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Origin of calcium-induced minimum in bulk compressional modulus of lipid membranes

Configurational entropy of adsorbed Ca^{2+}

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Addition of Ca^{2+} to a dipalmitoylphosphatidylcholine lamellar system decreases the bulk compressional modulus (increases compressibility) of the membrane (S. Aruga, R. Kataoka and S. Mitaku, Biophys. Chem. 21 (1985) 265). The bulk modulus was reported to show a minimum value at 10 mM Ca^{2+} within the temperature range 20–45 °C. In the present report, the occurrence of this minimum in the bulk modulus is explained quantitatively as a result of fluctuation in the number of Ca^{2+} adsorbed onto the lipid bilayer surface. From this theory, the change in apparent molal volume of Ca^{2+} upon surface adsorption is estimated to be 5.7 $cm^3\ mol^{-1}$, which appears to be a reasonable value. The number of adsorbed Ca^{2+} at the concentration where the bulk modulus assumes the minimum value is half of the number of allowable adsorption sites on lipid membranes. The configurational entropy of the adsorbed Ca^{2+} attains a maximum at the minimum point.

1. Introduction

Aruga et al. [1] measured ultrasonic velocity in a dipalmitoylphosphatidylcholine (DPPC) lamellar system and reported the presence of anomalies in Ca^{2+} effects upon the membranes. When plotted vs. Ca^{2+} concentration, the ultrasound velocity, V, expressed the limiting value $[V]$, defined as

$$[V] = \lim_{C \to 0} \frac{V - V_s}{V_s C}$$

(where C is the phospholipid concentration and subscript s represents the solvent) showed a minimum value at about 10 mM Ca^{2+}. Similarly, the limiting value of the ultrasound absorption, $[\mu]$, defined as

$$[\mu] = \lim_{C \to 0} \frac{\mu - \mu_s}{\mu_s C}$$

also manifested a maximum value at 10 mM Ca^{2+}. These authors [1] envisioned these anomalies as pseudo-critical phenomena occurring at the phase transition of the lipid membranes.

However, at 10 mM Ca^{2+}, the minimum value for the bulk modulus of the lamellar system was observed, at as low as 20 °C [1], far from the main transition temperature. The critical temperature of the DPPC monolayer was reported to be 44 °C

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from the π-A curve [2]. Therefore, the minimum in the bulk modulus may not be related to the pseudo-critical phenomenon associated with the phase transition of the membrane system.

In the present communication, a theory is proposed that explains the presence of a Ca^{2+}-dependent minimum in the bulk modulus. It will be shown that the fluctuation in the number of adsorbed Ca^{2+} at the phospholipid membrane surface can account for the volume fluctuation of the membrane system and the decrease in bulk modulus of the membrane. A quantitative comparison between the theory and experimental data will be presented.

2. Theory of the bulk modulus of lipid membranes

Before evaluating the phenomenon of a minimum in the bulk modulus, the pressure-volume relationship in a membrane system must be clarified. The compressibility κ of the system is defined as

$$\kappa = -V^{-1}\delta V/P \tag{1}$$

where V, δV and P are the volume of the system, the change in volume of the system caused by the applied pressure, and the hydrostatic pressure applied to the system, respectively. By definition, the bulk modulus, K, is κ^{-1}. The change in volume of the system, δV, has multiple causes. The major contribution to the compressibility is the volume change of the lipid membrane itself due to the application of pressure. By expressing the Ca^{2+}-induced volume change by δV_c, we rewrite κ as

$$\kappa = \kappa_0 - V^{-1}\delta V_c/P \tag{2}$$

where κ_0 is the compressibility of the membrane in the absence of Ca^{2+} and is expected to be large compared to that caused by adsorption of Ca^{2+}. Then, according to the definition of the bulk modulus, K is expressed as

$$K = K_0 + \Delta K \tag{3}$$

$$\Delta K = K_0^2 \delta V_c/(VP) \tag{4}$$

where K_0 is κ_0^{-1}.

