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PHASE TRANSITION IN CHARGED LIPID MEMBRANES

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Summary

Experimental results on the effect of electrostatics on bilayer phase transitions are compared with corresponding data for monolayers and the predictions of electrical double layer theory. The two substantial conclusions which emerge are that: (i) double layer theory based on a continuous surface charge distribution cannot explain all the relevant data, a situation which may be improved by taking into account the discrete nature of the surface charge distribution; (ii) the crystal - liquid crystal phase transition of charged bilayer membranes is always a continuous one which takes place through an intermediate state consisting of both fluid and frozen domains.

Introduction

The physical state of bilayer membranes is affected by electrostatic effects at the membrane surface. Träuble and Eibl [1] have measured the effect of changing pH and electrolyte concentration on the membrane phase transition temperature. As a general rule, it is found that the transition temperature is lowered for membranes with higher surface charge. Later experiments [2,3,4] provide most of the information presently available on the phase transition of charged lipid bilayers.

Clearly a change in bulk pH or electrolyte concentration will alter the electrical double layer free energy which in turn affects the statistical mechanical state of charged bilayers. This fact was exploited in analyses of the data, contained in the original report [1] and in subsequent refinements [4,5]. The free energy of a continuous electrical double layer was calculated and the resulting shift of the transition temperature was found to be in excellent agreement with experiment.

Despite this agreement, the problem is a much more complicated one. For, while the shift in transition temperature is easily explained by inclusion of the simple double layer free energy in the membrane description, such a theory cannot explain observed changes in the transition entropy. Results [2,3] of differential scanning calorimetry show that for charged bilayers the transition entropy is dramatically decreased. At the same time the transition itself becomes blurred.

More information on electrostatic effects on the membrane surface properties is available from corresponding studies on monolayers. Although such experiments provide the full functional dependence of the pressure-area curves instead of a single quantity ΔT_c (shift of transition temperature) they have never been correlated with the bilayer studies to yield a unified picture. In this paper we shall draw on all of the available experimental information to elucidate the nature of both the phase transition and of contributions to the electrostatic free energy of charged lipid membranes. The important departure from and extension of earlier theoretical analyses is that the electrostatic free energy is built into the complete mean field molecular partition function for the membrane instead of treating electrostatic head-group interactions as a perturbation of the hydrocarbon tail free energy.

1. Electrostatic free energy of the membrane

(i) Continuous surface charge

Before constructing the partition function for the whole assembly, it is necessary to have an expression for the electrostatic free energy per lipid molecule. We deal first with the case that the membrane surface charge is approximated by a continuum and recapitulate known results. Consider a planar membrane composed of a mixture of acidic lipids whose dissociation is described by the relation $AH \rightleftharpoons A^- + H^+$. The membrane is in equilibrium with a monovalent electrolyte solution at concentration n_1 molecules/ml and a prescribed pH. Γ, surface excess of A^- molecules per unit area; Γ_A, corresponding number of $AH + A^-$ molecules per unit area. The free energy per unit area, f, can then be written as

$$f = e \int_0^{\psi_0} \Gamma(\psi_0') \, d\psi_0' + \Gamma_A kT \ln\left(\frac{\Gamma_A - \Gamma}{\Gamma_A}\right) \tag{1}$$

Here ψ_0 is the surface potential, e, unit charge, k, Boltzmann's constant and T the temperature. This result was first derived by Payens [6] and has recently been rederived [4,5]. A rigorous derivation of Eqn. 1 is given elsewhere [7]. Straightforward integration of the non-linear Poisson-Boltzmann equation yields

$$\Gamma = -\left(\frac{2n_1 \epsilon kT}{\pi e^2}\right)^{1/2} \sinh\left(\frac{e\psi_0}{2kT}\right) \tag{2}$$

where ϵ is the dielectric constant of water. To complete the problem a further relation between Γ and ψ_0 is required. This follows from the condition for dissociation equilibrium

$$\frac{[A^-][H^+]}{[AH]} = K \tag{3}$$

where K is the dissociation constant and $[H^+]$ is the surface hydrogen ion concentration. In our notation Eqn. 3 becomes

