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LATERAL COMPRESSIBILITY OF LIPID MONO- AND BILAYERS

THEORY OF MEMBRANE PERMEABILITY

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Summary

The passive sodium permeability of pure lipid vesicles and dispersions has a large peak at the bilayer phase transition temperature. We discuss this anomaly in terms of density fluctuations, which can open up cavities in the headgroup region into which small ions can enter, and which may be large if bilayer conditions at the melting point are similar to those near the critical point which seems to exist in monolayers. We present two arguments, one thermodynamic and one microscopic, which suggest that the permeability is proportional to the lateral compressibility. We then calculate the lateral compressibility for two previously published theoretical models and compare the results with experiment.

Introduction

Although the permeability of biomembranes is largely dominated by specialized gates and pores associated with protein [1], the passive permeability of pure lipid bilayers is of interest insofar as it provides insight into the structure of the lipid components in biomembranes. The particular phenomenon that we think is especially worthy of attention is the anomalous peak in the permeability of sodium ions, P_{Na^+}, as a function of temperature at the main phase transition in pure lipids [2], shown in Fig. 1. This is significant because the obvious a priori guess would have been that P_{Na^+} should simply have a discontinuous increase as T is raised through the first order phase transition.

The anomalous peak in the permeability has been attributed to the formation of "domains" in bilayers, with enhanced diffusion occurring at the domain boundaries [2]. Taken literally, this explanation cannot be correct for pure single component lipids. The presence of domains implies three phase coexistence of fluid and solid bilayers as well as of excess water. By the Gibbs phase

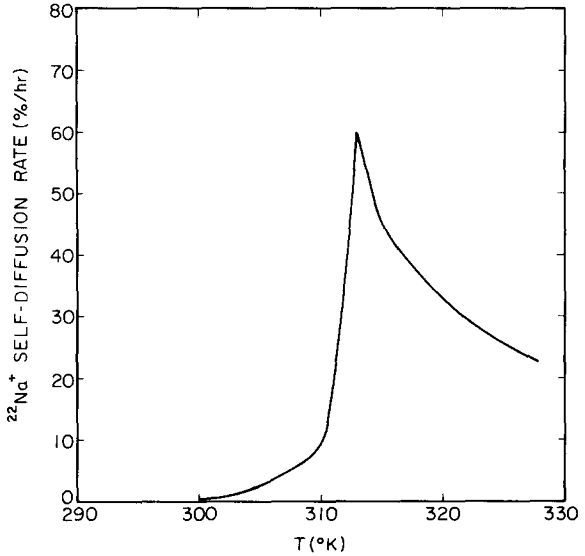

Fig. 1. A plot of the experimental data of Papahadjopoulos et al. [2] for self-diffusion of $^{22}Na^+$ from unsonicated vesicles of dipalmitoyl phosphatidylglycerol vs. temperature. For sonicated vesicles the curve is wider and not as sharp. Error bars are not shown here.

rule the transition at constant pressure and surface pressure must then occur at a single temperature. Although the actual transition is broadened, perhaps due to limited cooperativity [3], the width in pure lipids as measured by calorimetry and dilatometry is less than a degree [3,4], whereas the sodium permeability peak is spread over a 10° range. Some of the lipids in ref. 2 had about 6% impurities in the form of hydrocarbon chain lengths differing by two. From studies on the phase diagram of mixed phosphatidylcholines [5] such mixtures introduce a two phase region, but only over a temperature interval of about one degree, so the domain picture cannot account for the permeability peak.

A different suggestion is that the anomalous peak is due to enhanced fluctuations near the transition [6,7,8]. Systems undergoing first order transitions do not necessarily have enhanced fluctuations in the single phase regions unless they are near a critical point. According to several studies, lipid monolayers appear to have a critical point [10,11,12]. Comparison of monolayers and bilayers suggest that as temperature is varied the thermodynamic state of bilayers may come close to the critical point in monolayers [9,10]. In order to precisely achieve the critical state bilayers would require the application of a lateral pressure which has not been accomplished in the laboratory. Nevertheless, the proximity of the transition state in bilayers to the critical point in monolayers suggests that fluctuations may be responsible for the increased permeability in bilayers. Furthermore, the particular fluctuations will be fluctuations in lateral density associated with high lateral compressibility, as measured, for example, in lipid monolayers [10,11,12].

A rough picture is that lateral density fluctuations open short-lived cavities in the head group region of the bilayer into which ions enter. When the cavity closes, the ions are then trapped in the hydrocarbon region of the bilayer across which they can diffuse. We believe that this latter diffusion process is not normally the rate limiting process; in particular, the correlation functions relevant to this process computed by G.R. Allen and J.F. Nagle (unpublished) from Nagle's model, as well as those computed from Scott's model [13], do not show anomalous increases near the transition. In the present picture the rate-limiting process is the entry of the ions into the bilayer, which is facilitated when the lateral compressibility is high. However, sufficiently near a critical point this entry process could become fast enogh for the diffusion of ions across the hydrocarbon region to become rate limiting, thereby providing a cut-off to the maximum value of P_{Na^+}.

