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INTERACTION BETWEEN Ca^{2+} AND DIPALMITOYLPHOSPHATIDYLCHOLINE MEMBRANES

I. TRANSITION ANOMALIES OF ULTRASONIC PROPERTIES

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The effect of Ca^{2+} on a gel-to-liquid crystal transition as well as the mechanical properties of dipalmitoylphosphatidylcholine bilayers was studied by an ultrasonic technique. Transition temperature increased with increase in Ca^{2+} concentration, whereas the variation of ultrasonic anomalies indicated that dipalmitoylphosphatidylcholine bilayers exhibited maximum pseudocritical fluctuation at a Ca^{2+} concentration of about 10 mM. Hardening of dipalmitoylphosphatidylcholine membranes due to the Ca^{2+} binding was observed above 10 mM $CaCl_2$, suggesting the lateral compression of the lipid bilayer by bound Ca^{2+}. Long-range attraction between bound Ca^{2+} and the head groups of surrounding lipid molecules was proposed from these calcium effects.

1. Introduction

The distribution of Ca^{2+} in biological systems is kept constant by membrane systems, providing fundamental conditions for various important physiological functions such as muscle contraction [1] and nerve excitation [2]. Therefore, the effects of Ca^{2+} on membranes, particularly on acidic lipid bilayers, have been studied extensively [3–6]. Bilayers of neutral phospholipids, e.g., phosphatidylcholine, are also influenced by Ca^{2+} in solvents as revealed by X-ray diffraction [7] and calorimetry [8]. The variation of the lamella period by small-angle X-ray diffraction indicated clearly that Ca^{2+} bound to phosphatidylcholine membranes with a binding constant of 21 M^{-1} [9,10]. The intramembrane structure also changed due to the binding of Ca^{2+} [8,11,12]. However, the nature of the interaction between Ca^{2+} and phosphatidylcholine remains an open question.

The transition of dipalmitoylphosphatidylcholine is very nearly a second-order transition as revealed by ultrasonic studies [13,14]. Critical phenomena, i.e., critical softening, critical slowing down and the enhancement of relaxation strength, were observed and the anomalous types of behavior were explained by the Landau theory of phase transitions. Accordingly, its transition curves are influenced drastically by various kinds of perturbation [15–17]. Therefore, the variation of the transition anomalies due to Ca^{2+} binding should provide new insight into the calcium-phosphatidylcholine interaction. Furthermore, the calcium concentration dependence of the bulk modulus which is obtained from the ultrasonic velocity should also indicate the nature of the calcium-phosphatidylcholine interaction, because the bulk modulus is directly related to the density fluctuation of materials [18].

In an attempt to study the interaction between

Ca^{2+} and phosphatidylcholine membranes, we have measured in this work the ultrasonic anomalies of the lipid bilayer transition as well as the mechanical properties as a function of Ca^{2+} concentration. The results indicated that the critical fluctuation at the lipid transition became maximum at a Ca^{2+} concentration of about 10 mM, while the transition temperature increased monotonically. The bulk modulus also showed a biphasic change. It decreased below 10 mM Ca^{2+} and increased above the minimum point. These types of behavior were qualitatively well explained in terms of the long-range attraction between dipalmitoylphosphatidylcholine and bound Ca^{2+}, which is also suggested from the molecular information in the accompanying paper [12].

2. Materials and methods

2.1. Preparation of liposomes

Synthetic L-α-dipalmitoylphosphatidylcholine (DPPC) was purchased from Sigma and used without further purification. We prepared a dilute suspension of multilamellar liposomes in the same manner as in the previous reports [13,14]. The dry weight concentration of lipid in the suspension was approx. 3 mg/ml. The concentration of $CaCl_2$ in solvent was varied from 0 to 300 mM.

2.2. Ultrasonic measurements

Ultrasonic properties were measured at 3 MHz by a differential ultrasonic velocimeter [19]. The apparatus is equipped with a large thermostatted water bath which enables one to control the temperature of the twin cells to within ± 0.001°C. The accuracy of measurements was ± 0.7 cm/s for the ultrasonic velocity and $\pm 2 \times 10^{-4}$ for the absorption per wavelength, being precise enough to detect the change of lipid bilayers during the phase transition. We measured the ultrasonic properties of a liposome suspension with a certain Ca^{2+} concentration as a function of temperature in the range 20–55°C, stirring the sample just before each measurement in order to avoid sedimentation.

2.3. Data analysis

Since the ultrasonic properties depend upon both aqueous solvent and suspended membranes, we used limiting numbers of velocity and absorption per wavelength for data analysis. The limiting number of velocity $[V]$ and absorption per wavelength $[\mu]$ are defined as follows:

$$[V] = \lim_{c \to 0} \frac{V - V_s}{V_s c}, \tag{1}$$

$$[\mu] = \lim_{c \to 0} \frac{\mu - \mu_s}{c}, \tag{2}$$

in which c denotes the dry weight concentration of lipid and subscript s represents the solvent. The bulk modulus of lipid bilayers can be estimated from the limiting number of velocity by using the