$$\frac{\Gamma n_{H^+}\, e^{-e\psi_0/kT}}{\Gamma_A - \Gamma} = K \tag{4}$$

where n_{H^+} is the bulk hydrogen ion concentration. Together Eqns. 2 and 4 can be solved for ψ_0 and Γ. The result can then be substituted into Eqn. 1 to yield the free energy. The electrostatic contribution to the surface pressure Π_{el} can also be obtained [6] as:

$$\Pi_{el} = -e \int_0^{\psi_0} \Gamma(\psi_0')\, d\psi_0' \tag{5}$$

and in view of Eqn. 2 this becomes:

$$\Pi_{el} = 2 \times \left(\frac{2(kT)^3 n_1 \epsilon}{\pi e^2}\right)^{1/2} \cosh\left(\frac{e\psi_0}{2kT}\right) - 1 \tag{6}$$

This electrostatic pressure is plotted against area per lipid in Fig. 1. Because of the nature of the model, surface pressure contributions are additive, and these results can be compared directly with the measured pH dependence of monolayer Π-A data [8] [cf. inset Fig. 1]. One discrepancy is immediately obvious: the data show that for areas larger than about 50 Å^2 per molecule, surface pressure is independent of pH. We return to this point later.

At smaller areas per molecule, the predicted pH dependence is in qualitative agreement with observation [8]. Since surface pressure increases with increasing dissociation at smaller areas per molecule, the transition between the fluid

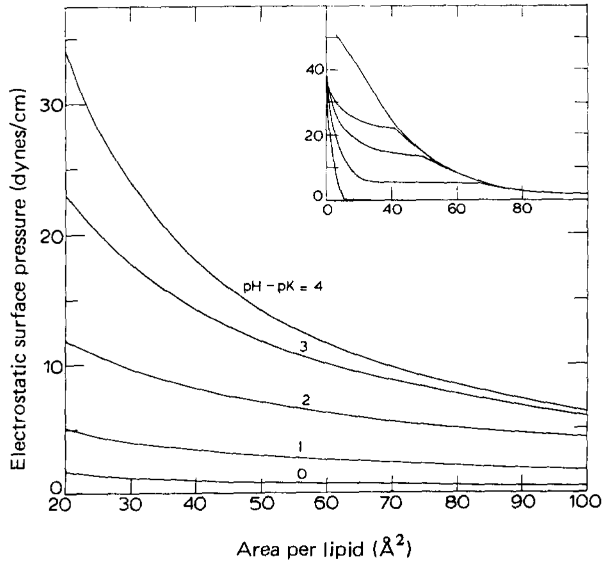


Fig. 1. Electrostatic contribution to monolayer surface pressure computed for the continuous double layer at different values of pH — pK. The inset to the figure shows typical behaviour of experimental pressure-area data for a wide range of subphase pH values (stearic acid, after ref. 8). In both Fig. 1 and Fig. 2, electrolyte is univalent at concentration 0.1 M and temperature is 300 K.

and the crystalline phase becomes less pronounced at higher pH values. This fact corresponds with the decrease in transition entropy observed for lipid bilayers at higher pH values [2,3]. As already remarked, the monolayer data are here very useful. Instead of the single number available in the membrane case, the complete form of the Π-A curves is available.

(ii) Application to study of the phase transition

The form of the electrostatic free energy when the surface charges are replaced by a continuum has now been established. Bearing in mind the limitations of this approximation in the regime of high areas per head-group, we can proceed to a preliminary study of the phase transition. Comparison with observation on charged bilayer membranes will then provide a clue to the nature of the actual phase transition and a guide towards the more complicated model explored in section 2.

