

Inter- and Intramembrane Interactions and Phase Transitions

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Abstract. A theory is presented which explains the effect of inter- and intramembrane interactions on phase transition temperature, T_c , in lipid bilayers. It was shown that the adsorption of Ca^{2+} ions by charged membranes produces a surface with alternating positive and negative charges. In this system, there is a strong electrical attraction between molecules resulting in shift of the phase transition temperature in the membrane. This shift is greater than that occurring due to monovalent cation adsorption. The effect is more pronounced due to the dehydrated contact between liposomes in the presence of Ca^{2+} ions. If the dielectric constant in the contact is close to 2, the phase transition temperature increases by approximately 160 K. Our results explain the experimental data obtained by Papahadjopoulos et al. The intermembrane interaction results in differences in phase transition temperature between different monolayers. The same effect may be produced by membrane curvature or another structural factors. Differences in transition temperatures between monolayers, and the geometrical coupling between them, result in non-isothermality of the phase transition in liposomes. The maximum values of the temperature interval within which the phase transition can be "smeared" was estimated. Theoretical conclusions were compared with literary data.

Key words: Ca^{2+} adsorption — Shift in transition temperature — Elastic stresses — Non-isothermality

Introduction

Molecules of lipids in biological and artificial membranes can undergo phase transition between crystalline (gel) and fluid states (Chapman 1975; Lee 1977; Ivkov and Berestovsky 1981). This phenomenon was analyzed by numerous authors (Marčelja 1974; Nagle 1976, 1980; Priest 1980; Scott and Cheng 1977; Scot 1981; Forsyth et al. 1977; Trauble and Eibl 1974; Copeland and Andersen 1981a, b) and the role of hydrophobic tails and polar heads of lipid molecules in phase transition was elucidated. The interest in polar heads arised from their sensitivity to changes in external media. External influences can trigger phase

transitions in membranes specifically over lipid heads and thus affect the biological functions of the cell.

Lipid ionization and cation adsorption shift the temperature of membrane phase transition, T_c . This shift results from changes in energy of electrical interactions between lipid heads. The conventional theory explains well this effect in adsorption of univalent cations (Copeland and Andersen 1981). It however fails in explaining the effect of divalent cations, namely Ca^{2+} , which can easily provoke both phase transitions in liposomes and their aggregation and fusion. Papahadjopoulos and coworkers (Portis et al. 1979; Wilschut et al. 1980; Düzgünes et al. 1981) demonstrated that the addition of Ca^{2+} (in concentrations exceeding 1 mmol/l) results in a shift of the transition temperature of phosphatidylserine (PS) membranes by more than 100 K. The respective shift in glycerolmonooleate membranes is 60 K (Copeland and Andersen 1981a, b).

Such great shifts of phase transition temperature cannot be simply explained by neutralization of the membrane by the cation adsorption. Copeland and Andersen tried to explain this phenomenon on the assumption that Ca^{2+} ion can build a bridge between two neighbouring lipid molecules in crystalline state. This explanation does not seem satisfactory because it postulates the existence of strong non-electric interactions between Ca^{2+} and the lipid heads.

There is another peculiarity of phase transitions in liposome membranes: they sometimes happen to be "smeared" within certain temperature intervals (Nagle 1980). Strictly speaking, it is not a first order transition and we shall call it a non-isothermal transition. Dael et al. (1982) investigated this phenomena in large (~ 100 nm) and small (~ 10 nm) lecithin liposomes. They found that phase transition in large liposomes was "smeared" over 15 K and in small liposomes over 30 K. This has remained unexplained since attempts to relate it to the nucleation of the crystalline clusters in the liquid phase (Kanehisa and Tsong 1978; Doniach 1978; Freire and Bibtonen 1978; Mc Cammon and Deutch 1975) can only explain a smearing interval of 2–3 K.

We believe that both phenomena can be understood taking into consideration the relationship of inter and intramembrane interaction. The membranes are known to form dense and dehydrated contact in the presence of Ca^{2+} ions (Portis et al. 1979). The establishment of such a contact in the presence of Ca^{2+} unavoidably results in a strong lateral constriction in contacting monolayers and thus it produces a substantial increase in the phase transition temperature, T_c .

The reason for the non-isothermality of phase transition might be a simple geometric coupling between two monolayers of a closed membrane. If the phase transition in the outer monolayer of a liposome starts at a higher temperature than that in the inner one, the accompanying shrinkage of the outer monolayer will be hindered by the inner one, leading to the smearing of the phase transition.