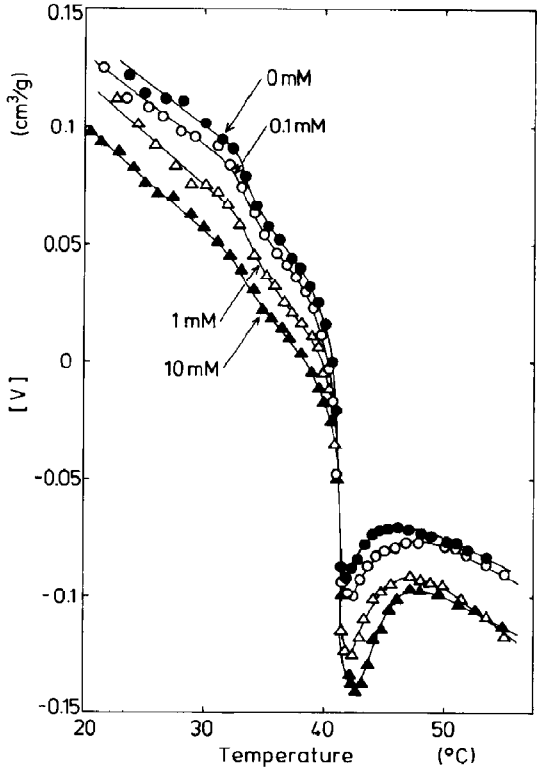

Fig. 1. Limiting velocity number of DPPC membranes at 3 MHz as a function of temperature for various Ca^{2+} concentrations below 10 mM: 0 mM (●), 0.1 mM (○), 1 mM (△) and 10 mM (▲).

following relationship [16];

$$[V] = \frac{1}{2}\left(\frac{K_m - K_s}{K_m} - \frac{\rho_m - \rho_s}{\rho_s}\right)\frac{1+\delta}{\rho_m}. \quad (3)$$

where, K denotes bulk modulus, ρ is the density, δ the weight of water hydrated to 1 g of membrane, and the subscripts, m and s, indicate membrane and solvent, respectively.

3. Results

We have measured the specific velocity, $(V - V_s)/V_s$, and the excess absorption per wavelength, $(\mu - \mu_s)$, at 3 MHz as a function of temperature and Ca^{2+} concentration. Since $(V - V_s)/V_s$ and

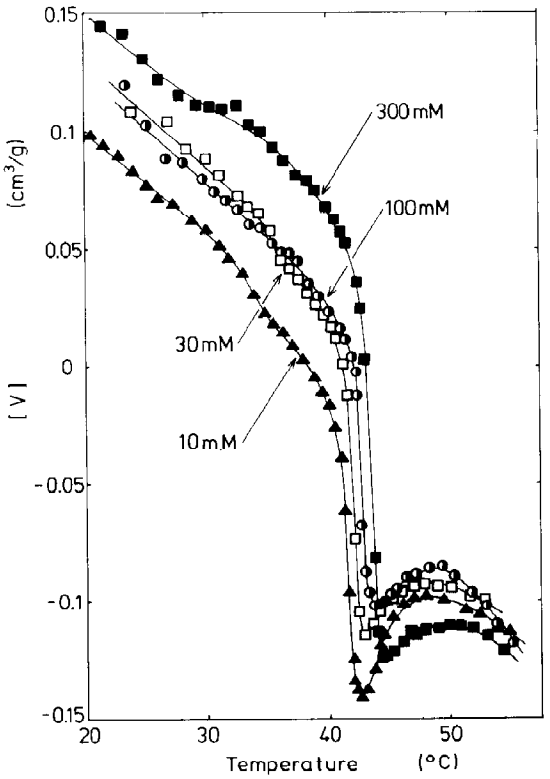

Fig. 2. Limiting velocity numbers of DPPC membrane at 3 MHz as a function of temperature for various CA^{2+} concentrations above 10 mM: 10 mM (▲), 30 mM (□), 100 mM (◑) and 300 mM (■).

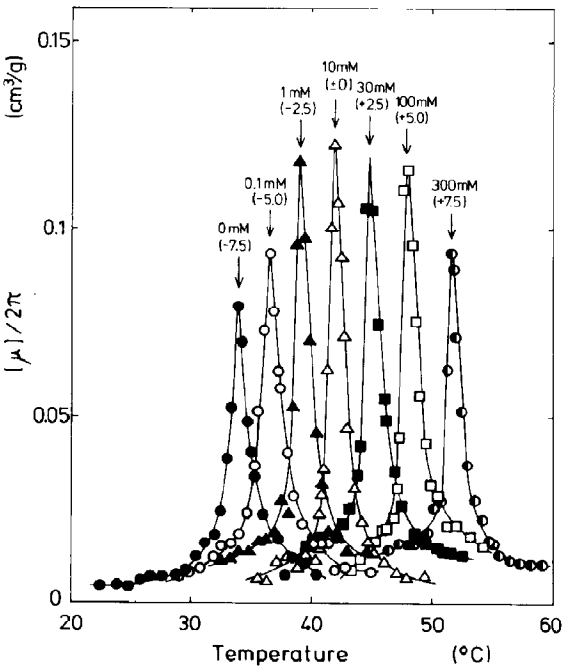

Fig. 3. Limiting numbers of ultrasonic absorption per wavelength at 3 MHz as a function of temperature for various Ca^{2+} concentrations: 0 mM (●), 0.1 mM (○), 1 mM (▲), 10 mM (△), 30 mM (■), 100 mM (□) and 300 mM (◑). Data are shifted along the temperature axis by the number degrees indicated in parentheses.