As a basis we use a model for hydrocarbon chain ordering introduced by one of us and described in detail in several recent publications [9—11]. This model treats exactly all conformations of a single hydrocarbon chain and uses a mean field approximation to describe the interaction between neighbouring chains. For uncharged hydrocarbon molecules [10] the model gives an excellent description of the thermodynamic properties. The approximation scheme is easily generalised to include the electrical double layer free energy. This energy depends on the area occupied by the molecule, and in mean field approximation we have in the notation of refs. 9—11

$$E(\Phi, P) = E_{\mathrm{int}} + E_{\mathrm{ch\text{-}ch}}(\Phi) + PA + f(A) \tag{7}$$

$$Z = \sum_{\substack{\text{all chain}\\ \text{configurations}}} \exp\left(-\frac{E(\Phi, P)}{kT}\right) \tag{8}$$

The first three terms in Eqn. 7 are not changed from previous work [9], and describe respectively the internal energy of a chain, chain-chain interaction and the effect of lateral pressure. The last term is the double layer free energy per molecule. The free energy, f, rather than other thermodynamic potentials is the appropriate term which enters into Eqn. 7. This is because the extensive thermodynamic variables specified in calculating the partition function, Z, are surface number, area per molecule, and temperature, and the system is in equilibrium with a bulk electrolyte at a fixed chemical potential. This specification is implicit in Eqn. 8, since the summation runs over chain conformations only.

Details of the computation are involved and are as in refs. 9—11. The computation produces shifts in transition temperatures which are in good agreement with previous work [4]. This is satisfying, as the earlier work was based on the approximation that the electrostatic effects can be treated as a perturbation of the hydrocarbon tail free energy. However as expected from the argument presented in Fig. 1, use of the continuous electrical double free energy Eqn. 1 merely shifts the phase transition without predicting a decrease in transition entropy.

(iii) Effect of discrete surface charges

We now argue that the problem of decreasing transition entropy with increasing pH can be resolved if the discrete nature of the surface charges is taken into account.

It is reasonable to expect that when the Debye screening length $\kappa^{-1} = \sqrt{(8\pi n_1 e^2/\epsilon kT)}$ is less than the average distance between A^- groups, i.e. $\kappa\Gamma^{-1/2} > 1$, then discrete charge effects should become important and the continuum approximation to the electrostatic free energy should begin to break down. (Typically, for 0.1 M univalent electrolyte solutions we have $\kappa^{-1} \approx 10$ Å. while for both monolayers and bilayers in the transition region we have 40 Å$^2 < \Gamma <$ 60 Å^2. Thus $\kappa\Gamma^{-1/2}$ is indeed of the order of unity and discrete charge effects may be expected to play a significant role.) To obtain some idea of the order of magnitude of discrete charge effects, consider first a system of point charges on a square lattice at the membrane surface. A square lattice is chosen for mathematical convenience only. Provided the dielectric constant of the solution ($\epsilon \cong 80$) is much greater than that of the membrane ($\epsilon \cong 2$), and if the Poisson-Boltzmann equation is linearised, then the equation for the electrostatic pressure which corresponds to Eqn. 5 can easily be shown to be [7]

$$\Pi_{\mathrm{el}} = \frac{e^2\Gamma^{3/2}}{\epsilon} \sum_{l,m=-\infty}^{\infty}{}' \frac{\exp[-\kappa(l^2 + m^2/\Gamma)^{1/2}]}{\sqrt{l^2 + m^2}}, \tag{9}$$

where the prime on the summation symbol indicates that the term in $l = m = 0$ is omitted. The charge density Γ is determined by Eqn. 2, where now the potential ψ_0 is given by

$$\psi_0 = -\frac{2e}{\epsilon}\Gamma^{1/2} \sum_{l,m=-\infty}^{\infty}{}' \frac{\exp[-\kappa(l^2 + m^2/\Gamma)^{1/2}]}{\sqrt{l^2 + m^2}} \tag{10}$$

This is the potential at a given charge due to all the other charges of the lattice as screened by the electrolyte. From Eqn. 9 we see that if $\kappa\Gamma^{-1/2} \gg 1$, then the electrostatic pressure will be exponentially damped at high surface areas per head-group. Although in the domain of very high surface areas linearisation of the Poisson-Boltzmann equation is surely a valid approximation, it can be shown that for the continuum limit the linearised and non-linearised forms for the pressure differ by a factor of up to 2 or 3 at surface areas of < 40 Å^2 for $\kappa^{-1} \approx 10$ Å. [The linearised form gives the larger pressure.] Consequently we expect that for the discrete charge case, the pressure Π_{el} Eqn. 9 obtained by linearisation will also be in error and that numerical results based on Eqns. 2—5 are only qualitatively correct. The non-linear discrete case is very difficult to solve, and in any event for a complete solution of the problem a self-consistent specification of the pair distribution function of head groups is required.

