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# DIVALENT CATION-INDUCED SURFACE TENSION INCREASE IN ACIDIC PHOSPHOLIPID MEMBRANES

## ION BINDING AND MEMBRANE FUSION

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**Chelation binding of divalent cations to phospholipid membranes may cause deformation in the headgroup regions of these lipid molecules. This deformation may be responsible for the observed large increase in surface tension of acidic phospholipid membranes induced by divalent cations. On the other hand, simple binding of monovalent cations without being followed by such a deformation of membrane molecules, does not result in a large surface tension increase in the membrane. A theoretical explanation for the above situation is given and the divalent cation-induced acidic phospholipid membrane fusion as well as other lipid membrane fusions are discussed in terms of the increased surface energy of membranes.**

It has been shown that divalent and polyvalent cations can induce fusion of acidic phospholipid membranes [1–6], whereas monovalent metal cations are not able to induce fusion of these lipid membranes [5,6] although they can cause aggregation of them [7]. It was also observed [6,8,30] that as the divalent or polyvalent cation concentration in the solution increases, the surface tension of the air/water interface coated by an acidic phospholipid monolayer increases considerably, probably by binding of these metal ions to the negatively charged sites of lipid polar groups. On the other hand, monovalent metal cations cannot cause the increase in surface tension of acidic lipid membranes as much as those induced by divalent or polyvalent cations [6,8], although such monovalent cations at high enough concentration can bind to the negatively charged sites of lipids to an extent as great as those by divalent or polyvalent cations [7,9–11].

Among other theories [13–15] proposed for the divalent cation-induced membrane fusion, we have recently proposed that the increase in surface tension of membranes is responsible for fusion of the two apposed lipid membranes [6,8].

In order to explain the observed increase in surface tension of the air/water interface covered by an acidic lipid monolayer [6,8], we would like to formulate the surface free energy, $F$, of the inferface of a total area, $S$, as follows:

$$F = F_0 + S\int_0^{\sigma} \psi_s \mathrm{d}\sigma + F_{\mathrm{chem}} \quad (1)$$

where on the right, the first term $F_0$ is the surface free energy of an unionized lipid monolayer coated air/water interface, the second and third terms represent the free energy change due to the formation of the Gouy-Chapman diffused double layer as the monolayer possesses fixed charges [21]. In the free energy of the double layer formation, the second term corresponds to the electrical free en-

ergy of the double layer formation, the second term corresponds to the electrical free energy part where $\psi_s$ is the surface potential and $\sigma$ the surface charge density (see Appendix), and the sum of the following three terms:

$$F_{chem} = F^b + F^s + F^c \quad (2)$$

where $F^b$ is the free energy change of the charged sites of lipid molecules due to binding of ions to them, $F^s$ the free energy due to the configurational entropy of the charged sites of membrane molecules resulting from a certain type of ion binding, and $F^c$ the free energy change due to the conformational change of lipid molecules caused by a special type of ion binding, for example, a chelation binding of divalent metal ion with two lipid molecules may cause such conformational changes (e.g., deformation) in the molecules. The first and second terms do not depend on the total area of the monolayer, whereas the third term will depend on the total area.

Then, Eqn. 1 can be rewritten by

$$F = F_0 + S\int_0^{\sigma} \psi_s d\sigma + F^b + F^s + F^c(S) \quad (3)$$

The surface tension of the interface, $\gamma$, is expressed as the derivative of the free energy, $F$, with respect to the total area, $S$:

$$\gamma = \frac{\partial F}{\partial S} = \frac{\partial F_0}{\partial S} - \int_0^{\psi_s} \sigma d\psi_s + \frac{\partial F^c}{\partial S} \quad (4)$$

where $\frac{\partial F_0}{\partial S} = \gamma_0$ the surface tension of an uncharged monolayer.

When metal ions bind to phospholipids in a manner of one phospholipid molecule to one metal ion (1:1 binding), the third term on the right (Eqn. 4) is zero, since lipid molecules would not change their conformation by this type of ion binding. In this case, the surface tension change due to ion adsorption is entirely that of the electrical free energy part (the second term). This electrical free energy term can be calculated using Eqns. A-1 and A-2 by knowing only the surface potential of the film and does not depend on the types of ion binding. The second term results in an increase in surface tension as both charge density and surface potential decrease in magnitudes upon adsorption of metal ions to the monolayer.

It should be noted that a possible dehydration occurring in the membrane polar group region caused by ion binding may contribute to increse in surface energy of the membrane. However, since the increase in surface energy due to the 1:1 binding does not seem to depend on the area of the membrane, such effect would not contribute to the surface tension of the interface of multi-component systems.