$(\mu - \mu_s)$ are linear functions of dry weight concentration of lipid (data not shown), we determined $[V]$ and $[\mu]$ by simply normalizing $(V - V_s)/V_s$ and $(\mu - \mu_s)$ by c, respectively.

Figs. 1 and 2 show the temperature dependences of $[V]$ in the Ca^{2+} concentration ranges 0–10 and 10–300 mM, respectively. The temperature dispersion of $[V]$ shows an anomalous dip in the vicinity of the lipid bilayer transition temperature, T_m, which is in accordance with previous measurements [13,14]. The temperature dependence of $[\mu]$ is shown in fig. 3 at various Ca^{2+} concentrations. $[\mu]$ values for all samples were also enhanced dramatically toward T_m.

These transition properties were influenced considerably by the presence of Ca^{2+}. The temperature of the velocity minimum and absorption peak increased with increasing Ca^{2+} concentra-

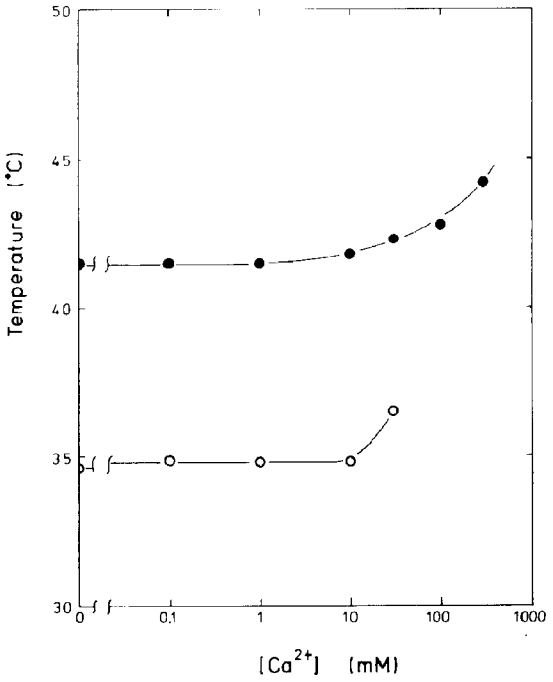

Fig. 4. Transition temperature of the main transition and the pretransition as a function of Ca^{2+} concentration. The main transition temperature was determined from the peak of ultrasonic absorption.

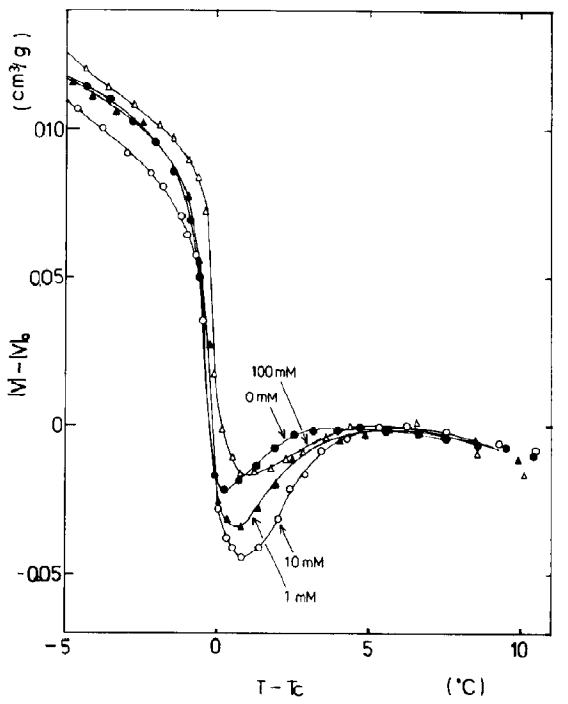

Fig. 5. Limiting numbers of velocity as a function of the temperature difference from the transition temperature. In order to compare the depth of the anomalous dip in the vicinity of the transition point, the data are shifted along the velocity axis so that the limiting velocity numbers at 5–10°C above the transition point become equal.

tion. Namely, the temperature of the gel-to-liquid crystal transition increased monotonically when the Ca^{2+} concentration was increased, as shown in fig. 4. Fig. 4 also shows the Ca^{2+} concentration dependence of the pretransition temperature, which was determined from the midpoint of the sigmoidal change of the ultrasonic velocity. The monotonic increase of the transition temperature is in good agreement with the previous report by Graddick et al. [8]. On the other hand, the depth of the dip of $[V]$ and the peak height of $[\mu]$ appeared to be greatest at a Ca^{2+} concentration of 10 mM. We have replotted the velocity data in fig. 5, so that the depth of the dip may be compared between transition curves for different Ca^{2+} concentrations. The limiting velocity numbers in the temperature region 5–10°C above the transition temperature were set equal to each other. The dip of $[V]$ became deeper, until the Ca^{2+} concentration exceeded 10 mM, and then became shallower again with increasing Ca^{2+} concentration. In accordance with this biphasic change in the ultrasonic velocity, the ultrasonic absorption exhibited the largest transition anomaly around 10 mM $CaCl_2$ (fig. 3).