Nonetheless, the simple lattice problem does demonstrate the essential point, that discrete effects will act to make Π_{el} independent of pH at large areas as is experimentally observed for monolayers (Fig. 1). Likewise, for discrete charges distributed on a lattice the electrostatic free energy will decrease exponentially with increasing area. Of course, the charge distribution in real membranes is neither completely random (where the continuum results apply) nor is it completely ordered. However, and in summary, this example strongly suggests that the negligible effect of pH variation on expanded monolayers [8] is at least partly attributable to the discrete nature of the surface charge. Once this is accepted, the reasons for change in transition entropy with pH for bilayers is clear. We shall not attempt to set up the inordinately more complicated generalisation of the model embodied in Eqns. 7 and 8 required to take account of discrete charge effects. The well-known deficiencies of the Poisson-Boltzmann equation make precise numerical predictions suspect. Further, even if good numerical estimates of transition entropies were obtained, the phase transition would be sharp and this is contrary to observation. We now address this problem.

2. Gradual phase transition: the Lαβ phase

The data from differential scanning calorimetry for dipalmitoyl-phosphatidylserine at higher pH clearly show a gradual crystal-liquid phase transition. Such a transition has been described in some detail before. Using X-ray scattering, Ranck et al. [12] have found that for phosphatidic acid the transition between the lamellar liquid crystal phase (Lα in the notation of ref. 12) and the lamellar phase (Lβ) is gradual. Similar conclusions follow from spin-label experiments [13]. The conclusion of refs. 12 and 13 is that during the gradual transition small domains of both Lα and Lβ phases exist in equilibrium. This new mixed phase is termed Lαβ. We show here that the competition between electrical double layer free energy and the boundary free energy associated with the formation of microdomains does indeed favour a gradual Lα—Lβ transition in charged membranes, as opposed to a spontaneous separation into two macroscopic phases.

The electrical double layer free energy is lower in the fluid phase, and this contribution shifts the phase transition of charged bilayers to a lower temperature. When the temperature is decreased to the new transition point, the bilayer does not suddenly freeze. Instead, a mixed state consisting of both fluid and frozen domains is formed. We shall consider a mixed state and estimate the decrease in double layer free energy and increase in the hydrocarbon chain free energy as compared to the free energy in the uniform phases. The results show that the formation of the mixed phase Lαβ is favourable.

In order to make an estimate of the decrease in free energy due to domain formation without undue mathematical complexity, we shall make the following approximations. The lipids will be assumed to be fully ionised, i.e. $\Gamma \to \Gamma_A$, and the Poisson-Boltzmann equation will be linearised. Making these approximations in Eqn. 1 we obtain:

$$f = \tfrac{1}{2} e\Gamma \psi_0 - kT\Gamma\{2.303(\mathrm{pH} - \mathrm{p}K)\} \tag{11}$$

Note that the first term of Eqn. 11 is positive. This point is discussed in more detail in refs. 4 and 5.

In order to determine the difference in free energy between the domain state and one where complete phase separation has taken place, it is sufficient to consider only the first term of Eqn. 11. The second term is irrelevant since the average charge density is the same for both states. Consequently, the energy per lipid of the phase separated state E_s is:

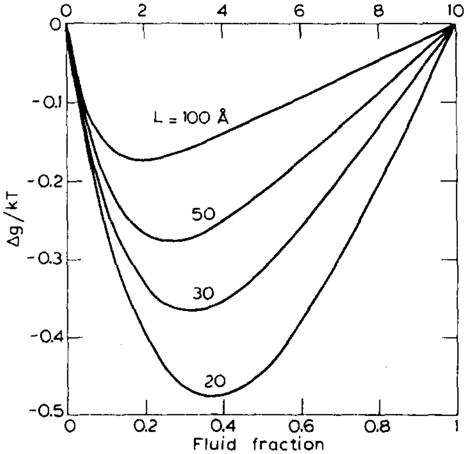
$$E_s = \frac{2\pi e^2}{\kappa\epsilon}\left(\frac{(1-c)}{A_1} + \frac{c}{A_2}\right) \tag{12}$$

Here κ^{-1} is the Debye screening length, A_1 is the mean area per frozen lipid, A_2 is the mean area per fluid lipid, and c is the fraction of fluid lipids.