Both these effects will be calculated in following sections.

Formulation of the problem

Consider a membrane of closed liposome consisting of molecules of the same kind, having a negative electric charge, $-e_0$. The lipid molecules in a membrane can be in two phase states — liquid and crystalline — which differ from each other by the area per molecule, a .

Denote all the quantities, pertaining to external and internal monolayers of membrane by the superscripts “ o ” and “ i ” respectively, and the quantities, pertaining to the molecule in liquid and crystalline (gel) phase state by the subscripts “ λ ” and “ γ ”. For instance, N^o and N^i are total numbers of molecules in the external and internal monolayers; N_γ^o and N_γ^i are the numbers of molecules in crystalline state in the respective monolayers.

To the thermodynamic equilibrium of the system, which is at a constant pressure, P , and temperature, T , there corresponds a minimum of the Gibbs free energy (Landau and Lifschitz 1976). The free energy per one molecule is expressed as $u + Pv - Ts$, where u , v and s are the energy, volume and entropy per molecule. The expression for the total free energy of the bilayer under consideration can be written as

$$\Phi = (N^o - N_\gamma^o) (u_\lambda^o + Pv_\lambda^o - Ts_\lambda^o) + N_\gamma^o (u_\gamma^o + Pv_\gamma^o - Ts_\gamma^o) + (N^i - N_\gamma^i) (u_\lambda^i + Pv_\lambda^i - Ts_\lambda^i) + N_\gamma^i (u_\gamma^i + Pv_\gamma^i - Ts_\gamma^i) \quad (1)$$

Obviously, the energy, entropy and volume per particle depend upon its area. Therefore, the variables defining the thermodynamic potential Φ are a_λ^o , a_γ^o , a_λ^i , a_γ^i , N_γ^o and N_γ^i . However, these variables are not independent. Let us suppose that the liposome radius is significantly in excess of the bilayer thickness. In this case one may write approximately:

$$(N^o - N_\gamma^o)a_\lambda^o + N_\gamma^o a_\gamma^o = (N^i - N_\gamma^i)a_\lambda^i + N_\gamma^i a_\gamma^i \quad (2)$$

For the sake of simplicity, we shall suppose, that the total numbers of molecules in the monolayers are identical: $N^o = N^i = N$

The minimum condition of the thermodynamic potential (1) gives the following expressions for phase transition temperatures in both monolayers:

$$T_c^o = \frac{(u_\lambda^o - u_\gamma^o) + P(v_\lambda^o - v_\gamma^o) - P(a_\lambda^o - a_\gamma^o) \frac{\partial v_\lambda^o}{\partial a_\lambda^o} - (a_\lambda^o - a_\gamma^o) \frac{\partial u_\lambda^o}{\partial a_\lambda^o}}{s_\lambda^o - s_\gamma^o - (a_\lambda^o - a_\gamma^o) \frac{\partial s_\lambda^o}{\partial a_\lambda^o}} \quad (3)$$

$$T_c^i = \frac{(u_\lambda^i - u_\gamma^i) + P(v_\lambda^i - v_\gamma^i) + P(a_\lambda^i - a_\gamma^i) \frac{\partial v_\lambda^i}{\partial a_\lambda^i} + (a_\lambda^i - a_\gamma^i) \frac{\partial u_\lambda^i}{\partial a_\lambda^i}}{s_\lambda^i - s_\gamma^i + (a_\lambda^i - a_\gamma^i) \frac{\partial s_\lambda^i}{\partial a_\lambda^i}} \quad (4)$$

and mechanical equilibrium equations

$$N_{\gamma}^o \left[\left(\frac{\partial u_{\gamma}^o}{\partial a_{\gamma}^o} - \frac{\partial u_{\lambda}^o}{\partial a_{\lambda}^o} \right) + P \left(\frac{\partial v_{\gamma}^o}{\partial a_{\gamma}^o} - \frac{\partial v_{\lambda}^o}{\partial a_{\lambda}^o} \right) - T \left(\frac{\partial s_{\gamma}^o}{\partial a_{\gamma}^o} - \frac{\partial s_{\lambda}^o}{\partial a_{\lambda}^o} \right) \right] = 0 \quad (5)$$

