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Phase Separation in Bilayer Lipid Membranes: Effects on the Inner Leaf Due to Coupling to the Outer Leaf

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ABSTRACT The combined effects of the tendency of cholesterol to order lipids in the liquid phase and the coupling between lipids in the two leaves of a bilayer are investigated theoretically utilizing a Landau free energy. We show that as a consequence of these combined effects, lateral phase separation in the outer leaf between cholesterol-rich and -poor liquids causes a similar, but weaker, phase separation in the inner leaf. Just as the areal density of lipids in the outer leaf increases in the cholesterol-rich regions, so the areal density of lipids also increases in the inner leaf. Thus, the areal density in the inner leaf varies spatially, reflecting spatial variations of the areal density in the outer leaf. This provides a mechanism for proteins attached to the inner leaf via a hydrocarbon tether to respond to variations in the composition of the outer leaf. We also note that the effect of coupling between the leaves should be observable in artificial bilayers.

INTRODUCTION

The hypothesis that the lipids in biological bilayers are not distributed uniformly, but that regions rich in saturated lipids and cholesterol float, like rafts, in a sea of unsaturated lipids has generated enormous interest. The consequences of the hypothesis, and the experimental evidence for and against it, are presented in recent reviews (3,13,14,19,20). While the existence of such regions in vivo is currently somewhat obscure, their existence in vitro is quite clear. Ternary mixtures of cholesterol and saturated and unsaturated lipids with compositions mimicking those of the outer leaflet of the plasma membrane exhibit phase separation over a considerable range of temperatures (1,2,22–24). Two of the phases observed are liquids. One, the liquid-ordered phase (8), is rich in cholesterol, which tends to order the lipids (16), while the other, the liquid-disordered phase, is cholesterol-poor. The lipids in this phase are less ordered. Below the chain melting transition of the saturated lipid, a third, saturated-lipid-rich gel phase appears. The observations of coexistence between the two liquid phases support the hypothesis that lipids are distributed nonuniformly in the plasma membrane. However, experiments on ternary systems with compositions that mimic those of the inner leaf of the plasma membrane show no phase separation whatsoever (25). The question then naturally arises as to what is expected to occur in a bilayer consisting of two leaflets of different compositions. It is this question that we address in this article with the aid of a Landau free energy.

Our assumptions are as follows. One leaflet, the outer leaflet, has a composition of cholesterol and lipids such that phase separation would occur between cholesterol-rich and cholesterol-poor liquids below some critical temperature, $T_{o,c}$, were the leaf not coupled to the inner leaf. In the inner leaf, phase separation would occur below some much lower critical temperature, $T_{i,c} \ll T_{o,c}$, were the inner leaf not coupled to the outer leaf. An interaction between cholesterol and lipids causes lipids in a cholesterol-rich phase to be more ordered. In accord with experimental observation (2,11,17), a coupling between lipids in the two leaflets is assumed. This could be due to interdigitation of chains or to the high frequency interchange of cholesterol between leaves (5–7). The gel phase is ignored as experimental evidence (18,21) clearly indicates that its existence is, at best, only indirectly related to lateral phase separation. Different theories also support this view (4,15).

Our results are as follows. When a phase transition occurs, it occurs at the same temperature in both leaves. The difference in cholesterol concentration between the coexisting phases in the outer leaf is, however, greater than the difference in cholesterol concentration in the inner leaf. The lipids in the cholesterol-rich region in the outer leaf become more ordered, and so their areal density increases. Because these lipids are coupled to the lipids in the inner leaf, the latter also become more ordered, but less so than those in the outer leaf. Hence, their density also increases in the same spatial region in which the density of lipids in the outer leaf increases.

The increase of the areal density of the inner leaf in the spatial region of the cholesterol-rich phase provides a mechanism by which those proteins within the cell that are attached to the inner leaf of the plasma membrane by an acyl chain can respond to the phase separation in the membrane. What our theory cannot provide are the answers to the questions of just how much the areal density of lipids in the inner leaf changes, and how sensitive the partition coefficient of a given protein is to these regions of different areal density.