There are two objectives to this paper. The first is to find a quantitative relationship between P_{Na^+} and the two dimensional isothermal lateral compressibility κ_T. The second is to calculate the lateral compressibility for two models of lipid bilayers and to compare the calculated κ_T to the experimental P_{Na^+}.

I. Relation between bilayer permeability and lateral compressibility

The conclusion of this section is that the sodium permeability P_{Na^+} should be roughly proportional to the isothermal lateral compressibility

$$\kappa_T = (-1/A)(\partial A/\partial \pi)_T \tag{1}$$

where A is the surface area/molecule and π is the surface pressure. Such a relation can be established in several ways. Let us employ an old thermodynamic construct used to establish the stability relation $\kappa_T \geqslant 0$ [14]. Consider a particularly simple spontaneous fluctuation in which the area of half the molecules increases by ΔA and the area of the other half decreases by ΔA. The free energy F_f of such an incipiently phase-separated state compared to the free energy of the homogeneous equilibrium state F_0 is

$$F_f = F_0 + (\tfrac{1}{2})(\partial^2 F/\partial A^2)(\Delta A)^2 + \text{higher order terms}\,. \tag{2}$$

Thus, from the definition of κ_T

$$\Delta F = F_f - F_0 = (\tfrac{1}{2})(\Delta A)^2/A\kappa_T\,. \tag{3}$$

The probability of such a fluctuation is proportional to $\exp(-\Delta F/kT)$. Thus, as κ_T becomes larger the system will support with equal probability larger fluctuations ΔA according to the scaling relation

$$(\Delta A)^2 \propto \kappa_T\,. \tag{4}$$

Next, we relate the permeability, P_{Na^+}, phenomenologically to the area by a series expansion about the area A_0

$$P_{Na^+} = C_0 + C_1\Delta A + C_2(\Delta A)^2 + \text{higher order terms}\,. \tag{5}$$

where C_0 is the permeability of the homogeneous state with uniform area A_0 with $\Delta A = 0$. Increasing A_0 results in a similar expansion to Eqn. 5 about the new A_0, but with slightly altered C_1 values. One may suppose that both C_1 and C_2 are positive, so C_0 of a homogeneous state with uniform area/molecule increases as A_0 increases. This gives a "normal" increase in permeability at T, and therefore A increases through the transition. In contrast for the fluctuating state, P_{Na^+} is the average of the two incipient phases, which have ΔA values of opposite sign in Eqn. 5, thereby giving

$$P_{\mathrm{Na,f}} = C_0 + C_2(\Delta A)^2 = C_0 + C_2'\kappa_{\mathrm{T}} \, . \tag{6}$$

This derivation is easily generalized to include an ensemble of fluctuations. Such a generalization gives the same result, namely Eqn. 6. The first term in Eqn. 6 increases monotonically with average area A_0. The anomalous part of the permeability is the second term, proportional to κ_{T}, which becomes large near a critical point, as will be shown in the next section.

A more microscopic examination uses the fluctuation theorem to relate the lateral compressibility to the pair correlation function $g(r)$

$$\kappa_{\mathrm{T}} \propto 1 + \int_0^{\infty} g(r)\,\mathrm{d}r \propto (\Delta A/A)^2 \tag{7}$$

Near a critical point $g(r)$ goes to zero more slowly with increasing r and this leads to large κ_{T}. Typically,

$$g(r) \propto \mathrm{e}^{-r/\xi} \tag{8}$$

for large r and the correlation length ξ, which is the measure of the range of the correlations, increases near the critical point. This formulation reveals a point of some concern. It is not apparent that a few very large regions of differing area ΔA (the large ξ regime) facilitates P_{Na^+} more than a large number of smaller regions with the same area difference ΔA (the smaller ξ regime) so long as the low density cavities are large enough to accomodate the ion i.e. $\xi > b$ were b is related to ionic diameter. We shall take $b = 40$ Å as a sufficiently large cavity that edge effects are negligible. This suggests that it might be more relevant to restrict one's attention to short range correlation functions, and that one should have

$$P_{\mathrm{Na}^+} \propto 1 + \int_0^{b} g(r)\,\mathrm{d}r \, . \tag{9}$$

Such a consideration is very important in the anomalous transport of electrons in Ni near the critical point [15]. We now argue that $b = 40$ Å is large enough for lipid bilayers so that there is little difference between Eqns. 7 and 9 unless one is closer to T_{c} than is likely to be the case for lipid bilayers.