$$(N^i - N_{\gamma}^i) \left[\left(\frac{\partial u_{\gamma}^i}{\partial a_{\gamma}^i} + \frac{\partial u_{\lambda}^i}{\partial a_{\lambda}^i} \right) + P \left(\frac{\partial v_{\gamma}^i}{\partial a_{\gamma}^i} + \frac{\partial v_{\lambda}^i}{\partial a_{\lambda}^i} \right) - T \left(\frac{\partial s_{\gamma}^i}{\partial a_{\gamma}^i} + \frac{\partial s_{\lambda}^i}{\partial a_{\lambda}^i} \right) \right] = 0 \quad (6)$$

$$N_{\gamma}^i \left[\left(\frac{\partial u_{\gamma}^i}{\partial a_{\gamma}^i} + \frac{\partial u_{\lambda}^i}{\partial a_{\lambda}^i} \right) + P \left(\frac{\partial v_{\gamma}^i}{\partial a_{\gamma}^i} + \frac{\partial v_{\lambda}^i}{\partial a_{\lambda}^i} \right) - T \left(\frac{\partial s_{\gamma}^i}{\partial a_{\gamma}^i} + \frac{\partial s_{\lambda}^i}{\partial a_{\lambda}^i} \right) \right] = 0 \quad (7)$$

Equations 3—7 should be solved simultaneously with Eq. (2).

It follows from Eq. (3), (4) that the phase transition temperature is determined by the energy of intramembrane interaction, u , per one molecule. Consequently, if intermembrane interaction can influence the intramembrane one, a change in transition temperature will occur. A second remarkable feature of Eq. (3), (4) is the dependence of the transition temperature in one monolayer on the energy derivative $\partial u / \partial a$. This term is determined by the intramembrane mechanical interaction between two monolayers, which can influence the phase transition. This interaction is accounted for by equilibrium equations (5)—(7).

Let us suppose that the energy per molecule is the sum of u_0 , which is independent of the area per molecule, of the energy of electric interaction, w and of the molecular deformation elastic energy, η .

We shall suppose that entropy change is stepwise (Fig. 1), and consequently: $\partial s / \partial a = 0$.

We shall consider the phase transition dependence on electric and elastic intramembrane interactions separately, the latter being associated with the mechanical coupling of two membrane monolayers. We shall demonstrate the triggering effect of intermembrane interaction in this phenomenon.

*Shift in the phase transition temperature,
resulting from the adsorption of divalent cations.*

The phase transition in membranes from the liquid to crystalline phase state is accompanied by a decrease in the area occupied by one lipid molecule. As a result, the phase transition in the case of attraction between the molecules, should occur at a higher temperature than in the case of non-interacting molecules. We shall demonstrate that the adsorption of one divalent cation per two charged lipid

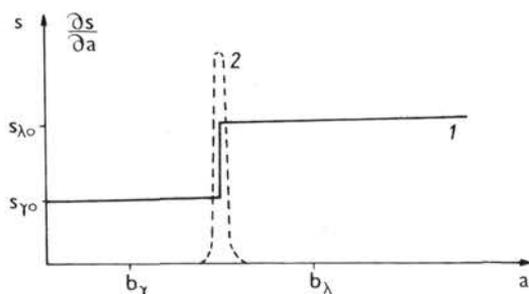


Fig. 1 The assumed dependence of the entropy s (curve 1) per particle on its area a , and the corresponding dependence of $\partial s/\partial a$ on the area a (curve 2).

molecules produces electric attraction in the system and consequently a major shift in transition temperature.

For the sake of simplicity, in this paragraph we shall neglect the effect of molecular deformations. One special case where this effect may be important is considered in Appendix A.

Transition temperature in the external monolayer can be obtained from Eq. (3):

$$T_c^o = T_{cn}^o + \frac{w_\lambda^o - w_\gamma^o}{s_{\lambda o} - s_{\gamma o}} + P \frac{v_\lambda^o - v_\gamma^o}{s_{\lambda o} - s_{\gamma o}}, \quad (8)$$

where $s_{\lambda o}$ and $s_{\gamma o}$ are the entropy per molecule in liquid and crystalline states, respectively; T_{cn}^o is the phase transition temperature in the external monolayer, corresponding to the case of a microscopically neutral membrane, i.e. a membrane consisting of neutral molecules. The respective result for the internal monolayer can be obtained from (8) by substituting "i" for "o".

It can be demonstrated that if the pressure is atmospheric, ($P=0.1$ kPa) the pressure comprising term in (8) can be neglected.