The magnitude of $[V]$ in the temperature region far from the transition point also varied systematically upon addition of Ca^{2+}. The bulk modulus of the DPPC membrane was calculated from $[V]$ by using eq. 3, assuming that the density of the DPPC bilayer is not influenced by the binding of Ca^{2+}. The extent of hydration of the membrane was assumed to be 0.4 g/g membrane [13]. The bulk modulus of a multilamellar membrane at 30°C is plotted in fig. 6 as a function of Ca^{2+} concentration together with the result for a sonicated unilamellar membrane for comparison. The bulk modulus of the membrane decreased below 10

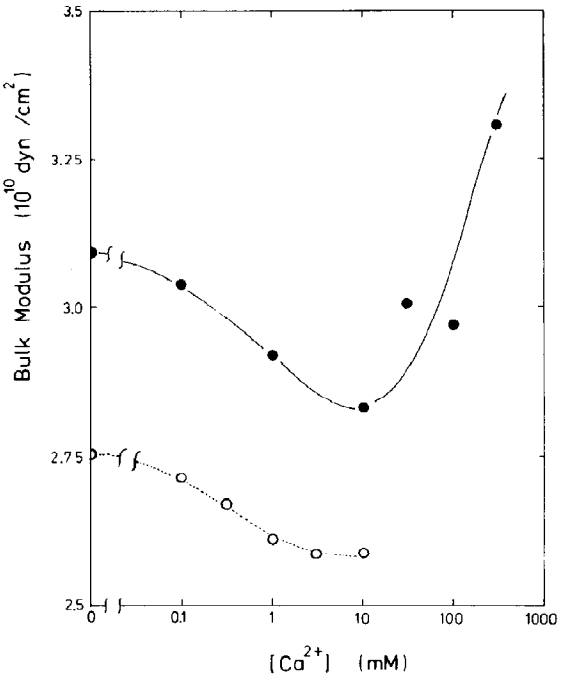

Fig. 6. Bulk modulus of DPPC bilayers calculated from [V] of multilamellar liposomes (●) and unilamellar vesicles (○) as a function of Ca^{2+} concentration.

mM and increased above the minimum point of 10 mM, suggesting a structural change due to Ca^{2+} even at temperatures far from the transition point.

4. Discussion

The experimental results can be summarized as follows: (1) The transition anomalies changed systematically due to the presence of Ca^{2+}. The dip of the velocity and the absorption peak were most significant at 10 mM Ca^{2+}. (2) The transition temperature became higher as the Ca^{2+} concentration was increased. (3) The bulk modulus of the DPPC bilayer below T_m had a broad minimum at 10 mM Ca^{2+}. These phenomena should be explained comprehensively by some interaction mechanism between Ca^{2+} and DPPC bilayers.

In order to elucidate the physical significance of the calcium effect on the transition anomalies, it is necessary to know beforehand the mechanism of the transition anomalies, i.e., we require clarification as to why the dip-like behavior of the velocity and sharp peak of absorption occur around the lipid phase transition. Therefore, we first review briefly the present knowledge of the lipid bilayer transition and then discuss the calcium-phosphatidylcholine interaction in detail.

4.1. Mechanism of ultrasonic anomalies

The anomalous change of various physical properties during the lipid bilayer transition may be classified into two sets of transition curves: a very sharp change at the transition point and rather broad anomalies. The sharp peak of the specific heat obtained by differential scanning calorimetry [20] and the sharp decrease of the order parameter found using a fluorescence anisotropy decay technique [21] belong to the former, while the anomalous behavior of permeability [22] and ultrasonic properties [13,14] are typical examples of the latter. From the early stages of investigation of the lipid bilayer transition, the sharpness of the transition curves of the thermal analysis and other physical properties was recognized [20] and attributed to the first-order nature of the phase transition: The melting of the hydrocarbon region occurs isothermally at the transition temperature.

The second-order characteristics, i.e., the enhanced fluctuation of membrane order, were suggested from the permeability anomaly near the transition point [23] and demonstrated clearly by ultrasonic relaxation measurements [14]. In the vicinity of the transition temperature, distinct relaxation with a relaxation time of tens of nanoseconds was observed, showing the maximum of the relaxation strength as well as the relaxation time at the transition point. Because this value of the relaxation time is much longer than the motional relaxation of a single hydrocarbon chain, it was concluded that the ultrasonic relaxation is associated with the order parameter fluctuation. Furthermore, analysis of the temperature dependence of the relaxation parameters in terms of the Landau theory of phase transitions indicated clearly that the transition temperature is different from, but very near, the pseudocritical temperature [14], i.e., the lipid bilayer transition is a weak first-order transition.