Then, the surface tension increase caused by divalent cation adsorption for the case of one lipid molecule to one ion (1:1) bining can be calculated using Eqn. 4: the increased surface tension [$\Delta\gamma = \gamma_2$ (1 mM $CaCl_2$, 100 mM NaCl) $- \gamma_1$ (100 mM NaCl, no $CaCl_2$)] of a phosphatidylserine monolayer (65 $Å^2$ per molecule) formed on the 0.1 M NaCl subphase by the addition of 1 mM $CaCl_2$ was calculated to be 1.87 dyn/cm;

$$\Delta\gamma = \gamma_2 - \gamma_1 = -\left(\int_{\sigma}^{\psi_s} d\psi\right)_2 + \left(\int_{\sigma}^{\psi_s} d\psi\right)_1 \quad (5)$$

Here, the surface potential used in the above calculation was obtained from the surface potential measurements for a phosphatidylserine monolayer and using the following binding constants of ions for the phosphatidylserine membrane: 30 $M^{-1}$ for $Ca^{2+}$ and 0.6 $M^{-1}$ for $Na^+$ [22].

On the other hand, the experimental value of surface tension change for the same monolayer was about 8 dyn/cm at the same environmental solution change (from 0 mM $Ca^{2+}$ to 1 mM $Ca^{2+}$). This observed large change in surface tension can not be explained by assuming the 1:1 binding mode as shown above, but it may be explained by introducing a special binding of divalent cations to phospholipid molecules; one divalent cation may bind two phospholipids by chelating their two polar headgroups. The existence of this binding mode has been suggested by earlier workers [6,23,24]. This mode of binding would deform the headgroup regions of the phospholipid molecules and render the hydrocarbon chains of the lipid molecules to be revealed more to the aqueous phase. Since the hydrocarbon/water interfacial tension (50 dyn/cm) [25] is greater than the lipid membrane/water interfacial tension (zero to a few dyn/cm) [26], this type of binding, if it

occurs, should increase the interfacial tension of the film.

In such a case, $F^c(S)$ in Eqn. 3 may be written as

$$F^c(S) = 2N_2\omega\left(\frac{S}{M}\right) \tag{6}$$

where $N_2$ is the number of 2:1 binding (two lipid molecules to one divalent cation), $M$ the number of lipid molecules of the monolayer and $\omega$ the free energy change per metal ion bound lipid molecule due to deformation of the lipid molecule caused by chelation binding of divalent cations.

If we assume that the increase in the hydrocarbon/water interface per lipid molecule by this binding mode (2:1 binding) is proportional to the available area per lipid molecule, we have:

$$\omega = \beta\gamma_{o/w}(A - A_0) \tag{7}$$

Then, the contribution of the third term in the Eqn. 4 to the surface tension is:

$$\frac{\partial F^c(S)}{\partial S} = \frac{\partial 2N_2\omega\left(\frac{S}{M}\right)}{\partial S} = \frac{2N_2}{M}\beta\gamma_{o/w} \tag{8}$$

where $\beta$ is the percentage of the increase lipid hydrocarbon area revealed to the aqueous phase per lipid molecule due to the chelation binding of divalent cation, $\gamma_{o/w}$ the interfacial tension of the oil/water interface, $A(=S/M)$ the area per lipid molecule (65 Å$^2$) and $A_0$ the area per lipid molecule at a state corresponding to the collapse pressure of the monolayer (42 Å$^2$). Since the observed surface tension change of the phosphatidylserine monolayer at 1 mM $Ca^{2+}$ in 0.1 M NaCl subphase solution is approximately 8.0 dyn/cm [7], and the contribution from the electrical double layer formation is 1.9 dyn/cm (the value on the dotted line at 1 mM $Ca^{2+}$ in Fig. 1), the term $2\beta\gamma_{o/w}N_2/M$ contributes about 6.1 dyn/cm increase to the observed surface tension.

It is not certain, however, how much percent of the total phospholipids in the monolayer is in this chelation binding with divalent cation. In fact, one lipid to one divalent cation binding also seems to exist, which is evidenced by the reversal of the surface charge of the membrane at a high divalent cation concentration (approx. 100 mM $CaCl_2$)