To evaluate the volume change, δV_c, caused by Ca^{2+} adsorption at the membrane/solvent interface, formulation of the thermodynamic average of δV_c is required. The additive enthalpy term, δH, for the membrane system caused by adsorption of Ca^{2+} under hydrostatic pressure, P, is written as

$$\Delta H = P\delta V \tag{5}$$

δV_c is then interpreted as the thermodynamic average, defined by

$$\delta V_c = \langle \delta V \rangle = \langle \delta V \exp(-P\delta V/kT) \rangle_0 \tag{6}$$

where $\langle X \rangle_0$ signifies the ensemble average of the physical quantity X in the absence of hydrostatic pressure. The other symbols have the usual thermodynamic meanings. Linear irreversible thermodynamics considers only the first-order effect of the external pressure [3]. Then, the exponent of eq. 6 is expanded only up to the first-order term with respect to P.

$$\delta V_c = -\langle \delta V^2 \rangle_0 P/kT \tag{7}$$

where the fact that the value of $\langle \delta V \rangle_0$ is zero has been used.

Inserting eq. 7 into eq. 4, the change in bulk modulus ΔK caused by Ca^{2+} adsorption becomes

$$\Delta K = -\langle \delta V^2 \rangle_0 K_0^2/(VkT) \tag{8}$$

When the bulk modulus of the lipid membrane in the absence of Ca^{2+} is known, the change in bulk modulus caused by addition of Ca^{2+} can be estimated. Eq. 8 states that when the volume fluctuation has a maximum value, the bulk modulus must attain a minimum value.

The volume fluctuation of a lipid membrane, caused by adsorption of Ca^{2+}, is now considered. To our knowledge, there has been no report on the volume fluctuation of adsorbed molecules in membranes or estimation of the fluctuation of the number of adsorbed species. Adsorption of ligands such as anesthetics and ions, onto phospholipid membranes has been shown to reach limiting numbers [4,5].

A rigorous treatment of Ca^{2+} binding to membranes has been described by Ohshima et al. [5], the electrostatic and van der Waals interactions having been calculated in order to estimate the

interaction forces between bilayer membranes. The present study is concerned mainly with the volume fluctuation. The consequence of the previous theory [5] will be applied here and the fluctuation will be described with the aid of a simple treatment.

Let N, N_a, and N_0 be the number of Ca^{2+} adsorbed, the average value of N, and the total number of Ca^{2+} in the system, respectively. As remarked previously [5], the ion binding approximately follows the Langmuir adsorption isotherm. The number of maximum allowable adsorption sites is denoted by N_{max}. The partition function, Z, of the total system is written as

$$Z = \sum_{N=0}^{\infty} Z_N \tag{9}$$

where Z_N is the partition function of the system, written explicitly as

$$Z_N = \frac{1}{(N_0 - N)!}(pf)_b^{N_0 - N}\binom{N_{max}}{N}(pf)_a^N \tag{10}$$

where

$$(pf)_b = \lambda^{-3}\delta v_w N_w \tag{11}$$

and

$$(pf)_a = \lambda^{-3}\delta v_a \exp(-\Delta f'/kT) \tag{12}$$

are the molecular partition functions of a Ca^{2+} in the bulk aqueous solution and a Ca^{2+} adsorbed onto the membrane, respectively. The factors λ, δv_w, δv_a, N_w, and $\Delta f'$ are the de Broglie wavelength of a Ca^{2+}, the free volume of a water molecule, the free volume of an adsorbed ion, the number of water molecules in the system and the free energy change of an ion transferred from the aqueous phase to the membrane surface in which the translational entropy is not included, respectively. When fluctuation of the number adsorbed is not considered, Z can be expressed as

$$Z \approx Z_N \tag{13}$$

and the free energy, F_a, of the system in this case is defined by

$$\begin{aligned} F_a &= -kT \ln Z_N \\ &= -(N_0 - N_a)kT \ln\{e\lambda^{-3}\delta v_w N_w/(N_0 - N_a)\} \\ &\quad - kT\{N_{max} \ln N_{max} - N_a \ln N_a \\ &\quad - (N_{max} - N_a)\ln(N_{max} - N_a)\} \\ &\quad - N_a kT \ln(\lambda^{-3}\delta v_a) + N_a \Delta f' \end{aligned} \tag{14}$$

The number, N_a, is determined by minimizing F_a with respect to N_a as follows.