The anomalous dip of velocity in figs. 1 and 2 and the peak of absorption in fig. 3 are also distinct indications of the fluctuation of membrane structures [13,14], providing an easy and useful method for monitoring the change in transition properties. The bulk modulus, K, which may be determined directly from the ultrasonic velocity generally shows an inverse relationship to the density fluctuation $\langle \delta\rho^2 \rangle$ in a semi-microscopic volume ν,

$$\frac{1}{K} = \frac{\nu \langle \delta\rho^2 \rangle}{kT\rho^2} . \tag{4}$$

Therefore, the dip of velocity,i.e., the peak of the bulk modulus, represents the enhanced structural fluctuation, whereas the peak of absorption is due to the increase of the structural relaxation strength in lipid bilayer systems [14].

Based upon these previous works, we note the following points for the discussion of calcium effects.

(1) The dip of the velocity and the peak of absorption are due to the pseudocritical fluctuation of membrane order which indicates the second-order characteristics of the lipid bilayer transition. Therefore, the depth of the velocity minimum as well as the height of the absorption should represent the magnitude of the critical fluctuation around the transition temperature.

(2) The transition temperature is defined by the singular points of the ultrasonic anomalies: the minimum of the velocity and the maximum of the absorption. Because the temperature dependence of the velocity shows a general tendency to decrease with increasing temperature, the minimum point of the velocity is shifted to slightly higher temperature than the peak of absorption. Therefore, we define the transition temperature by the maximum point of ultrasonic absorption. The gel-

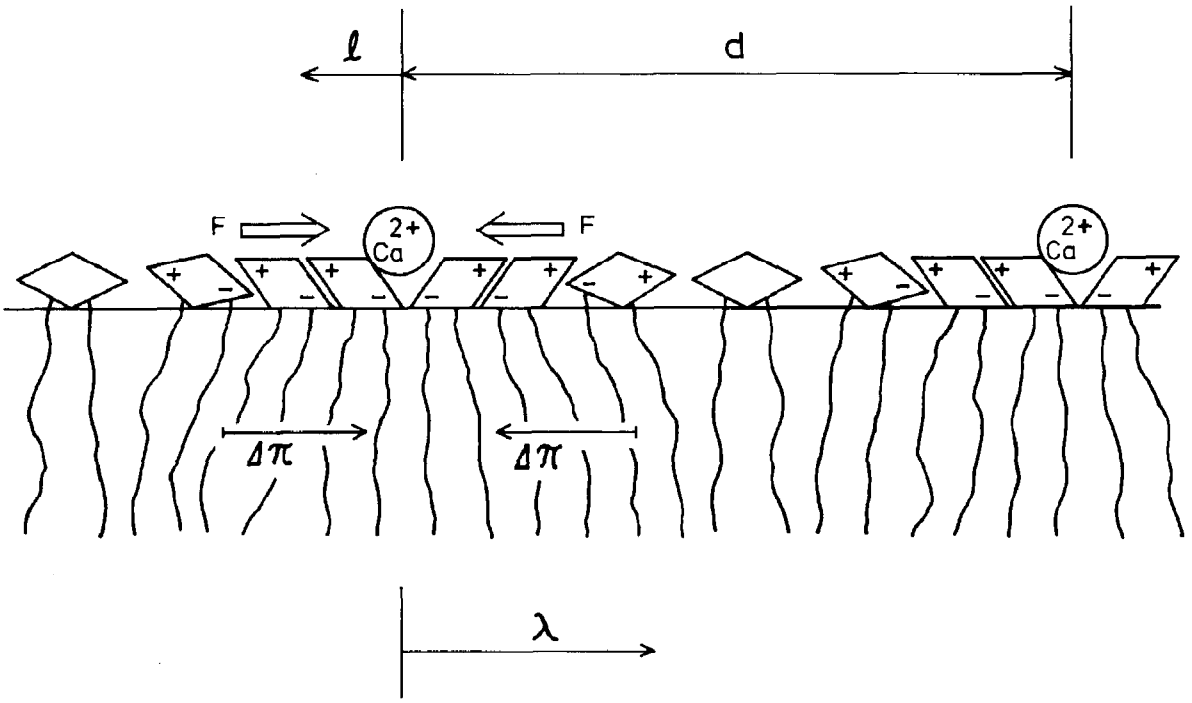

Fig. 7. Schematic model of the calcium effect on phosphatidylcholine membrane. d, l and λ are the average distance between bound Ca^{2+}, Debye screening length and characteristic length of calcium effect on hydrocarbon region, respectively. A bound Ca^{2+} attracts polar head groups of surrounding phosphatidylcholine molecules leading to increased lateral pressure, $\Delta\pi$.

to-liquid crystal transition temperature in fig. 4 was determined according to this definition.

4.2. Calcium effects on transition properties

The lipid bilayer transition is very nearly a second-order phase transition, as mentioned above. Therefore, the influence on this critical phenomenon is important in elucidating the calcium-phosphatidylcholine interaction. As shown in fig. 5, the anomalous dip of $[V]$ is deepest at the Ca^{2+} concentration of about 10 mM. Corresponding to this anomaly of the velocity, the peak of absorption around the transition temperature had a maximum at a Ca^{2+} concentration of about 10 mM. These biphasic changes in the transition anomalies indicate that the critical phenomenon was greatest at 10 mM Ca^{2+}: The gel-to-liquid crystal transition is of weak first order in the absence of Ca^{2+} and approaches a critical point as the Ca^{2+} concentration is increased to 10 mM.