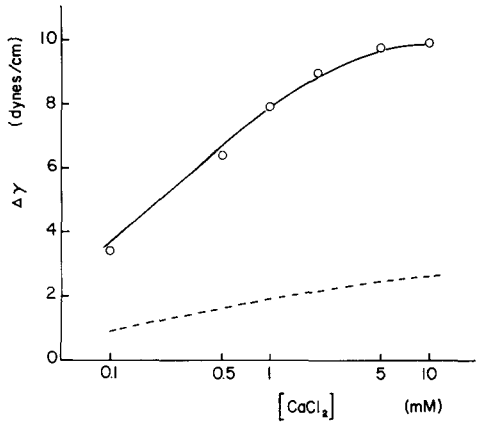


Fig. 1. Experimental and theoretical values of surface tension change in a phosphatidylserine monolayer (65 Å$^2$ per molecule) formed at the air/water interface, 23°C, with respect to the subphase $CaCl_2$ concentration. Subphase salt solution: 0.1 M NaCl/2 mM Hepes/0.01 mM EDTA (pH 7.0) containing various concentrations of $CaCl_2$. $\Delta\gamma \equiv \gamma_2$(100 mM NaCl + $x$ mM CaCl) − $\gamma_1$(100 mM NaCl). ○, experimental data [8]; ———, Theoretical values for the 2:1 (two lipids to one calcium ion) binding mode; - - - - - -, Theoretical values for the 1:1 (one lipid to one $Ca^{2+}$) binding mode (or no deformation of lipid molecules). Binding constants used for $Na^+$ and $Ca^{2+}$ to the phosphatidylserine membrane were 0.6 $M^{-1}$ for $Na^+$ and 30 $M^{-1}$ for $Ca^{2+}$ [22].

from the study of electrophoretic mobility of acidic phospholipid vesicles [27]. However, the observed increase in surface tension of a phosphatidylserine monolayer also indicates clearly the existence of the two phospholipids-one divalent cation binding mode because if all divalent cation bindings were the 1:1 mode binding, the surface tension change would be much smaller than the observed values as shown in Fig. 1.

If we assume that the binding mode of divalent cation in the low $Ca^{2+}$ concentration range is only the 2:1 binding (two lipid molecules to one divalent cation), the previous study [6] indicates that about 64% of the total lipids are bound with $Ca^{2+}$ at 1 mM $Ca^{2+}$/100 mM NaCl. From this, we can estimate $\beta$ to be 0.19. This means that the increased area of hydrocarbon chain per lipid molecule, revealed to the water phase caused by divalent cation chelation, is about 4.4 Å$^2$ for a monolayer of 65 Å$^2$ per molecule. Or, the exposure of, on an average 2.8 Å$^2$ of hydrocarbon surface per lipid molecule to the aqueous phase would correspond to the increase in surface tension of 6

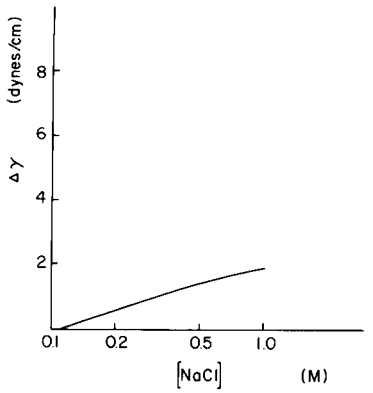


Fig. 2. Theoretical results of surface tension change of the same monolayer as in Fig. 1. Subphase salt: various NaCl concentrations containing 2 mM Hepes, 0.01 mM EDTA (pH 7.0). $\Delta\gamma \equiv \gamma(x \text{ M NaCl}) - \gamma(0.1 \text{ M NaCl})$. ———, Theoretical values.

dyn/cm. This finding compares well with those obtained in the membrane expansion study of lipid monolayers in relation to membrane fusion [6,7]. By using the obtained value for $\beta$ at 1 mM $Ca^{2+}$ and knowing the fraction, $f^{Ca}$, of bound lipids to the total lipids at different $Ca^{2+}$ concentrations [22], we can calculate the surface tension contribution arising from the conformational change of lipids, $2\beta\gamma_{o/w}N_2/M$, as a function of $Ca^{2+}$ concentrations. The theoretical results agree quite well with the experimental results (see Fig. 1).

Contrary to the divalent cations, monovalent metal cations do not cause such an increase in surface tension of acidic lipid monolayers which is demonstrated experimentally [6,8] as well as theoretically (Fig. 2), probably because they do not form the chelation binding with lipid molecules, although the previous study indicates that monovalent cations at high concentration can bind to the negatively charged sites of lipid molecules and consequently reduce the surface charge density of the membrane to the same extent as those caused by divalent cations [7,10]. At these concentrations of divalent cations, however, they can induce fusion of acidic lipid membranes [5,6], whereas monovalent metal cations do not induce fusion but only cause aggregation of these membranes [7]. As an exceptional case of monovalent cation, $H^+$ can induce acidic lipid membranes [28,29], and, indeed, $H^+$ does increase the surface tension of acidic lipid membranes as much as divalent cations do (Ref. 23, and Ohki, S., unpublished data) probably by inducing the conformational changes of the membrane molecules.

