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PROPERTIES OF BILAYER MEMBRANES IN THE PHASE TRANSITION OR PHASE SEPARATION REGION

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Summary

The increase in passive permeability of bilayer membranes near the phase transition temperature is usually explained as caused by either the increase in the amount of 'boundary lipid' present in the membrane, or by the increase in lateral compressibility of the membrane. Since both the amount of 'boundary lipid' and the lateral compressibility show a similar anomaly near the transition temperature, it is difficult to distinguish experimentally between the two proposed mechanisms.

We have examined some details of both of the proposed pictures. The fluid-solid boundary energy, neglected in previous work, has been computed as a function of the domain size. For a single component uncharged lipid bilayer, the results rule out the existence of even loosely defined solid domains in a fluid phase, or vice versa. Thermodynamic fluctuations, which are responsible for anomalous behaviour near the phase transition temperature, are not intense enough to approximate the formation of a domain of the opposite phase.

Turning next to lateral compressibility of bilayer membranes we have considered two-component mixtures in the phase separation region. We present the first calculation of lateral compressibility for such systems. The behaviour shows interesting anomalies, which should correlate with existing and future data on transport across membranes.

Introduction

In 1973, Papahadjopoulos et al. [1] observed that the passive permeability of bilayer membranes has a pronounced maximum at the phase transition temperature of bilayer lipid chains. This observation has aroused considerable experimental and theoretical interest, and after a few years the proposed

Abbreviations: DPPC, dipalmitoyl phosphatidylcholine; DMPC, dimiristoyl phosphatidylcholine.

explanations for this effect have settled for one or the other of the following two hypotheses: (1) The increase in permeability is associated with 'boundary lipid', i.e. it arises because of the mismatch in packing of lipid chains at the boundary between solid and fluid domains [1—5], or (2) the increase in permeability is correlated with the increase in lateral compressibility of the bilayer, i.e. the increase in permeability is associated with the increase in fluctuations of the cross-sectional area of lipid chains near the phase transition temperature [6—8].

The two mechanisms are not entirely different if a 'boundary lipid' picture is extended by adopting a very loose concept of a frozen domain in an otherwise fluid bilayer or vice versa. However, the picture then becomes ambiguous, and a poorly defined domain should rather be referred to as a local fluctuation in the order of the lipid environment.

The conceptual clarification of the physical principles involved in the permeability anomaly at the lipid phase transition is very important as a part of a broader range of problems where lipid environment controls membrane function [10]. We have therefore examined some details of both of the proposed mechanisms. In the following sections we discuss in turn the concept of domains near the phase transition, and lateral compressibility of membranes formed from binary mixtures of lipids. We conclude by rejecting the domain picture for neutral, single-lipid bilayers and suggesting a few relevant experiments.

Domain boundary energy

The formation of a stable domain state near the phase transition temperatures of dispersions of pure, single-component lipids is opposed by the Gibbs phase rule [8]. However, when lipid head groups are charged, strong surface effects are introduced. The balance between the electrostatic and the phase boundary energy then favours a gradual phase transition via the domain state [9].

While no direct evidence supports the idea of formation of stable domains near the phase transition of single-component neutral bilayer, it is possible to imagine [5] a dynamic equilibrium with rapid formation and decay of 'domain' structures. Such a concept would be appropriate and useful if most of the thermodynamic fluctuations near the phase transition temperature, reflected e.g. in the specific heat anomaly [5], lead to the formation of a well-defined domain of the opposite phase. This question is examined here by the computation of the free energy change associated with the formation of a solid domain in a fluid bilayer just before the phase transition temperature is reached.

The computation has been performed using the model of lipid chain ordering in bilayer membranes described previously [11]. Lipid molecules are arranged on the sites of a two-dimensional hexagonal lattice, and various thermodynamic quantities are computed separately for each site. A domain of frozen lipids is simulated by setting the molecules occupying a selected number of sites into the frozen state, and allowing the remainder of the system to equilibrate.