To show the effect of a small deviation from T_{c} let us consider the Ornstein-Zernike expression for $G(r)$ [16] for a two dimensional system

$$G(r) = \frac{C\,\mathrm{e}^{-r/\xi}}{r^{1/2}} \tag{10}$$

where C is a constant. The "classical" theory of critical phenomena requires that ξ have the form [17]

$$\frac{1}{\xi} \approx \frac{\left|\dfrac{T - T_{\mathrm{c}}}{T_{\mathrm{c}}}\right|}{\xi_0} \rightarrow \frac{\xi}{\xi_0} \approx \left|\frac{T - T_{\mathrm{c}}}{T_{\mathrm{c}}}\right|^{-1/2} \tag{11}$$

where ξ_o is a characteristic length for the system. Thus, in this approximation the range of the correlations is given by ξ. For this range to be 40 Å, taking the interparticle distance ξ_o to be 5 Å (consistent with X-ray spacings), we find that

$$t = \frac{T - T_c}{T_c} = \tfrac{1}{64} \tag{12}$$

so that the system must be within less than 5°C of the critical temperature. For b smaller, the system could be correspondingly farther from the critical temperature. Furthermore, the correlation length will not diverge unless the lateral pressure is equal to the critical pressure, π_c. In fact, one may also perform a simple manipulation of the critical exponent expressions [17] to show that the correlation length diverges at $T = T_c$ as

$$\xi/\xi_0 \approx \left|\frac{\pi - \pi_c}{\pi_c}\right|^{1-\delta/2\delta} \tag{13}$$

where δ is the exponent which describes the critical isotherm. For the purpose of estimation we use the classical value $\delta = 3$, and we find that $\xi = 40$ Å gives $|(\pi - \pi_c)/\pi_c| \approx 0.002$ or $|\pi - \pi_c| \simeq 0.1$ dyne/cm at $T = T_c$. Thus, in the region near the bilayer melting point the range of correlations is not likely to be greater than the size of a typical cavity. It is, therefore, reasonable to assume that the portion of the bilayer permeability which depends upon fluctuations is proportional to the lateral compressibility. The good qualitative agreement between the calculations in the next section and the experiments supports this conclusion. It is also significant that the above considerations may be applied to other bilayer permeants by considering different values of b in Eqn. 9. Our arguments imply that larger permeants should have sharper peaks in P vs. T plots because longer range correlations are needed to open up the associated cavities, and the system must therefore be closer to the critical point. For larger permeant molecules the proportionality factor in Eqn. 9 should be smaller so that the peak in a P vs. T plot is not necessarily higher, only narrower. This agrees with the conclusions of Jacobson and Papahadjopoulos [18] who review a number of other transport and equilibrium properties that show anomalous peaks at the transition.

II. Model calculations

In Fig. 2 we plot the lateral compressibility vs. temperature at various lateral pressures, π, for Nagle's model [9]. This is a two-dimensional model of very long chains with a form of rotational isomerism appropriate for the lowered dimensionality. The calculation is exact for the excluded volume effect and the rotational isomerism, and the longer range van der Waals attractive interactions are taken into account by a simple density dependent term of the form

$$U_{vdW} = -a\rho^{3/2}\,. \tag{14}$$

Because the excluded volume interaction is infinitely hard, κ_T is zero below the transition in the maximum density state. As T approaches T_c from above with $\pi = \pi_c$, κ_T diverges as $(T - T_c)^{-1/2}$. This kind of transition is called a 3/2