On the other hand, the transition temperature showed a monotonic increase due to the addition of Ca^{2+}. The temperature of the main transition increased from 41.4 to 44.2°C at 300 mM $CaCl_2$. It should also be pointed out that the calcium effect appears at a considerably low concentration, i.e., the transition temperature at 10 mM $CaCl_2$ is already 0.4°C higher than that in pure water. The contrasting calcium effects on the critical behavior and transition temperature should be explained by a calcium-phosphatidylcholine interaction, because there are no molecular species in the sample other than $CaCl_2$ and phosphatidylcholine.

It was previously proved by Ohshima et al. [10] that Ca^{2+} binds to phosphatidylcholine membranes with a considerably large binding constant of 21 M^{-1}. Furthermore, as revealed by the comparison between calcium and magnesium effects in the accompanying paper, the bound species of Ca^{2+} are the main cause of the change in physical properties of DPPC membranes [12]. Then, what happens in the hydrocarbon region when Ca^{2+} is bound to the head group of a lipid molecule? The lipid-Ca^{2+} complex is charged positively and produces a surrounding electric field, which is shielded by the electric double layer of small ions in the solvent. Because the head groups of phosphatidylcholine molecules contain zwitterions, the positive charges of the lipid-Ca^{2+} complex exert a long-range attraction on the surrounding lipid molecules as illustrated in fig. 7. Due to the electric field resulting from the positive charge, the zwitterions of the head group must be aligned with the negative charge pointing toward the bound Ca^{2+}, at least statistically, when they are within the range of the Debye length, l. This alignment of the head groups leads to the attractive force between lipid molecules. When the ionic concentration in the solvent is low, the attraction by this direct mechanism is long range.

In addition to this mechanism, there may be an indirect attractive effect of Ca^{2+} on the surrounding lipids. The lipid bilayer structure is formed by various kinds of attractive interactions between lipid molecules: van der Waals force, hydrophobic interaction in the hydrocarbon region and electrostatic interaction in the polar region. The characteristic length of the combination of these interactions is estimated to be 1.5 nm [24]. Therefore, the electrostatic attraction between bound Ca^{2+} and the polar head group of an adjacent lipid molecule may influence indirectly the structure of the lipid molecules far from the Ca^{2+} with a coherence length of about 1 nm. This indirect mechanism of the attractive effect should be important at high ionic concentration in the solvent where the electrostatic force is exerted over a range no longer than the molecular dimension.

The DPPC bilayer/Ca^{2+} system is, therefore, characterized by three kinds of characteristic length: the average distance, d, between bound Ca^{2+}; the Debye screening length l; and the distance, λ, in which the hydrocarbon structure is influenced by direct as well as indirect effects of the bound Ca^{2+}. We estimate d, l and λ, in order to study whether such long-range attraction may cover the surface of membrane. The Debye screening length l of a salt solution is given by

$$l = \left(\frac{4\pi l^2 n_0}{\epsilon kT} \sum_i \nu_i Z_i^2 \right)^{-1/2}, \tag{5}$$

in which n_0 is the number of salt molecules per unit volume, and ν_i and Z_i are the number and valence of the i-th atom in a salt molecule, respec-

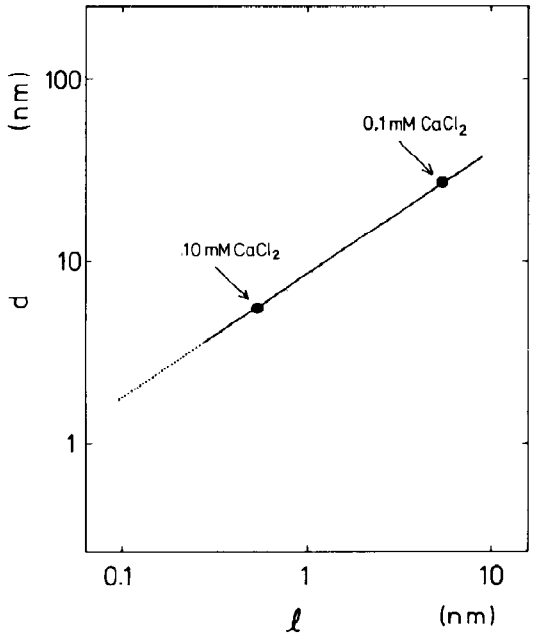

Fig. 8. Relationship between calculated characteristic length, d and l for calcium-DPPC system. A binding constant of 21 M^{-1} [10] and the area of a phosphatidylcholine molecule of 0.5 nm^2 were used for the calculation.

tively. For example, the Debye length of 1 mM $CaCl_2$ solution is 1.7 nm which is long enough with respect to the molecular dimension.