In Fig. 1 the change in the Gibbs free energy ΔG associated with the formation of a solid domain in the fluid phase is shown as a function of the domain

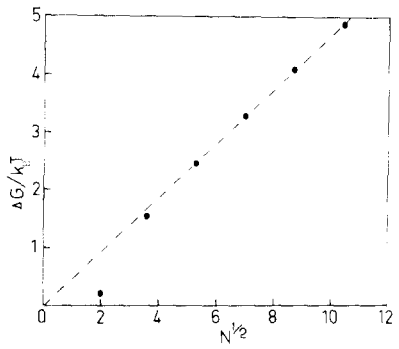


Fig. 1. The change in free energy, ΔG, associated with the formation of a domain of N molecules of solid in the bulk fluid phase. k_B is Boltzmann's constant and T the absolute temperature.

size. The results are computed at the phase transition temperature of the model system, $T = 24.12°C$. The free energies of the bulk fluid and the bulk solid lipid molecules are therefore equal, and ΔG is the free energy of the fluid-solid boundary. For the roughly circular domain shapes which were selected, ΔG should scale with $N^{1/2}$ where N is the number of molecules forming the domain. Since change of lipid order between the fluid and the solid phase is gradual [9,11], N is determined by taking the dividing line approximately half-way between the fluid and the solid phase.

The computation shown in Fig. 1 uses lipid chain length of 12 carbon atoms. The ΔG corresponding to the chains of 16 carbon atoms should be roughly twice as large as the values shown in Fig. 1. If the domain is formed in both halves of a bilayer, the values should be doubled again. Reading from Fig. 1, we estimate e.g. that for a dipalmitoyl phosphatidylcholine bilayer the free energy of formation of a frozen domain is $9k_BT$ (domain size 25 molecules) or $12\,k_BT$ (domain size 50 molecules).

The free energy difference associated with thermodynamic fluctuations in an equilibrium system is typically of the order of the thermal energy, k_BT. In statistical mechanics, fluctuations where the free energy difference is an order of magnitude higher, say $10\,k_BT$ are certainly exceedingly rare. For most practical purposes therefore such fluctuations may be safely ignored.

From the above argument, we conclude that thermodynamic fluctuations near the phase transition of single-component, uncharged lipid bilayers are not intense enough to create well-defined domains of the opposite phase. The 'domains' found from the general statistical mechanics argument by Freire and Biltonen [5] are not frozen patches: rather they could be thought of as correlated regions of the bilayer where local order deviates slightly from the equilibrium value. 'Domains' obtained from the Ising model considerations by Tsong et al. [4] are only a consequence of applying the Ising model, which allows for just two different states at each site. They cannot be taken to mean frozen or fluid domains in a lipid bilayer *.

* This conclusion is in agreement with the small average cluster size calculated by Kanehisha and Tsong ((1978) J. Am. Chem. Soc. 100, 424–432). We thank Dr. Tsong for a correspondence regarding this and related points.

Lateral compressibility of bilayers formed from binary mixtures

Several research groups have reported an increase in transport across the membrane at temperatures corresponding to the coexistence of both solid and fluid phases of lipids in the membrane. Linden et al. [6] have proposed that the increase is due to the large lateral compressibility of bilayers in the phase separation region. Similarly [12], the compressibility of single-component bilayers is enhanced near the phase transition temperature, and this should be reflected in higher transport rates over a small temperature range. This latter case was examined in more detail by Doniach [7] and by Nagle and Scott [8].

How large is the increase in lateral compressibility in the phase coexistence region? What is the shape of the anomaly? For bilayer membranes, these questions cannot be answered experimentally. We have therefore combined the information available from related studies on both monolayers and bilayers to obtain an insight into the problem.

Lateral phase separation in bilayer membranes is normally studied as a function of temperature, and the coexistence region shown in temperature-composition (T-x) phase diagrams. To consider lateral compressibility, the formalism should include the lateral pressure (Π) as a second thermodynamic variable. Lipid chains in bilayers are subject to an intrinsic lateral pressure [13] which depends strongly on the interactions at the polar head-water interface and cannot be conveniently controlled *. However, monolayer studies provide complementary data, where T is held constant and Π is continuously varied.

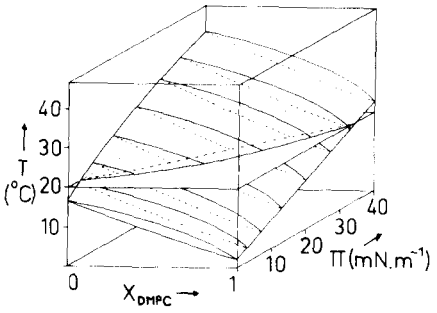
As an illustration of the general principles involved, we have constructed the Π-T-x phase diagrams assuming ideal mixing between the two components of the binary mixture. The formalism used in e.g. a review article by Lee [16] is easily extended to accommodate lateral pressure as another independent variable. In that case, the chemical potential difference between the solid and the fluid phase of the component A is (cf. Eqn. 24 of Ref. 16)