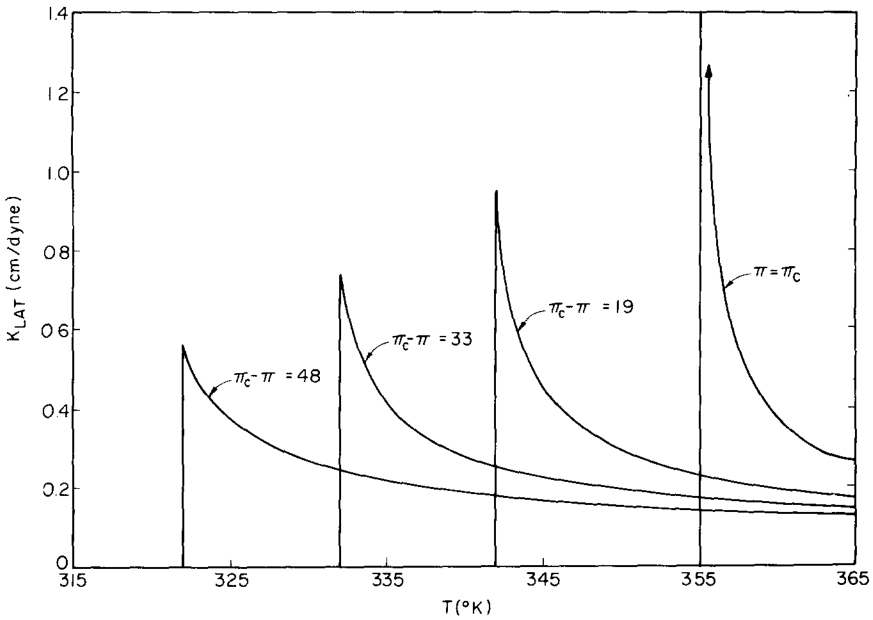

Fig. 2. A plot of κ_T vs. T at various lateral pressures (in dynes/cm) for Nagle's model. This is the basic model of ref. 9 without headgroup interactions and with $E_{att} = -a\rho^{3/2}$, $a = 1.84$ kcal/mol.

order critical point [9]. For bilayers which have a first order transition and miss the critical point one has $\pi < \pi_c$. For $\pi \neq \pi_c$, κ_T does not become infinite but does have a sharp maximum at the transition as shown in Fig. 2 for $\pi < \pi_c$. (Similar behavior occurs for $\pi > \pi_c$.) The relative height of the maximum in κ_T decreases as $|\pi - \pi_c|$ increases. However, even when $\pi_c - \pi = 48$ dynes/cm, the peak in κ_T is sharp enough to account for the experimental anomaly in P_{Na^+} and the peak has a half-width comparable to the observed 10° wide peak in P_{Na^+}. Finally, the theory gives a very pronounced asymmetry to κ_T around the transition which is similar to the observed asymmetry in P_{Na^+} shown in Fig. 1.

In Fig. 3 we plot κ_T versus T at various pressures for Scott's model [13]. In this model the correct dimensionality of a bilayer system is retained, but the steric repulsive contributions to the free energy are calculated in a modified Flory approximation. As in Nagle's model, a density-dependent free energy of the same form, Eqn. 5, is added to simulate the van der Waals attractions. The curves in Fig. 3 qualitatively resemble those in Fig. 2 except the approximate model has a non-zero κ_T below the transition temperature at each pressure. Comparison of the curves in Fig. 3 with the data of Papahadjopoulos et al. [2] shown in Fig. 1 for unsonicated vesicles shows good agreement in the shapes of the curves. At the critical pressure the approximate model compressibility diverges as $(T - T_c)^{-1}$, as is the case for all classical models [16,17]. In fact, the data of Fig. 3 should closely resemble κ_T calculated from mean field models, such as that of Mačelja [19].

The theory presented here can accomodate the fact that the height of the peak in the permeability vs. T plot seems to depend rather strongly upon the

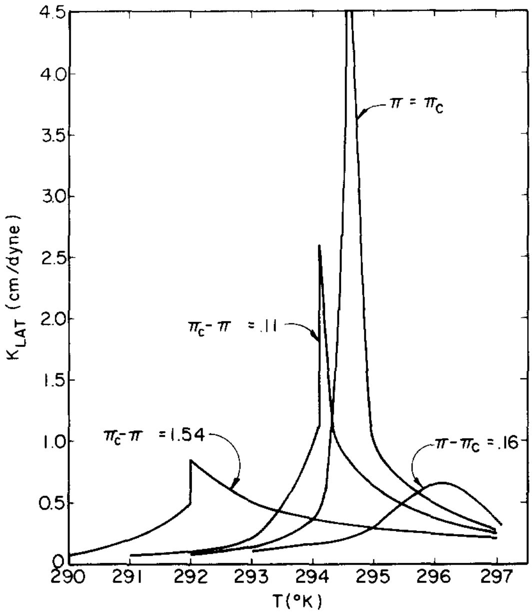

Fig. 3. A plot of κ_T vs. T for Scott's model. The model used is the chain model of ref. 13 without head-group interactions, and with the same chain parameters as those used in this reference.

length of the hydrocarbon chains [20]. One may expect that for the different lipids studied, the bilayers at their melting temperatures may not be in precisely the same thermodynamic states relative to their associated monolayer critical points. That is, $|\pi - \pi_c|$ may be different for the different systems. If this is the case than the correlation length at T_c will vary following to Eqn. 13, and the permeability will vary accordingly. We note that the variation in peak height with $|\pi - \pi_c|$ in Figs. 2 and 3 reflect these conclusions.

In summary, our calculations support the notion that the thermodynamic state of a membrane is important to its functional properties. Furthermore, our results suggest that membranes, or at least parts of membranes may exist in a near-critical state in which fairly large-scale fluctuations in lipid density exist. Such fluctuations should allow for cavity formation, and perhaps protein interaction and/or conformational change.

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