We follow the treatment by Ohshima and Mitsui [9] in the calculation of d. We solve the following equations self-consistently for each Ca^{2+} concentration.

$$K_b = \frac{\xi N_A}{(1-\xi) n_0 \exp(-2e\psi_0/kT)}, \quad (6)$$

$$\sigma = \left[\frac{\epsilon kT}{2\pi}\sum_i n_0 \nu_i \{\exp(-Z_i e\psi_0/kT) - 1\}\right]^{1/2}, \quad (7)$$

$$\sigma = \frac{2e\xi}{A}, \quad (8)$$

in which ξ is the ratio of bound calcium to total lipid concentration, K_b the binding constant of Ca^{2+}, ψ_0 the surface potential, A the area per molecule and N_A Avogadro's number. Here, $\nu = 1$ and $Z_i = 2$ for Ca^{2+} and $\nu = 2$ and $Z_i = 1$ for Cl^-. Eq. 6 describes the binding of Ca^{2+}. Eq. 7 represents the electric double layer and eq. 8 is a relation between the calcium binding and the surface charge density. The average distance was calculated from ξ with the equation:

$$d = 1.075\, A/\xi. \quad (9)$$

The factor of 1.075 was derived, assuming a hexagonal array of bound Ca^{2+}. We have assumed in the calculation that $K_b = 21$ M^{-1} and $A = 0.5$ nm^2.

The relationship between l and d is shown in fig. 8. Both l and d decrease with increasing Ca^{2+} concentration. d is longer than l by some factor. However, because the screening of the electric field occurs only in aqueous solvent and the hydrocarbon region contains few ions to shield the electric field, λ must be longer than l. Furthermore, due to the indirect mechanism of the long-range attractive force, λ becomes larger than l particularly at high ionic concentrations. Therefore, it should be reasonable to assume that λ is compara-

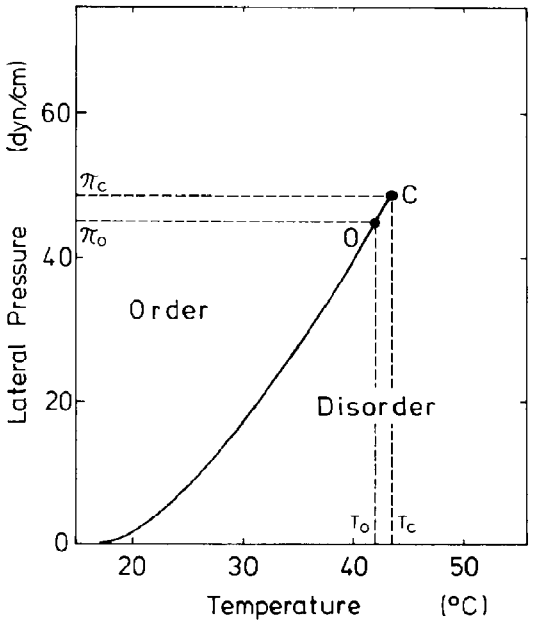

Fig. 9. Schematic phase diagram of DPPC monolayer which has been replotted from ref. 24. Points C and O denote the critical point and transition point of the lipid bilayer without Ca^{2+}, respectively.

ble to $d/2$, although the analytical estimation of λ is not as easy as that of l and d.

As shown schematically in fig. 7, the attraction of the polar head groups to the bound Ca^{2+} leads necessarily to elevated lateral pressure in the hydrocarbon region. The lateral pressure in such a case is not uniform but highest at the position of the bound Ca^{2+} and decreases as the distance from the ion becomes larger. Therefore, the average lateral pressure must increase when the Ca^{2+} concentration is raised, because the distance d between bound Ca^{2+} decreases with increasing Ca^{2+} concentration.

The influence of lateral pressure on the transition behavior has been studied experimentally using the monolayer technique. As far as the main transition is concerned, the transition behavior varies significantly according to the lateral pressure, as shown schematically in fig. 9 [24]. There is a critical point C, where $T_c = 42$–44°C and $\pi_c =$ 47–50 dyn/cm [25–27]. The phase transition is first order at the lateral pressure below π_c, whereas it is second order when $\pi = \pi_c$. The transition curves become smoother when $\pi > \pi_c$. Therefore, the critical phenomenon should be enhanced when the lateral pressure is increased from π_0, which is a little lower than π_c, to the critical lateral pressure, π_c. After the maximum of the critical fluctuation at π_c, the transition anomalies become smaller again, i.e., the transition anomalies due to the critical phenomenon show a biphasic change. On the other hand, it is apparent that the transition temperature becomes higher monotonically, when the lateral pressure is increased. As a consequence, the increase of the average lateral pressure due to the attractive force between a bound Ca^{2+} and surrounding lipids may cause simultaneously a maximum of the critical phenomenon at a certain calcium concentration as well as a monotonic increase of the transition temperature, if the lateral pressure of the lipid bilayer in the absence of divalent cations is a little lower than π_c. This picture of the calcium effect on the transition properties appears physically sound because of several lines of evidence: Firstly, the free volume for a molecule in the hydrocarbon region is more restricted at high Ca^{2+} concentration than in pure water, in accordance with the increased lateral

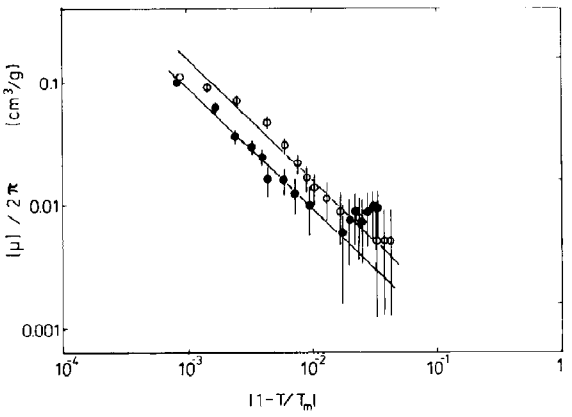

Fig. 10. Limiting numbers of absorption per wavelength of multilamellar liposomes in 10 mM $CaCl_2$ as a function of $|1 - T/T_m|$ above (○) and below the transition temperature, T_m (●). Solid lines are drawn, assuming a critical exponent of 1.