Consider a membrane in the phase L$\alpha\beta$, where the frozen lipids are in square patches of side L, surrounded by fluid lipids. The patches themselves are on a square lattice. (A square patch is chosen only for computational convenience.) The expression for the energy per lipid of the mixed state, E_d, is quite complicated, and is derived in the Appendix. In Fig. 2 the gain in electrostatic free energy of domain formation $\Delta g = (E_s - E_d)/kT$ at T = 300 K is plotted as a function of the fluid fraction c for various values of L.

To estimate the hydrocarbon chain free energy in the domain state, a definite spatial distribution of frozen and fluid domains is assumed. The order parameter of lipid molecules forming the core of the domain is kept fixed (see Fig. 3), while the order parameter between the domain cores is computed following closely the method described in ref. 11. The computation of thermodynamic functions is then straightforward, and as a final result the average increase in free energy per lipid molecule is obtained.

Throughout the computation the electrical double layer free energy Eqn. 1 with (pH — pK) = 5 (almost completely ionized lipids) has been added as another contribution to the energy of a lipid molecule. Assuming no domain formation and a chain length of 12 carbons, this results in a decrease of the transition temperature from 25.2°C to 1.4°C. Except for this shift in the transition



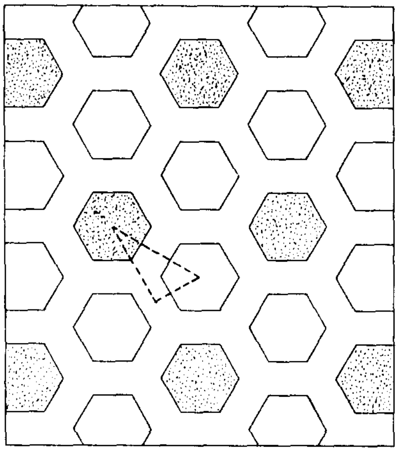


Fig. 2. Decrease in electrostatic free energy associated with the formation of the intermediate state L. Domain size is shown in the figure.

Fig. 3. Illustration of the method of computation of hydrocarbon chain free energy. Solid and fluid domain cores are respectively shown as open and shaded hexagons. The order is computed self-consistently in the region between the hexagons. The lattice may be expanded to vary the fraction of fluid chains. Due to the symmetry of the problem, the actual computation is only carried out for the triangular region shown by dashed lines.

TABLE I

FREE ENERGY OF THE SYSTEM USING FRACTION OF CHAINS IN FLUID STATE 0.67

Temperature (°C)	Excess free energy per lipid (kT)
5	0.03
3	0.04
1	0.07
−1	0.10
−3	0.12

temperature, the energy of domain formation is almost independent of this additional term. The free energy of the system with different number and size of fluid domains at different temperatures has been examined. Some typical results are shown in Tables I and II. It should be noted that, since model calculations are done with chain length of 12 carbons (calculated transition entropy 6.2 kcal/mol), to scale the results to chain length of 16 carbons all the values in Tables I and II should roughly be doubled. Because the change of order between fluid and frozen domains is continuous, the definition of the fraction of chains in the fluid state is not unique. The values in Table II are obtained by defining the fluid fraction c through the average order parameter $\bar{\eta}$

$$c = \frac{(\eta_{\text{cryst.}} - \bar{\eta})}{(\eta_{\text{cryst.}} - \eta_{\text{fluid}})}$$

Comparing the values in Fig. 2 and Tables I and II, we conclude that the formation of the domain state L$\alpha\beta$ is favoured. It should be noted that linearisation of the Poisson-Boltzmann equation results in an over-estimate of the continuum electrostatic free energy by a factor of, at most, 2. Neglect of discrete charge effects have given a further minor source of over-estimate in the regime of interest also. Further for the more usual situation involving lipid chain length of 16 carbon atoms, the hydrocarbon free energy should be roughly doubled. When these sources of error are taken into account the conclusion still appears to be valid. We conclude that Lα-Lβ phase transition of charged lipid bilayers is therefore never a one-step, sharp phase change. Even for bilayers composed of a single lipid species, such a transition proceeds gradually, through the formation of the intermediate domain state L$\alpha\beta$.