$$\frac{\partial F_N}{\partial N_a} = kT \ln\left(\frac{N_w}{N_0 - N_a}\right) + kT \ln\left(\frac{N_a}{N_{max} - N_a}\right) + \Delta f = 0 \tag{15}$$

where

$$\Delta f = \Delta f' + kT \ln(\delta v_w/\delta v_a) \tag{16}$$

is the free energy change of an ion transferred from the aqueous solution to the lipid membrane and is assumed to include the electrostatic interaction. By solving eq. 15, N_a becomes

$$N_a = N_{max}\left(\frac{x}{1+x}\right) \tag{17}$$

where

$$x = \frac{N_0 - N_a}{N_w}\exp\left(-\frac{\Delta f}{kT}\right) \tag{18}$$

Eqs. 17 and 18 have the characteristics of a typical Langmuir-type adsorption. The usual studies on adsorption phenomena require only information on physical quantities that are related to the above mentioned average number of adsorbed agents.

Because the present study is concerned with the fluctuation of the adsorbed number, N, around the average value, N_a, the summation in eq. 9 must be evaluated precisely. Because fluctuation of N is very small, Z_N can be written

$$Z_N = Z_{N_a} \exp\left[-\frac{1}{2kT}\left(\frac{\partial^2 F}{\partial N^2}\right)_{N=N_a}(N - N_a)^2\right] \tag{19}$$

By taking the derivative of eq. 15 again with respect to N_a, the second derivative of the free energy, F, at $N = N_a$ becomes

$$\frac{\partial^2 F_N}{\partial N^2} = kT\frac{N_{max}}{N_a(N_{max} - N_a)} + kT\frac{1}{N_0 - N_a} \tag{20}$$

The last term, $kT/(N_0 - N_a)$, is generally very small compared with the other because the concentration of lipids is usually much lower than that of the aqueous solvent, and the number of adsorbed ions is much less than that of free ions in the bulk aqueous phase. For this reason, this term is neglected in the following treatment.

Let Δv and v_0 be the volume change of Ca^{2+} when it is adsorbed from the aqueous solution onto the lipid membrane and the molecular volume of the phospholipid, respectively. Then, δV is written as

$$\delta V = (N - N_a)\Delta v \tag{21}$$

Therefore, the ensemble average of the volume fluctuation in eq. 7 becomes

$$\langle \delta V^2 \rangle_0 = \Delta v^2 \langle (N - N_a)^2 \rangle_0 \tag{22}$$

If the number of allowable adsorption sites, N_{max}, is equal to half of the number of phospholipid molecules, the original volume of the phospholipid membrane, V, is equal to $2N_{max}v_0$. From this and eq. 22, eq. 8 turns out to be

$$\Delta K = -\langle (N - N_a)^2 \rangle_0 K_0^2 \Delta v^2 / (2N_{max} v_0 kT) \tag{23}$$

The ensemble average of the fluctuation $\langle (N - N_a)^2 \rangle_0$ in eq. 23 is evaluated by use of the partition function, Z_N

$$\langle (N - N_a)^2 \rangle_0 = \sum_{N=0}^{N_{max}} (N - N_a)^2 Z_N / \sum_{N=0}^{N_{max}} Z_N \tag{24}$$

The summation in eq. 24 is performed by using eqs. 19 and 20.

$$\sum_{N=0}^{N_{max}} Z_N = Z_{N_a} \sum_{N=0}^{N_{max}} \exp\left[-\frac{N_{max}(N - N_a)^2}{2N_a(N_{max} - N_a)} \right] = Z_{N_a} \left[\frac{2\pi N_a (N_{max} - N_a)}{N_{max}} \right]^{1/2} \tag{25}$$

and

$$\sum (N - N_a)^2 Z_N / \sum Z_N = N_a (N_{max} - N_a)/N_{max} \tag{26}$$

Inserting eq. 26 into eq. 23, the final expression for the change bulk modulus, ΔK, becomes

$$\Delta K = -X(1 - X)\frac{(K_0 \Delta v)^2}{2v_0 kT} \tag{27}$$

where

$$X = N_a / N_{max} \tag{28}$$

is the packing fraction of adsorbed Ca^{2+} in the phospholipid membrane, its value being given by eqs. 17 and 18.