$$\mu_A^{(S)0} - \mu_A^{(L)0} = -(\Delta H_A)_{T,\Pi}(1 - T/T_A) + (\Delta a_A)_{T,\Pi}(\Pi - \Pi_A) \quad (1)$$

with a similar expression for the component B. Δa_A is the change in the molecular cross-sectional area at the phase transition of the pure component A, and the second term on the right-hand side of the Eqn. 1 describes [13] the change in chemical potential associated with the change in the lateral pressure acting on lipid chains. ΔH_A is the melting enthalpy of component A. With the assumption that $(\Delta a)_{T,\Pi}$ is unchanged by small changes in Π (analogous to the assumption [16] that $(\Delta H)_{T,\Pi}$ is independent of T) Eqn. 1 is essentially symmetric in its dependence on T and Π. The solidus and the fluidus curves in the Π-x plane thus form a shape similar to the 'cigar' formed in the T-x plane.

An example of the Π-T-x phase diagram corresponding to ideal mixing in binary lipid systems is shown in Fig. 2. The diagram, corresponding to mixtures of dipalmitoyl phosphatidylcholine (DPPC) and dimiristoyl phosphatidylcholine (DMPC), has been calculated using the monolayer data [17] on the

* To some extent, intrinsic lateral pressure can be controlled by varying the pH of the aqueous phase [14,15].



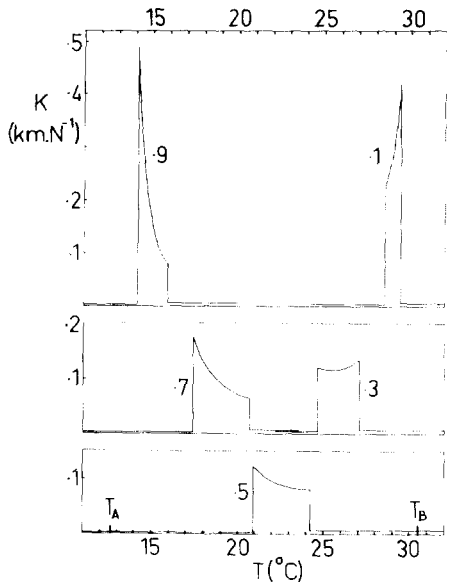


Fig. 2. The phase diagram for a mixture of DMPC and DPPC. The intersections of several isobaric planes with the fluidus surface (———) and solidus surfaces (- - - - -) are shown. The plane T = 20°C is also drawn to show the 'cigar'-shaped figure in the Π-x phase diagram. If a system is specified by a point X', Π', T' which lies above the fluidus surface, it is an homogeneous fluid, if X', Π', T' lies below the solidus surface, it is an homogeneous solid. If it lies between the two it separates into fluid and solid phases whose compositions are specified by the intersection with, respectively, the fluidus and solidus surfaces of a line through X', Π', T' parallel to the x-axis. Ideal mixing is assumed and the data are from Ref. 17.

Fig. 3. Isothermal compressibility as a function of temperature for different mixtures of DMPC and DPPC. The fractions printed on each curve are those of DMPC. These curves are strictly applicable to a monolayer at Π = 20 mN · m^{-1}, which lateral pressure approximates that in a bilayer, and T_A and T_B are, respectively, the melting temperatures of DMPC and DPPC in such a monolayer. Since $(T_B - T_A)$ is nearly independent of Π (see Fig. 2) and is almost the same for monolayers and bilayers, the curves can be applied to different systems simply by displacing them to match the new values of T_A and T_B.

melting enthalpies and the dependence of melting temperatures on pressure *.

The isothermal compressibility $\kappa = -(1/a)\partial a/\partial\Pi$ is now easily evaluated using the average area/molecule.

$$a = x_F(x_A^F a_A^F + x_B^F a_B^F) + (1 - x_F)(x_A^S a_A^S + x_B^S a_B^S) \quad (2)$$

(where x_F is the total fluid fraction) and standard expressions from Ref. 16. In Fig. 3 we have plotted the compressibility as a function of temperature of a monolayer comprising different mixtures of DMPC and DPPC. The pure fluid phase of the Π-a isotherm was fitted to the monolayer data [17] using the van der Waals form equation and the pure solid phase was fitted assuming a small constant compressibility. The fluid above the phase separation temperature is twice or three times more compressible than the solid below the phase separation. In the separation region however, the compressibility is higher by an order

* Since the equivalence between mono- and bilayers is only approximate (see e.g. Discussion in Refs. 17 and 18) the phase diagrams of Fig. 2 should be applied to bilayers cum grano salis.