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THEORY

The Landau theory for this problem will be constructed in three steps. First, we consider the outer leaflet by itself, uncoupled from the inner leaflet. We assume that at biologically relevant temperatures, it can undergo a phase separation into cholesterol-rich and cholesterol-poor phases. We include an interaction between the cholesterol and the lipid chain order so that the lipids in the cholesterol-rich region are more ordered than those in the cholesterol-poor regions. Next, the inner leaflet, still uncoupled to the outer leaflet, will be added. We assume any phase separation that it undergoes occurs at a lower temperature than that of the outer leaflet. Interactions between cholesterol and chain order are included in the description of the inner leaflet. Finally, the two leaflets are coupled via their chain ordering; i.e., the occurrence of chain ordering in the outer leaflet induces chain ordering in the inner leaf due to interdigitation or to the exchange of cholesterol between the leaves (5–7). We shall find that when the outer leaflet undergoes phase separation and the lipids in the cholesterol-rich region increase their order, the coupling between them and the lipids in the inner leaflet causes the latter to increase their order as well. Finally, the interaction between the chain order of the lipids in the inner leaflet and cholesterol causes the regions with more ordered lipids to become more cholesterol-rich. Hence, the tendency of the outer leaflet to order propagates through the entire system with physical manifestation in the inner leaflet as well.

The starting point for the description of the outer leaflet is the consideration of a two-dimensional layer consisting of three components: a high-melting-point phospholipid (hmp), typically having relatively long saturated chains; a low-melting-point phospholipid (lmp), typically having unsaturated chains; and cholesterol. For a given phospholipid, the melting temperature corresponds to the liquid-gel, or chain melting, transition. The liquid-liquid phase transition in this outer leaflet is characterized by separation into one phase rich in lmp lipid, and another rich in both cholesterol and hmp lipid. Because cholesterol tends to order lipids, the cholesterol-rich liquid phase is denoted (8) liquid-ordered (lo), while the other liquid phase is denoted liquid-disordered (ld). The increased order of the lo phase is reflected in the smaller number of *gauche* bonds and, therefore, the greater thickness of the leaflet. As the hydrophobic bulk density is essentially constant, the lo phase is characterized by a greater areal density, or smaller area per molecule.

We simplify our study by restricting ourselves to temperatures above the chain-melting temperature of the hmp lipid so that there can be no regions of three-phase (gel, lo, and ld) coexistence. As a consequence, we can, without loss of generality, consider the system to be effectively one of two components, and suppress the difference in concentration of hmp and lmp as a relevant variable. We consider, therefore, a two-component system of cholesterol and lipid without distinguishing the lipids themselves. Upon phase separation into two liquid phases, the cholesterol-rich phase is the lo phase, while the cholesterol-poor phase is ld.

As is well known for two-component systems, the competition between the entropy of mixing and attractive interactions between like-components leads to liquid-liquid phase separation at sufficiently low temperature. With the cholesterol concentration in the outer leaflet denoted by x_o and the lipid concentration by $1-x_o$, there will be a critical point of this liquid-liquid phase separation at temperature $T_{o,c}$ and concentration $x_{o,c}$.

To describe the liquid phases, we utilize two order parameters. They are taken to be $\phi_o \equiv x_o - x_{o,c}$, the deviation of the cholesterol concentration from the critical concentration, and ψ_o, the relative increase in thickness of the outer leaflet over its value at the critical point (9,10). The Landau free energy is a power-series expansion in the order parameters. Linear terms can always be eliminated by redefining the order parameters (12), so that the leading terms are quadratic in them. Once the quadratic form is diagonalized, the effect of the smaller, higher order terms will be described.

To quadratic order, then, the Landau free energy per unit area is

$$F_o(\phi_o, \psi_o) = a_o\phi_o^2 + b_o\psi_o^2 - \Gamma_o\phi_o\psi_o. \tag{1}$$

Here the ϕ_o^2 term, with a_o a linear function of temperature vanishing at $T_{o,c}$, describes the instability of a uniform system to liquid-liquid phase separation as $T_{o,c}$ is approached. Stability of the liquids is provided by a quartic term to be discussed later. Were the gel transition of the hmp lipid of importance to our discussion, we would have to include terms of higher order in ψ_o to describe it (10). As it is not, we restrict ourselves to temperatures higher than that of the gel transition with the consequence that b_o can be treated as a constant. The term proportional to $\Gamma_o > 0$ is the lowest-order interaction between chain order and phase separation. That Γ_o is positive reflects the fact that an increase in cholesterol concentration increases the order of lipids in the liquid phase.