3. Comparison with experimental data and discussion

In the previous section, equations for the change in bulk modulus, caused by adsorption of additive molecules, have been derived. Eqs. 27 and 28 can account for the effect of adsorbed Ca^{2+} on the bulk modulus of the phospholipid membrane. When the packing fraction X, defined by eq. 28, reaches 1/2, the bulk modulus is expected to show a minimum value. Physically, this minimum phenomenon originates from the fact that the configurational entropy of the adsorbed ions at the membrane becomes maximum and the fluctuation of the number of ions adsorbed also becomes maximum when the adsorption sites are half filled.

The present theory is now compared with experimental data on the bulk modulus of the phospholipid membrane [1]. As shown in fig. 1, the bulk modulus of the multilamellar membrane in the absence of Ca^{2+} is about 3.1×10^9 N m^{-2} and the depression of the bulk modulus at about 10 mM Ca^{2+} at 30 °C amounts to about 2.6×10^8 N m^{-2}. Thus, if we assume $X = 1/2$, $v_0 = 1000$ Å^3, $K_0 = 3.1 \times 10^9$ N m^{-2} and $\Delta K = -2.6 \times 10^8$ N m^{-2}, the apparent volume change of Ca^{2+} caused by adsorption on the phospholipid membrane/water interface becomes

$$|\Delta v| = 5.7 \text{ cm}^3 \text{ mol}^{-1} \tag{29}$$

which appears to be a reasonable value. In this estimation, the large value of the bulk modulus at 300 mM Ca^{2+} was not considered. The large bulk modulus may originate from a systematic increment in the bulk modulus as a function of in-

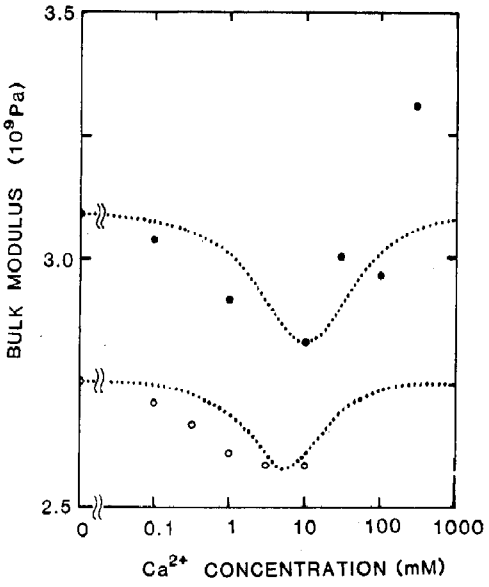

Fig. 1. Bulk modulus of DPPC membranes calculated from the ultrasonic study [1] in multilamellar liposomes (●) and unilamellar vesicles (○) as a function of Ca^{2+} concentration. (·····) Estimated values of the bulk modulus according to the present theory.

creased Ca^{2+} concentration. Although a clear minimum point has not been observed for unilamellar vesicles, the absolute value of Δv is estimated to be

$$|\Delta v| = 4.1 \text{ cm}^3 \text{ mol}^{-1} \tag{30}$$

The dotted lines in fig. 1 denote the estimated change in the bulk moduli as a function of Ca^{2+} concentration, according to the theory. The theoretical curves have narrower valleys than the experimental data, but the agreement is reasonably good. The cause of the difference in the bulk moduli between multilamellar and unilamellar vesicles is unclear. It may arise from the difference in vesicle curvature between the two.

Next, we investigate whether the allowable adsorption sites for Ca^{2+} are half-occupied at about 10 mM Ca^{2+} concentration, where the bulk modulus showed a minimal value. In other words, the validity of the derived apparent binding constant for Ca^{2+}, 100 dm^3 mol^{-1}, is examined. Ohshima et al. [5] evaluated the binding constant of Ca^{2+} to DPPC membranes by taking into account the electrostatic potential in analyzing the X-ray diffraction anomalies that are associated with the change in Ca^{2+} concentration. They [5] determined the apparent binding constant, K_{app}, at 10 mM $CaCl_2$ to be about

$$K_{app} = C_{Ca} \exp(-2e\phi/kT)$$
$$= 1.6 \pm 0.7 \text{ dm}^3 \text{ mol}^{-1} \tag{31}$$

where e is the elementary electronic charge and ϕ the electric potential at the membrane/solvent interface. The value in eq. 31 is small compared with the present expected value, 100 dm^3 mol^{-1}.