For further considerations it is necessary to define the electric energy per molecule, w . We shall consider two different cases. First, in the case of a charged membrane, one can demonstrate (Scott 1981) that

$$T_c^o = T_{cn}^o + 2 \frac{k T_{cn}^o}{s_{\lambda o} - s_{\gamma o}} \ln \frac{a_\gamma}{a_\lambda}. \quad (9)$$

The difference between the entropies, $s_{\lambda o} - s_{\gamma o}$, for a PS molecule is 77.3 J/mol K (Portis et al. 1979). Using the value of a_γ and a_λ equal to 0.48 nm² and 0.65 nm² respectively (Trauble and Eibl 1974) and putting $T_{cn}^o = 305$ K (Boggs 1980), we find, that $\Delta T_c = T_c - T_{cn}^o = -15$ K, which is in good agreement with the experimental data of -17 K for PS (Boggs 1980). Now let us consider a second case.

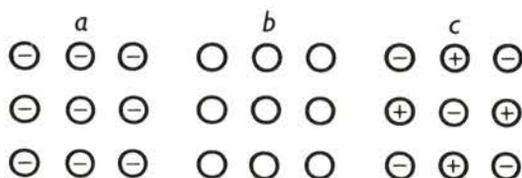


Fig. 2 The charge lattice of lipid molecules. (a) The initial membrane. (b) The membrane with adsorbed univalent cations which have compensated for the total charge. (c) The membrane with adsorbed divalent cations which have compensated for the total charge and produced a chess-board-type lattice.

Supposed there are divalent Ca^{2+} or Mg^{2+} cations in the membrane bathing solution capable of being adsorbed by the membrane, neutralizing its total surface charge. It may be inferred that after the complete neutralization half of the molecules will remain negatively charged and the other half (by which the divalent cations were adsorbed) will change the sign of their charge to the opposite one. (Analysis shows that this assumption leads to the lowest estimate for electrical energy). As a result a square lattice is obtained resembling a chess-board with staggered positive and negative charges (Fig. 2c). The energy of such a lattice of the chess-board type is determined by the known formula $(1/2)\sum\varphi_i e_o$, where e_o is the charge, and φ_i is the micropotential at the i -th site. The micropotential can be calculated by the improved cut-off disc method (Kozlov and Markin 1982), summing the potentials produced by the lattice of positive and negative charges (Appendix B). As a result

$$w \approx -7 \times 10^{-2} \frac{e_o^2}{\epsilon \epsilon_o \sqrt{a}} \quad (10)$$

is obtained.

Substituting Eq. 10 into Eq. 8 yields

$$T_c^c = T_{cn}^c + \frac{1}{s_{\lambda o} - s_{\gamma o}} \frac{0.07 e_o^2}{\epsilon \epsilon_o} \left(\frac{1}{\sqrt{a_\gamma}} - \frac{1}{\sqrt{a_\lambda}} \right) \quad (11)$$

A numerical estimate of the second term of Eq. 11 (on the right) shows that if the membrane with "chess-board" charges is exposed to the electrolyte solution ($\epsilon = 80$), a positive shift of the phase transition temperature results; for $a_\lambda = 0.65 \text{ nm}^2$, $a_\gamma = 0.48 \text{ nm}^2$ (Portis et al. 1979) and $s_{\lambda o} - s_{\gamma o} = 77.3 \text{ J/mol.K}$ (Portis et al. 1979) this shift is $\Delta T_c = 4 \text{ K}$.

Thus, if the charge of the PS membrane in contact with the electrolyte solution is neutralized by divalent cations, its transition temperature differs from T_c in a charged membrane by 19 K, this value is the sum of 15 K and 4 K, due to the neutralization of total charge and appearance of a lattice of the chessboard type, respectively.

Of particular interest is the case when the chess-board lattice resulting from the adsorption of divalent cations by a membrane, enters a dehydrated contact between membranes having a low dielectric constant (of about 2). In this case the shift of the transition temperature given by Eq. 11 approximately equals $\Delta T_c \approx 160$ K. This result explains the experimental data of Papahadjopoulos et al. (Portis et al. 1979; Düzgünes et al. 1981).

Non-isothermality of the phase transition

In this section the effect of mechanical intramembrane interaction between two monolayers of a closed liposome membrane on the phase transition shall be considered. We demonstrate that the coupling between the monolayers results in non-isothermality of the phase transition. It means that the phase transition does no longer occur abruptly and becomes "smeared" over a certain temperature interval.