pressure [12]. Secondly, the actual lateral pressure in the lipid bilayer is considered to be only a little lower than the critical lateral pressure, because the lipid bilayer transition temperature, 41.5°C, is very near the critical temperature of the monolayer, and the difference between the pseudocritical temperature and the transition temperature of the lipid bilayer is also smaller than 1°C [14]. Thirdly, the maximum of the transition anomalies due to the critical phenomenon was observed at a Ca^{2+} concentration of 10 mM. The transition temperature at this Ca^{2+} concentration was 41.8°C , in good agreement with the critical temperature obtained using the monolayer technique. Finally, the anomalous increase of the ultrasonic absorption at 10 mM Ca^{2+} was described well by a critical exponent of 1 which is in accordance with the molecular field theory [14,24], as shown in fig. 10. Consequently, a DPPC membrane in 10 mM $CaCl_2$ is very near a critical point and the critical temperature of this membrane must be equal to about 42°C.

4.3. Calcium effects on mechanical properties

The bulk modulus of a DPPC membrane at temperatures far from the transition point also exhibited a biphasic change. The bulk modulus of multilamellar lipid bilayers decreased smoothly

from 3.1×10^{10} dyn/cm^2 in pure water to 2.8×10^{10} dyn/cm^2 at 10 mM $CaCl_2$. The membrane had then become hardened. The bulk modulus of unilamellar lipid bilayers was smaller than multilamellar liposomes. However, the shape of the Ca^{2+} concentration dependence in the unilamellar vesicles was parallel to that in the multilamellar liposomes.

We should be careful, however, in the discussion of the bulk modulus of complex material such as lipid bilayers. It is well known that the binding of Ca^{2+} to phosphatidylcholine membranes changes greatly the intermembrane interaction, leading to a drastic transition of the membrane spacing [7,10]. In principle, such a long-range interaction may also affect the ultrasonic properties. However, it may be concluded from fig. 10 that the intermembrane interaction makes little contribution to the ultrasonic properties, i.e., to the bulk modulus of membranes determined from $[V]$, because of the following reasons; firstly, the bulk modulus changed quite smoothly in the calcium concentration range of several millimolar, where the intermembrane spacing shows discontinuous change. Secondly, the variation of the bulk modulus of unilamellar vesicles is quite similar to that of multilamellar liposomes, indicating that the semimicroscopic shape of a membrane system does not affect the ultrasonic properties. Therefore, the bulk modulus actually represents the mechanical property of membranes, which include hydrocarbons, head groups and hydrated water.

Consequently, which kind of mechanism causes the biphasic change in the bulk modulus at temperatures far from the transition temperature? We have as yet no definite answer to this question. However, the most plausible mechanism seems as follows: The binding of calcium induces an electric field not only in the direction perpendicular to the membrane but also in the plane of the membrane as discussed in section 4.2. This electric field causes necessarily the rearrangement of zwitterionic head groups of phosphatidylcholine molecules, resulting in the change in the physical properties of the hydrocarbon region as well. Since the hydrocarbons are compressed by the attractive interaction in the polar region, the bulk modulus of the hydrophobic region must increase due to the calcium binding even when the influence of the transition anomalies is negligibly small. The increase of the bulk modulus at high Ca^{2+} concentration is probably due to this effect. On the other hand, the change in the bulk modulus of the polar region is not easy to estimate. The initial decrease of the bulk modulus at lower Ca^{2+} concentration seems to indicate that the bulk modulus of the polar region decreases. More detailed discussion will be possible if we study the calcium effect using selective labelling of the polar group.

4.4. Concluding remarks

The model in fig. 7 seems to explain all the qualitative features of the calcium effects on the lipid bilayer transition: The binding of Ca^{2+} to the lipid bilayer induces an increase of the average lateral pressure on the hydrocarbon region. The elevated lateral pressure leads simultaneously to a monotonic increase of the transition temperature and to the maximum critical phenomenon around 10 mM $CaCl_2$, because the transition of the lipid bilayer in the absence of Ca^{2+} is weakly first order. However, several questions remain to be answered. First, our discussion was based upon the the assumption that all significant calcium effects are due to the binding of Ca^{2+} to the polar head group of phosphatidylcholine molecules. Secondly, the contribution of the hydrocarbon region and polar head groups to the change in the bulk modulus appears different. These two questions are answered in the accompanying paper experimentally by using dynamic fluorescence anisotropy measurements. Thirdly, the lipid bilayer with a certain amount of Ca^{2+} was regarded as a single phase throughout the gel-to-liquid crystal transition in our discussion, but it is possible that some change occurs in the binding constant during the transition. This problem will be treated theoretically in the forthcoming paper.

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