There are a number of reports on the binding constants of Ca^{2+} to lipid membranes [6–12]. Kimizuka and Koketsu [6] used a radiotracer method and estimated the binding constant of Ca^{2+} to lecithin monolayers to be 3.47×10^3 dm^3 mol^{-1} at a bulk pH of 8.2. Yamanaka et al. [9] studied metal ion effects upon the surface tension of various lipid monolayers. The abrupt reduction [9] in interfacial tension at Ca^{2+} concentrations of less than 1 mM suggests that the binding constant is more than 100 dm^3 mol^{-1}.

Lis et al. [13] measured the multilamellar lattice spacing by means of X-ray diffraction, and concluded that the association of Ca^{2+} with phospholipid membranes could not be described simply in terms of an association constant or a characteristic surface potential. Ohshima and Ohki [14] also reported that the binding mechanism of Ca^{2+} with a phosphatidylserine monolayer is rather complex, and estimated the binding constant to be of the order of several tens of dm^3 mol^{-1}. Thus, the reported binding constants range between 1 and 1000 dm^3 mol^{-1}. This wide variation may be caused by differences in the membrane systems, i.e., monolayer, unilamellar and multilamellar vesicles, or in the experimental technique.

The present theory analyzes the isothermal bulk modulus, whereas the ultrasonic method measures the adiabatic bulk modulus. Nevertheless, the qualitative tendency of the minimum phenomena in bulk moduli may not differ too greatly between the two approaches.

In this report, we have analyzed the experimental data only at 30 °C. As mentioned in section 2,

the minimum in the bulk modulus occurred between 20 °C (the minimum temperature where the measurements were made) and about 45 °C, which is slightly above the main transition temperature of the membrane. The disappearance of the minimum in the bulk modulus at high temperatures provides a clue as to the origin of this phenomenon. When the membrane is completely in the liquid-crystalline state at high temperature, the binding model for Ca^{2+} on the lattice, which we assumed, may no longer hold. The mode of Ca^{2+} binding to a liquid-crystalline membrane may not be the same as that to a solid-gel membrane. In this context, we have reported an atypical Langmuir adsorption for inhalation anesthetics on phospholipid monolayers, where the adsorption sites increased according to the increase in number of adsorbants [15].

The effect of fluctuation of the number of Ca^{2+} adsorbed onto lipid membranes may not be restricted to membrane compressibility. Divalent metal ions affect many other physical properties of membranes, such as thermotropic phase transition, phase separation in binary mixtures, vesicle fusion, membrane permeability, etc. For instance, Papahadjopoulos et al. [16–18] reported that Ca^{2+} and Mg^{2+} induced highly cooperative phenomena in phosphatidylserine membranes, such as an increase in the rate of diffusion of Na^{+} through the membrane. A major change in the rate of diffusion occurred at a Ca^{2+} concentration where the adsorbable sites for Ca^{2+} are almost fully occupied. However, the rate of diffusion increased steeply when the Ca^{2+}/phospholipid mole ratio reached 1/2. This result may be related to fluctuation of the number of Ca^{2+} adsorbed. Smaal et al. [19] also reported a nonselective permeability increase in phosphatidylcholine/phosphatidate mixed membranes evoked by Ca^{2+}. The bulk Ca^{2+} concentrations that showed an increase in diffusion in these studies, however, were about one order of magnitude lower than those in the present study. These differences are apparently caused by the use of negatively charged phospholipid membranes compared to zwitterionic phosphatidylcholine membranes. The negative surface potential is expected to condense cations in the Gouy-Chapman electrical double layer according to the Boltzmann distribution law.

It is known that membrane permeability increases at the phase-transition temperature of a lipid. There have been efforts to explain this permeability increase by assuming the coexistence of gel and liquid-crystalline domains in the membrane and structural incompatibility at the boundary region [20–22]. These models have been criticized by Nagle [23] on the grounds that they analyzed a two-dimensional phenomenon as a one-dimensional problem. Mitaku et al. [24] reported that the bulk modulus of phosphatidylcholine liposomes reached a minimum value at the phase-transition temperature, and treated the order-disorder phase transition as being a nearly critical phenomenon. The permeability anomaly at the phase transition suggests a critical phenomenon. The available data on the Ca^{2+} effect on membrane permeability demonstrate that membrane permeability is closely related to membrane compressibility, and support the above view.

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