It is necessary to postulate the expression for the energy of elastic deformation of the membrane. This energy per one lipid molecule is given by the formula analogous to the Hooke law (Israelachvili et al. 1977):

$$\eta = \Gamma \cdot \frac{(a-b)^2}{b}, \quad (12)$$

where b is the area per molecule in unstressed state (we shall call it equilibrium area); Γ is the proportionality coefficient (approximately 5×10^{-6} J/cm²) (Israelachvili et al. 1977).

The substituting of Eq. (12) into (3)—(7) gives the relationship between the numbers of molecules in the crystalline state in each monolayer, and the temperature:

$$T(N_\gamma^o) = T_{co}^o + \frac{\Gamma}{s_{\lambda o} - s_{\gamma o}} \cdot \frac{N_\gamma^i - N_\gamma^o}{N} \cdot \frac{(b_\lambda - b_\gamma)^2}{b_\lambda}, \quad (13)$$

$$T(N_\gamma^i) = T_{co}^i - \frac{\Gamma}{s_{\lambda o} - s_{\gamma o}} \cdot \frac{(N_\gamma^i - N_\gamma^o)}{N} \cdot \frac{(b_\lambda - b_\gamma)^2}{b_\lambda}. \quad (14)$$

Here, T_{co}^o and T_{co}^i are the transition temperatures in external and internal monolayers in unstressed state respectively. An analysis of expressions (13), (14) indicates, that if $T_{co}^o = T_{co}^i$, the phase transition in the membrane is isothermal (Fig. 4). Consider the case where $T_{co}^o \neq T_{co}^i$. This can take place, for example, if the intermembrane interaction mentioned above has changed the transition temperature of the external monolayer. It can also occur if the external and internal monolayers consist of different kinds of lipids. Moreover this effect can be expected even for monolayers of the same composition as they are bent in the opposite (in the topological sense) directions (Fig. 3). In all these cases the overall phase



Fig. 3 The opposite effect of the membrane curvature on molecules of the external and internal monolayers.

transition should unavoidably become non-isothermal. Assume for certainty that $T_{co}^o > T_{co}^i$ i.e., the crystallization of lipids in the external monolayer starts at higher temperatures as compared with the internal one; consequently, a situation is possible when all the molecules of the internal monolayer are in the liquid state, $N_{\gamma}^i = 0$, whereas some of the molecules of the external monolayer N_{γ}^o , have already gone over into the crystalline state (Fig. 4a). It is clear that in the outer monolayer the number of molecules in the crystalline state depends on temperature, T , since

$$N_{\gamma}^o = (T_{co}^o - T)N \frac{s_{\lambda o} - s_{\gamma o}}{\Gamma} \frac{b_{\lambda}}{(b_{\lambda} - b_{\gamma})^2}. \quad (15)$$

It follows from Eq. (15) that the phase transition is developing continuously as the temperature varies. When additional molecules in the external monolayer pass to crystalline state, the area of this monolayer tends to be reduced since the resistance of the internal monolayer is extended and that of the internal one compressed. This results in a decrease in and smearing of the transition temperature, T_c , in the external monolayer, and in an increase in and smearing of T_c in the internal one.

The temperature range in which the non-isothermal phase transition occurs depends on the relation between T_{co}^o and T_{co}^i (Fig. 4). Eq. (13), (14) suggest that if

$$T_{co}^o - T_{co}^i \geq \frac{2\Gamma}{(s_{\lambda o} - s_{\gamma o})} \frac{(b_{\lambda} - b_{\gamma})^2}{b_{\lambda}} \quad (16)$$

(for PS this would mean $T_{co}^o - T_{co}^i \geq 34$ K) the phase transition shall occur in two stages separated by a "silent" interval (Fig. 4a). As the temperature decreases, the crystallization of molecules of the external monolayer occurs first (Fig. 4a, α , β); the crystallization of molecules of the internal monolayer develops at lower temperatures (Fig. 4a, γ , δ). The "smearing" interval of the phase transition in each of the monolayers represents approximately 17 K for PS.

If the inequality (16) does not hold, the phase transitions in the monolayers partially overlap (Fig. 4 b—d). The Eq. (13), (14) show that in this case, at $T = (T_{co}^o - T_{co}^i)/2$, a discontinuous crystallization of molecules in both monolayers occur (Fig. 4c, δ , β). This result seems to correspond to experimental data (Dael et al. 1982).

