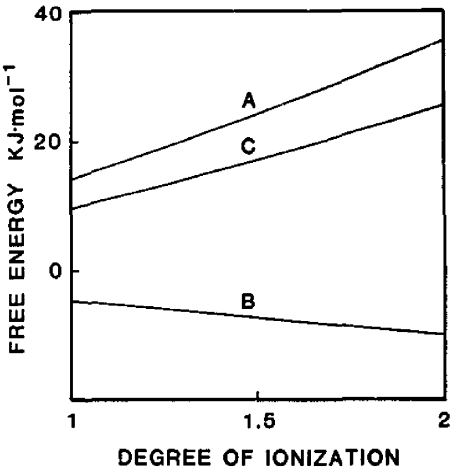

Fig. 4. The relationship between electrostatic free energy (G^{el}) and the degree of ionization. (A) The first term in Eqn. 13, (B) the second term in Eqn. 13, and (C) electrostatic molar free energy. $f = 60$ Å^2, $T = 320$ K, and the salt concentration = 0.1 M.

to $\ln(f_A/f_B)$, Eqn. 28 is nearly equivalent to Eqn. 25. If the enthalpy change of uncharged phosphatidic acid vesicle membrane were measurable, their equations (Eqns. 27 or 28) would be applicable to analysis of the pH dependence of the phase transition temperature of acidic phospholipids. Below pH 3, however, phosphatidic acid membranes are reported to be in the tube-like hexagonal phase (H_{II}) [17]. Hence, the experimental value of the enthalpy change for the phase transition of uncharged phosphatidic acid membrane below pH 4 may be irrelevant to the order-disorder transition.

The enthalpy changes of the phase transitions at temperatures of 324 K and 301 K were about 29 and 15 kJ · mol^{-1}, respectively (Fig. 2). These ΔH values correspond to the singly charged and doubly charged dimyristoylphosphatidic acid vesicles, respectively. The entropy changes for the phase transition of the singly and doubly charged dimyristoylphosphatidic acid bilayers are calculated to be 90 and 50 J · mol^{-1} · K^{-1}, respectively. The ratios (f_A/f_B) of the areas per phospholipid molecule for each phase are calculated to be 0.659 and 0.806 for the singly and doubly charged dimyristoylphosphatidic acid vesicles, respectively.

The relationship between the degree of ionization obtained by Eqn. 26 and the ratio of areas

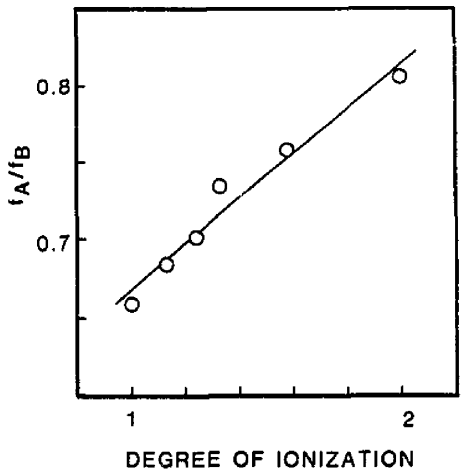

Fig. 5. The relationship between f_A/f_B and the degree of ionization, calculated by Eqns. 24 and 26. The line is $f_A/f_B = 0.523 + 0.145\alpha$, $r = 0.982$.

(f_A/f_B) obtained by Eqn. 24 is shown in Fig. 5. Notice that the relation is almost linear ($f_A/f_B = 0.523 + 0.145\alpha$, $r = 0.982$).

The curve in Fig. 2 is the computer-generated theoretical line drawn between the phase transition temperature and the enthalpy change, calculated by Eqns. 24 and 26, with the assumption that the relationship between the degree of ionization and the area ratio (f_A/f_B) is linear as shown in Fig. 5. The difference between the present experimental enthalpy change and the calculated values are within 10%. The decrease in the enthalpy change above 323 K shown in Fig. 2 may be caused by the membrane conformational change between bilayer and hexagonal phase.

Jähnig [6], Jähnig et al. [12], Blume and Eibl [5], and Eibl and Blume [18] proposed that the shift in the transition temperature was represented by the sum of the charge-induced shift (equivalent to the shift derived by Träuble et al.[2]) and the area-induced shift (the tilt induced shift, or the shift caused by the change in the area per phospholipid molecule) for each state. Also, the decrease in the ionization-induced area change was attributed to the changes in the internal energy and the van der Waals interactions caused by tilting of the hydrocarbon chain. In the present theory, the shift of the phase transition temperature by the ionization of acidic phospholipid membrane is described by Eqn. 25. In deriving this equation, the area-induced shift in the transition temperature was explicitly included. Despite this difference, Eqn. 25 showed basically the same structure to Träuble's equation (Eqns. 27 or 28). The decrease in the enthalpy change is related to the change in the area occupied by the phospholipid molecule, as shown by Eqn. 18. The area change initiates the change in the tilt angle.

The difference in estimating the area per phospholipid molecule for each state leads to contradictory conclusions. The disagreement of the conclusion reached by Träuble and Eibl [1] and Träuble et al. [2] to those of Jähnig [6], Jähnig et al. [12], Blume and Eibl [5], and Eibl and Blume [18] may be caused by the difference in the estimated area per phospholipid molecule.

The present study analyzed the relationship between the surface charge density and the main phase-transition by the surface area ratio between solid-gel and liquid-crystalline states and the enthalpy change at the transition. The derived equation is simple and encompasses the equations derived by Träuble et al. [2] and Jähnig [6]. The agreement between the theory and the experiment was excellent.

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