TABLE II

FREE ENERGY OF THE SYSTEM AT 1°C

Fraction of chains in fluid state	Excess free energy per lipid (kT)
0.07	0.007
0.17	0.017
0.33	0.056
0.67	0.066
0.83	0.026
0.91	0.016

Appendix

In this appendix the expression for the energy per lipid of the domain state, E_d, is derived.

Consider a planar membrane on the plane $z = 0$. Then the potential $\psi(\vec{r})$ must satisfy the linearised Poisson-Boltzmann equation, viz.:

$$\nabla^2\psi - \kappa^2\psi = 0 \tag{A.1}$$

with the boundary condition:

$$\left.\frac{d\psi}{dz}\right|_{z=0} = -\frac{4\pi\sigma(\vec{\rho})}{\epsilon}, \tag{A.2}$$

where σ is the surface charge density and $\vec{\rho}$ is a two-dimensional vector in the x-y plane. Let $\sigma(\vec{\rho})$, and therefore $\psi(\vec{\rho}, z)$ be periodic functions in the z-y plane, with reciprocal vectors $\vec{q}$; then

$$\psi(\vec{\rho}, z) = \sum_{\vec{q}} \tilde{\psi}(\vec{q}, z)\, e^{i\vec{q}\cdot\vec{\rho}}$$

$$\sigma(\vec{\rho}) = \sum_{\vec{q}} \hat{\sigma}(\vec{\rho})\, e^{i\vec{q}\cdot\vec{\rho}} \tag{A.3}$$

Consequently, in view of Eqns. A.1—A.3:

$$\psi(\vec{\rho}, 0) = \frac{4\pi}{\epsilon} \sum_{\vec{q}} \frac{\hat{\sigma}(\vec{q})\, e^{i\vec{q}\cdot\vec{\rho}}}{(\kappa^2 + q^2)^{1/2}}$$

The energy per unit cell is then given by:

$$\frac{1}{2}\int_{\text{cell}} \sigma(\vec{\rho})\, \psi(\vec{\rho}, \text{o})\, d\vec{\rho} = \frac{2\pi}{\epsilon}\text{Area}_{\text{cell}} \sum_{\vec{q}} \frac{[\hat{\sigma}(\vec{q})]^2}{(\kappa^2 + q^2)^{1/2}} \tag{A.4}$$

Let each unit cell consist of a square patch of frozen lipid of side L, surrounded by fluid lipids; the entire cell being a square of side a, then:

$$E_d = \frac{e^2 L}{A_1}\,\frac{2\pi}{\epsilon}\left(\frac{q}{R}\right)^2 \frac{1}{\left(1 + \frac{A_1}{A_2}\left(\left(\frac{a}{L}\right)^2 - 1\right)\right)} \sum_{l,n=-\infty}^{\infty} \frac{[I(l, n)]^2}{[(\kappa a)^2 + 4\pi^2(l^2 + n^2)]^{1/2}}, \tag{A.4}$$

where $I(l, n)$ is defined by

$$\begin{aligned} I(l, n) &= 4\left(1 - \frac{A_1}{A_2}\right)\frac{\sin(n\pi L/a)}{2n\pi}\,\frac{\sin(l\pi L/a)}{2l\pi} && l \neq 0, n \neq 0,\\ &= 4\left(\frac{L}{2a}\right)\left(1 - \frac{A_1}{A_2}\right)\frac{\sin(l\pi L/a)}{2l\pi} && l \neq 0, n = 0,\\ &= \left(1 - \frac{A_1}{A_2}\right)\left(\frac{L}{a}\right)^2 + \frac{A_1}{A_2} && l = 0, n = 0. \end{aligned} \tag{A.5}$$

Eqns. A.4 and A.5 were used in obtaining the results shown in Fig. 2.

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