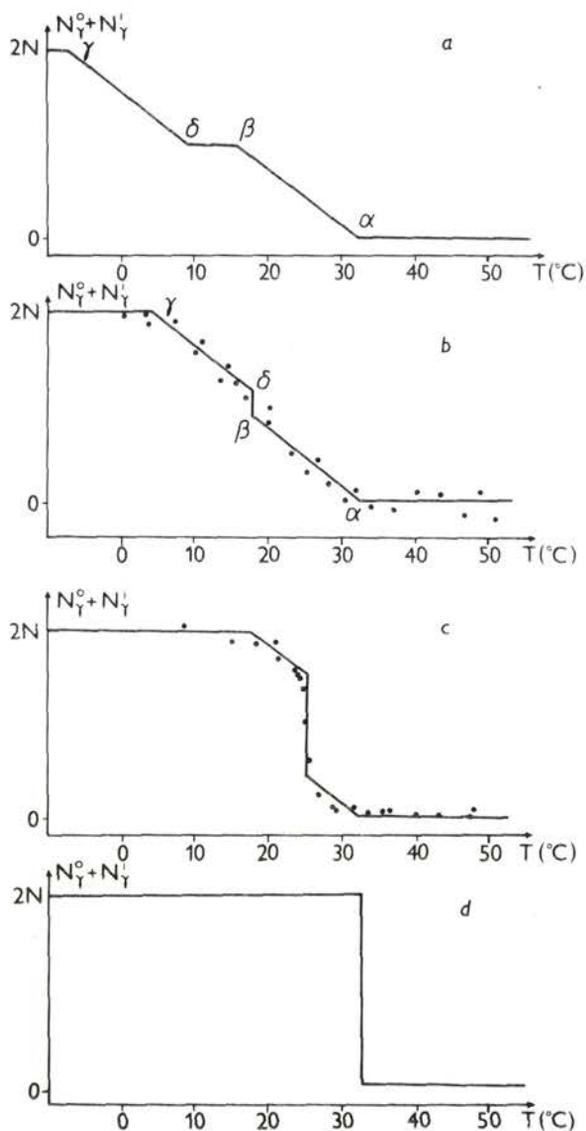


Fig. 4 The temperature dependence of the total number of particles in the crystalline state, $N_{\gamma}^c + N_{\gamma}^i$. The experimental data of Dael et al. (18) are denoted by points. The curves were plotted from Eq. (42), (43) on the assumption that $b_A = 0.65 \text{ nm}^2$, $s_{\lambda_0} - s_{\nu_0} = 56.3 \times 10^{-22} \text{ J/K}$ and $\Gamma = 5 \times 10^{-6} \text{ J/cm}^2$; $T_{\infty}^c = 32 \text{ K}$. (a) $T_{\infty}^c - T_{\infty}^i = 45 \text{ K}$. The segment $\alpha\beta$ corresponds to the crystallization of molecules of the external monolayer, and the segment $\gamma\delta$ to the crystallization of the internal monolayer, $\beta\delta$ is the "silent" interval. (b) $T_{\infty}^c - T_{\infty}^i = 30 \text{ K}$, (c) $T_{\infty}^c - T_{\infty}^i = 15 \text{ K}$. At $T = (T_{\infty}^c + T_{\infty}^i)/2$ a sudden change in $N_{\gamma}^c + N_{\gamma}^i$ occurs. (d) $T_{\infty}^c = T_{\infty}^i$. The phase transition is of isothermal nature.

Let us consider the dependence of the liposome heat capacity on temperature, as predicted by this theory. The expression for the amount of heat released during the crystallization:

$$dQ = T dS = T(s_{\lambda_0} - s_{\gamma_0}) \frac{d(N_{\gamma}^o + N_{\gamma}^i)}{dT} dT$$

gives for the heat capacity:

$$C_p = \frac{dQ}{dT} = T(s_{\lambda_0} - s_{\gamma_0}) \frac{d(N_{\gamma}^o + N_{\gamma}^i)}{dT} \quad (17)$$

The function $C_p(T)$, is illustrated in Fig. 5. The discontinuous character of the dependence $C_p(T)$ results from the assumption of a stepwise dependence of the entropy, s , per molecule on its area a . For the actual continuous dependence of s on a the curve for the heat capacity will be continuous (Fig. 5).

Discussion

The effect of inter- and intramembrane interactions on phase transitions in a bilayer lipid membrane was considered.