It is straightforward to obtain the equilibrium state of the leaflet by minimizing $F_o(\phi_o,\psi_o)$ with respect to both ψ_o and ϕ_o. Minimizing first with respect to ψ_o, we obtain $\psi_{o,\min}(\phi_o) \equiv (\Gamma_o/2b_o)\phi_o$. This indicates that the change in orientational order at a given concentration is proportional to the deviation of that concentration from the critical one. Substitution of this result into the expression for F_o yields

$$F_{o,\min}(\phi_o) \equiv F_o(\phi_o, \psi_{o,\min}(\phi_o)), = [a_o - \Gamma_o^2/4b_o]\phi_o^2. \tag{2}$$

As the liquid-liquid critical point occurs at the temperature at which the coefficient of ϕ_o^2 vanishes, and as the coefficient a_o is linear in temperature, the interaction between concentration and ordering in this single leaf shifts the critical point upwards from what it would have been in its absence.

The inner leaflet is dealt with in a similar fashion. Assuming for the moment that this inner leaflet does not couple to the outer leaflet, one finds by analogy that the inner leaflet also undergoes its own liquid-liquid phase transition. However, because the inner leaflet contains different lipids, the critical temperature and critical cholesterol concentration will be different from those of the outer leaf. Thus, the order parameter describing deviations from the critical concentration will be different, and we denote it ϕ_i. Similarly, there will be a separate order parameter, ψ_i, describing the lipid order in the inner leaf. So too will the coefficients in the Landau free energy, $F_i(\phi_i, \psi_i)$ for the inner leaf, differ from those of the outer leaf, and we denote the former a_i, b_i, and Γ_i:

$$F_i(\phi_i, \psi_i) = a_i\phi_i^2 + b_i\psi_i^2 - \Gamma_i\phi_i\psi_i. \tag{3}$$

Minimization with respect to ψ_i yields

$$F_{i,\min}(\phi_i) \equiv F_i(\phi_i, \psi_{i,\min}(\phi_i)), = [a_i - \Gamma_i^2/4b_i]\phi_i^2. \tag{4}$$

The system of two uncoupled leaves has two transitions. There is a critical point in the outer leaf whose temperature is given by the condition $[a_o(T) - \Gamma_o^2/4b_o] = 0$, and one in the inner leaflet given by the condition $[a_i(T) - \Gamma_i^2/4b_i] = 0$. Without loss of generality, we can assume that $[a_i - \Gamma_i^2/4b_i]$ vanishes at a lower temperature than does $[a_o - \Gamma_o^2/4b_o]$. We now turn on the coupling between the two leaves and discuss its effects.

Coupling between the two leaves can be provided by interdigitation of the lipid tails from each leaf, and from the exchange of cholesterol between the leaves (5–7). These considerations suggest modeling the coupling by a contribution to the free energy of $F_c(\psi_o, \psi_i) = -\Omega\psi_o\psi_i$, with $\Omega > 0$, by which increased ordering in one layer promotes such an increase in the other. The total Landau free energy is now

$$\begin{aligned} F(\phi_o, \phi_i, \psi_o, \psi_i) &= F_o(\phi_o, \psi_o) + F_i(\phi_i, \psi_i) + F_c(\psi_o, \psi_i), \\ &= a_o\phi_o^2 + b_o\psi_o^2 - \Gamma_o\phi_o\psi_o \\ &\quad + a_i\phi_i^2 + b_i\psi_i^2 - \Gamma_i\phi_i\psi_i \\ &\quad - \Omega\psi_o\psi_i. \end{aligned} \tag{5}$$

Were the lipids in the two leaves capable of sufficient exchange to bring about equilibrium with respect to their composition, the lipid chemical potential in the two leaves would be equal, and this equality would provide a relation between the two compositional order parameters ϕ_o and ϕ_i. However, as is well known, the compositions in the two leaves are kept out of equilibrium by specialized proteins. Thus, the two compositional order parameters are taken to be independent, and we minimize the free energy accordingly.