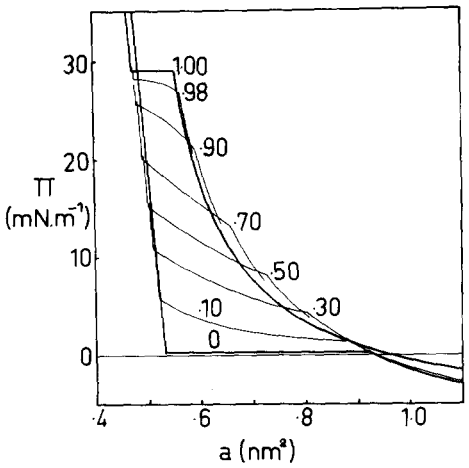


Fig. 4. Calculated Π-a isothermus at 16°C for monolayer mixtures of DMPC and DPPC at the air-water interface. The fraction of DMPC is indicated for each curve. The data from Ref. 17 have been used assuming a van der Waals equation of state for the fluid and a small constant compressibility for the solid.

of magnitude. The peaks in the compressibility are particularly pronounced when the fluidus or the solidus line on the phase diagram is close to horizontal. For example, an equimolar mixture whose components had a closer melting temperature, or a larger melting enthalpy than DMPC and DPPC, would produce a compressibility curve with two peaks and a marked trough between them.

Theoretical Π-a isotherms for binary mixtures of DMPC and DPPC calculated with identical assumptions are shown in Fig. 4. Such diagrams have an advantage in allowing for easy comparison between theory and experiment. For example, data on binary mixture monolayers at an oil-water interface [19] are qualitatively similar to the isotherms of Fig. 4. The similarity between typical experimental Π-a isotherms for single-component monolayers and isotherms of Fig. 4 for binary mixtures with a small concentration of one component suggests that the non-zero slope in the coexistence region may in some cases be due to contamination by a different species.

Discussion

The basic question which we have addressed is identification of the mechanism responsible for an increase in transport across the membrane in the phase separation or phase transition region. We have found that the lipid chain interior of single-component bilayers would not lead to the formation of domains near the phase transition temperature. Domains can then only be formed as a result of interactions at the polar head-water interface. We know [9] that bilayers made up of charged lipids do break up into domains in the phase transition region. In this view, comparison of ionic permeability anomalies exhibited by neutral and charged bilayers would be indicative of the role played by the domain formation.

Our most interesting results are the lateral compressibility anomalies shown in Fig. 3. In real systems, the shapes of the anomalies would be rounded, and

therefore closely resemble the specific heat anomalies measured for binary mixtures of lipids [20]. It should be noted that the values of κ obtained here are typically an order of magnitude higher than the corresponding values for single-component bilayers *. Anomalies shown in Fig. 3 should be compared to the anomalies in transport determined experimentally. In some cases (e.g. Fig. 1 of Ref. 6 or Fig. 3 of Ref. 12) the similarity is striking.

The present calculation could easily be extended to various models of non-ideal mixing [16]. In some cases, it may be easier to test various non-ideal mixing theories against Π-a isotherms or Π-x phase diagrams rather than T-x phase diagrams.

A more speculative application of our results is to enzyme activity over the phase separation region. Many authors have discussed the temperature dependence of enzyme-catalysed reactions in terms of the fluidity of the enzyme's lipid environment [10,21]. Where activity is presumed limited to a certain range of membrane lipid fluidity (Raison, J.K., Berry, J.A., Armond, P.A. and Pike, C.S., unpublished results), it is possible (and rather easier to model) that the limitation is a certain minimum compressibility.

The importance of a change in order of magnitude in the compressibility to the mechanical properties of a membrane is also worthy of consideration. In the phase separation region, bending a membrane need create only a small change in lateral pressures in the inner or outer monolayer since the molecular area can more easily change to fit the new geometry. An organelle which is already spherical may even swell a little at the expense of relatively little energy in response to, say, an osmotic stress.

We have implicated physical properties, chiefly the compressibility, in two physiological processes commonly associated with low temperature damage in organisms: enzyme inactivation [22] and electrolyte leakage [23]. This relationship is reported in more detail elsewhere [24].

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* The values of κ reported in Ref. 8 are not realistic. The highest values indicated by monolayer experiments or our own model computations [13] are of the order of 20 $m \cdot N^{-1}$.

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