The first main result of the theory presented herein is the explanation of a drastic (in excess of 100 K) increase in the transition temperature in PS liposomes in the presence of Ca^{2+} ions (Portis et al. 1979). As demonstrated in the investigations of Papahadjopoulos et al. (Portis et al. 1979; Wilschut et al. 1980; Düzgünes et al. 1981) Ps membranes in the presence of Ca^{2+} ions (>1 mmol/l) form dehydrated contacts. It is reasonable to suppose that in the region of the contact the dielectric constant of the medium is small. If the ϵ in the contact region is close to 2, a strong intramembrane attraction between the molecules, forming a chess-board-type lattice of charges appear resulting in an increase in the transition temperature in the membrane by approximately 160 K.

It may be argued that Ca^{2+} ions are not directly adsorbed by the polar head of the lipid molecule and are located between two charges, forming "bridges" (Copeland and Andersen 1981a, b). In this case another lattice of charges is formed, which is somewhat different from that described above. In such a lattice, however, effective attraction similar to that discussed here will also exist, due to the presence of dissimilar charges.

The second main results of this theory is the new explanation of the non-isothermality of the phase transition in liposomes.

The geometrical coupling between monolayers in closed membranes results in a mechanical intramembrane interaction during the phase transition. The resulting elastic stresses in the bilayer produce the non-isothermality of the phase transition. An estimate has shown that the maximum temperature range within which the

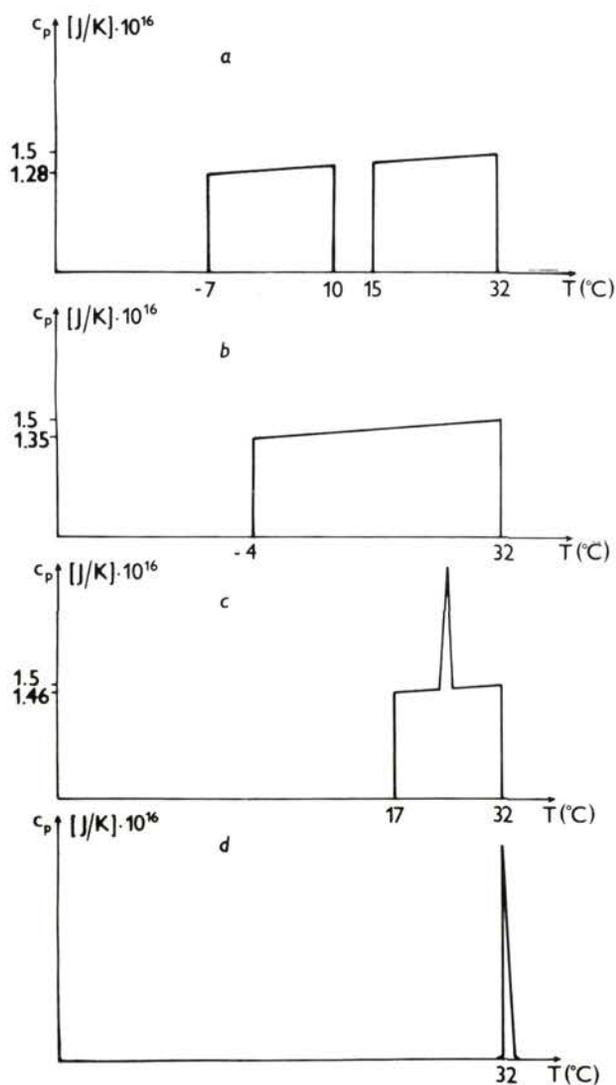


Fig. 5 The dependence of the liposome heat capacity on the temperature T plotted from Eq. (42), (43) and (47) on the assumption that $b_x = 0.65 \text{ nm}^2$, $b_y = 0.48 \text{ nm}^2$, $(s_{x0} - s_{y0}) = 56.3 \times 10 \text{ K}^{-1} \text{ J}$; $\Gamma = 5 \times 10^{-6} \text{ J/cm}^2$ and $N = 7700$, $T_{\infty}^0 = 32 \text{ K}$. (a) $T_{\infty}^0 - T_{\infty}^1 = 39 \text{ K}$. (b) $T_{\infty}^0 = 34 \text{ K}$. There is no temperature interval between the crystallization processes in the two monolayers. (c) $T_{\infty}^0 - T_{\infty}^1 = 15 \text{ K}$; (d) $T_{\infty}^0 = T_{\infty}^1$.

phase transition in one monolayer of the PS liposome may develop is approximately 17 K (Fig. 4).