It is straightforward to minimize $F(\phi_o,\phi_i,\psi_o,\psi_i)$ with respect to ψ_o and ψ_i, and to obtain

$$\psi_{o,\min}(\phi_o,\phi_i)=\frac{2b_i\Gamma_o}{D}\phi_o+\frac{\Omega\Gamma_i}{D}\phi_i, \tag{6}$$

and

$$\psi_{i,\min}(\phi_o,\phi_i)=\frac{\Omega\Gamma_o}{D}\phi_o+\frac{2b_o\Gamma_i}{D}\phi_i, \tag{7}$$

where $D\equiv 4b_ob_i-\Omega^2$.

This last equation is the most important result of the calculation. It shows that a consequence of compositional ordering in the outer leaf, i.e., $\phi_o\neq 0$, is not only that there is lipid ordering in the outer leaf, $\psi_{o,\min}\neq 0$ (Eq. 6), but also that there is lipid ordering in the inner leaf, $\psi_{i,\min}\neq 0$ (Eq. 7). Further the reason is clear in this equation: a compositional ordering in the outer leaf, ϕ_o, interacts with the lipid ordering in the outer leaf with a strength Γ_o, and this ordering of the lipids in the outer leaf couples to the lipid ordering in the inner leaf with a strength Ω. There are additional, smaller effects on the lipid ordering of the inner leaf due to the fact that, as we shall see, a small compositional order is also induced in the inner leaf, $\phi_i\neq 0$ which interacts with the lipid order there with a strength Γ_i.

Proceeding, we substitute these expressions into $F(\phi_o,\phi_i,\psi_{o,\min},\psi_{i,\min})$ and obtain

$$\begin{aligned}F_{\min}(\phi_o,\phi_i)&\equiv F(\phi_o,\phi_i,\psi_{o,\min}(\phi_o,\phi_i),\psi_{i,\min}(\phi_o,\phi_i))\\&=c_o\phi_o^2-\frac{\Omega\Gamma_o\Gamma_i\phi_o\phi_i}{D}+c_i\phi_i^2,\end{aligned} \tag{8}$$

with $c_o=a_o-b_i\Gamma_o^2/D$ and $c_i=a_i-b_o\Gamma_i^2/D$. This free energy is put into diagonal form

$$F_{\min}(\sigma,\tau)=d_2\sigma^2+f_2\tau^2 \tag{9}$$

by an orthogonal transformation from the old order parameters, ϕ_o and ϕ_i, to linear combinations of them, σ and τ,

$$\phi_o=\sigma\cos(\theta)-\tau\sin(\theta),\phi_i=\sigma\sin(\theta)+\tau\cos(\theta). \tag{10}$$

One finds

$$\begin{aligned}d_2&=\frac{c_i+c_o-Q}{2},\\f_2&=\frac{c_i+c_o+Q}{2},\\\cos(2\theta)&=\frac{c_i-c_o}{Q},\\\sin(2\theta)&=\frac{\Omega\Gamma_o\Gamma_i}{DQ},\\Q^2&\equiv(c_i-c_o)^2+\left(\frac{\Omega\Gamma_o\Gamma_i}{D}\right)^2.\end{aligned} \tag{11}$$

Note that when the coupling between leaves, Ω, is small, the angle θ is small and the new order parameters σ and τ are almost equal to the old order parameters ϕ_o and ϕ_i, respectively.

We now add higher-order terms to the free energy to stabilize the order parameters when they become nonzero. It is unimportant for our purposes what the particular form is. In general, there will be cubic terms, but for our purposes we will add only the most transparent, quartic terms, so that the free energy per unit area becomes

$$F(\sigma,\tau)=d_2\sigma^2+f_2\tau^2+d_4\sigma^4+f_4\tau^4, \tag{12}$$

where we have dropped the subscript *min*. This free energy is diagonal in its two order parameters, and is therefore easily analyzed.