These observations seem to confirm an importance of chelation binding of divalent (or polyvalent) cations for the increase in membrane surface energy, and such an increase in surface energy of the membrane is likely to be responsible for the observed divalent cation-induced lipid membrane fusion. This interpretation (increased membrane surface energy or tension) for membrane fusion accommodates many of the features observed in lipid membrane fusion by different fusion agents; membrane expansion in the increased temperature [30,31] or osmotic pressure gradient-induced membrane fusion [8,18]; phase defects in the membrane caused by different membrane molecular constituents [16] or by lowering of the temperature [17]; membrane micellization due to the incorporation of short chain fatty acids [12]; a phase boundary region between two different phases caused by divalent cations [13] local dehydration, enhanced cation binding [14], $Ca^{2+}$ transmembraneous complex [14,32], etc. All these events are associated with the increase in surface energy of the membranes locally or overall.

The discussion of the present theory made in relation to membrane fusion has so far been based on the overall averaged (time as well as space-wise) quantities such as the observed surface tension of membranes.

However, the actual membrane fusion phenomena may involve dynamical as well as local interaction processes of the two interacting membrane surfaces. Therefore, it is possible that the quantities obtained from the surface monolayer studies in order to correlate with those corresponding to vesicle membrane fusion may be different. Also, it should be noted that the physical state of a lipid monolayer may be different from that of a half of a lipid bilayer [33].

Therefore, our analysis of membrane fusion is meaningful only in relative quantity. In spite of these complicated factors, however, we believe that the basic physical principle of the theory (the increased hydrophobicity or increased free energy of the surface membrane) to explain membrane fusion phenomena is unchanged. When the mem-

brane fusion process involves the above mentioned factors (local or dynamical event) the application of the theory should be modified according to each situation.

It has been pointed out that the dehydration of membrane surface may be responsible for membrane fusion [2,20,32]. The dehydration of membrane surfaces is indeed intimately related to the degree of the increase in surface energy or increase in surface hydrophobicity. In this regard, it is interesting to note whether the dehydration is a major important factor for membrane fusion or not. It has been reported by some [34] that $Li^+$ can dehydrate the membrane surface of a phosphatidylserine membrane in a similar degree as divalent cations do. However, the fact that $Li^+$ does not induce phosphatidylserine membrane fusion at any concentration, suggests to us that the dehydration of the membrane surface only is not an adequate factor for membrane fusion. For example, as long as the hydrophilic groups at the membrane surface are not altered to more hydrophobic nature, it is possible that membrane fusion may not occur even if all water molecules are dehydrated from the membrane surface regions where two membranes are closely in contact.

Although monovalent cations per se do not induce fusion of phosphatidylserine vesicles at neutral pH regardless of their concentration, different monovalent cations seem to modulate divalent cation induced membrane fusion in their own specific ways [35].

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## Appendix

The relationship between surface charge density and its associated surface potential, which is necessary to calculate the free energy of the electrical double layer, is given by ($t = 23°C$)

$$\sigma = \frac{1}{273} \cdot \left( \sum_i C_j^b \left[ \exp\left( - \frac{ez_j\psi_s}{kT} \right) - 1 \right] \right)^{1/2} \qquad (A\text{-}1)$$

where $\sigma$ is the surface charge density, $C_j^b$ the bulk concentration of the $j$th ionic species, $z_j$ the valency of the $j$th ionic species, and $\psi_s$ the surface electrical potential.

Electrical free energy term in Eqn. 4 can be calculated by using the expression for $\sigma$ given by Eqn. A-1:

$$\int_0^{\psi_s} \sigma d\psi_s' = \frac{1}{273} \left[ \int_0^{\psi_s} \left( \sum_j C_j^b \left[ \exp\left( - \frac{ez_j\psi_s'}{kT} \right) - 1 \right] \right)^{1/2} d\psi_s' \right] \qquad (A\text{-}2)$$

The fraction, $f^{Ca}$, of the number of the $Ca^{2+}$ bound lipids to the total number of lipid is given by

$$f^{Ca} = \frac{K_2 C_2^b e^{-2e\psi_s/kT}}{1 + K_1 C_1^b e^{-e\psi_s/kT} + K_2 C_2^b e^{-2e\psi_s/kT}} = \frac{2N_2}{M} \qquad (A\text{-}3)$$

where $K$ is the binding constant of an ion, the suffixes 1 and 2 refer to $Na^+$ and $Ca^{2+}$, respectively, $N_2$ the number of the bound $Ca^{2+}$ and $M$ the total number of lipid molecules of the monolayer.

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