This is in a good agreement with results of Dael et al. Fig. 4 illustrates the correspondence of our theoretical conclusions and the experimental data of Dael et al. It may be concluded that in terms of the present theory, the value of $T_{\infty}^{\circ} - T'_{\infty}$ equals 15 K and 30 K for large and small liposomes, respectively.

The present theory has put forward a number of questions. First of all, it would be interesting to calculate how the membrane curvature influences the phase transition temperature, T_{∞} . We shall investigate this question in a future paper.

According to our information, there are few papers on the non-isothermality of phase transition in membranes. It is worth to investigate in detail how the smearing interval of the phase transition depends on the liposome size. Another important question is the non-isothermality of the phase transition in liposomes having monolayers of different composition, because just this is the case in the cells. In a mixed membrane the phase transition in the inner and outer monolayer might be separated by a "silent" interval, as illustrated in Fig. 4a. It would be also interesting to check the calorimetric predictions of this theory presented in Fig. 5.

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Appendix A

Bilayer collapse

Consider a chess-board-type lattice of the charges of lipid molecules with adsorbed divalent cations (Fig. 2). In such a system the equilibrium equations (5)–(7) can be presented as follows:

$$\begin{aligned} \frac{0.04}{\Gamma} \frac{e_o^2}{\epsilon \epsilon_o} \frac{1}{(a_x)^{3/2}} + 2 \frac{a_x - b_x}{b} &= 0 \\ \frac{0.04}{\Gamma} \frac{e_o^2}{\epsilon \epsilon_o} \frac{1}{(a_y)^{3/2}} + 2 \frac{a_y - b_y}{b_y} &= 0 \end{aligned} \quad (\text{A1})$$

The contribution of the first term on the left of Eq. (A1) depends on the value of the dielectric constant.

At low ϵ the attraction between molecules in the lattice becomes appreciable.

An analysis shows that, if the elastic energy of molecules in the crystalline state is expressed by Eq. (12) and the dielectric constant of the medium is less than 20, the elastic forces cannot counter-balance the attraction between molecules leading unavoidably to membrane collapse. This means that the stability of the planar structures may be perturbed, and micelles may arise in the system.

In the present paper we have been aware that the elastic energy of particles

obeys the Hooke law (12) only at small deformations and that the elastic forces increase much more with a large compression of monolayers (Rand et al. 1980); this may result from the steric interaction between molecules, or from the repulsion of their dipole moments. This means that under the action of external forces the molecules can be compressed to a certain degree only. Beyond these limits an even very considerable force can cause only a slight change in the area. It follows (Rand et al. 1980) that the area per molecule cannot, in fact, change more than by 7–8 per cent. In this case the shift in the phase transition temperature is well described by expression (11).

Appendix B

The micropotential in the chess-board-type lattice

Consider a square lattice with staggered positive and negative charges equalling in modulus e_0 (Fig. 2c).

Let us find a micropotential produced at the point of residence of one charge (we choose it positive to be sure) by all other charges of the lattice. It is convenient to find the potentials produced by all positive and by all negative charges separately.

The potential produced by the lattice of positive charges will be sought by the modified cut-off method (Kozlov and Markin 1982) which consists in an exact summation of the charges, which are the nearest neighbours of the point considered, while the remaining charges are "smeared" and the integration is performed over them. As a result, we get

$$\varphi_+ = \frac{e_0}{\sqrt{2}\pi\epsilon\epsilon_0\rho} - \frac{e_0}{4\epsilon\epsilon_0} \frac{1}{\rho^2} \int_{\frac{\rho}{\sqrt{2}}}^{\infty} \frac{d\rho}{\sqrt{\frac{5}{\pi}\rho^2}} \quad (\text{B1})$$

where ρ is the distance between the charges in the chess-board type lattice (Fig. 2c).

The potential produced by negative charges is calculated by the same method:

$$\varphi_- = -\frac{e_0}{\pi\epsilon\epsilon_0} \frac{1}{\rho} - \frac{e_0}{4\epsilon\epsilon_0} \frac{1}{\rho^2} \int_{\frac{\rho}{\sqrt{2}}}^{\infty} \frac{d\rho}{\sqrt{\frac{4}{\pi}\rho^2}} \quad (\text{B2})$$

The addition of expression (B1) and (2) yields

$$\varphi = \varphi_+ + \varphi_- \approx -0.14 \frac{e_0}{\epsilon\epsilon_0\rho} \quad (\text{B3})$$

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