RESULTS

This free energy still describes two transitions; the first occurs at the temperature at which $d_2(T)=0$, the second at the temperature for which $f_2(T)=0$. For weak coupling, these are very close to the temperatures at which the outer and inner leaves in the uncoupled systems would undergo their transitions. Below the higher of the two transitions at which $d_2(T)=0$, σ becomes nonzero,

$$\sigma=\pm\left(\frac{-d_2}{2d_4}\right)^{1/2}, \tag{13}$$

and below the lower temperature, τ is similarly nonzero. The phase diagram of the system is shown in Fig. 1. We show there both the σ- and τ-axes as well as the ϕ_o- and ϕ_i-axes, which are rotated by the angle θ with respect to them. In principle, the angle θ will be slightly temperature-dependent, but we ignore this in the figure.

To plot a phase diagram, as in Fig. 1, we must choose the parameters in our free energy. There are nine, but one of them can be eliminated, as we are not interested in the absolute value of the free energy. If the transition temperature at which σ becomes nonzero is denoted T_σ, then we can write $d_2=g(T-T_\sigma)$, where g is a constant, and consider the free energy $F(\sigma,\tau)/g$. In the expression for this quantity, all the coefficients, which are now divided by g, are expressed

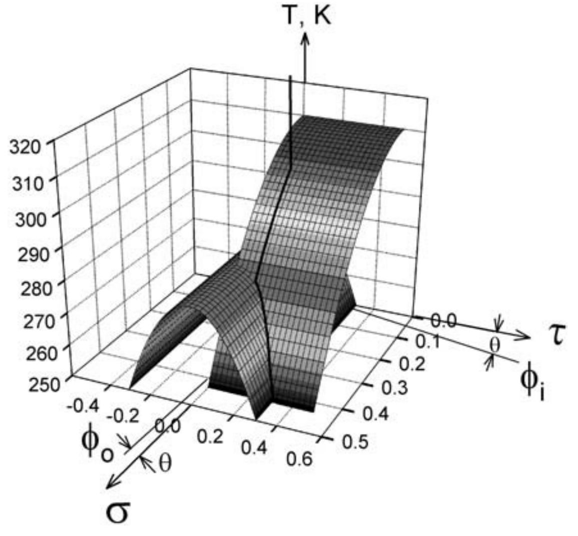

FIGURE 1 Phase diagram of the system of coupled leaves in the space of temperature, T, and uncoupled concentration variables τ and σ. The axes of the original concentration variables in the inner and outer leaves, ϕ_i and ϕ_o, are also shown. They are rotated by the angle $\theta=4.64°$ from the τ,σ axes. A representative experimental path is shown in the black line. Two transitions occur: the first when the upper sheet of transitions is encountered at 300 K, and the second when the seam connecting the sheets of transitions is encountered at ~275 K.

in degrees K. In the case of Fig. 1, we have chosen the interactions between concentrations and order parameters in the two leaves to be the same, $\Gamma_i = \Gamma_o$, and the coefficients of the squares of the order parameters in the two leaves to be the same, $b_i = b_o$. For purposes of illustration, we have taken the higher transition temperature, the one at which σ becomes nonzero, to be 310 K, with $d_2/2d_4 = (T - 310)/320$, and the lower transition at which τ becomes nonzero, to be 280 K with $f_2/2f_4 = (T - 280)/320$. The mixing angle is $\theta = (1/2)\sin^{-1}(\Omega\Gamma_o\Gamma_i/DQ) = 4.64°$. Finally we have taken the ratios $\Gamma_o/b_o = \Gamma_i/b_i = 0.75$, $\Omega/b_o = \Omega/b_i = 1.56$, and $b_i/g = b_o/g = 40$ K.

Let us consider what happens in the system of coupled leaves as the temperature is lowered. The concentrations of the two leaves are fixed by their initial compositions and, in general, both ϕ_o and ϕ_i are nonzero; that is, the concentrations in the two leaves are not, in general, equal to the concentrations at which a second-order phase transition would occur. Thus, in Fig. 1, the system descends along a line of constant ϕ_o and ϕ_i, or essentially, constant σ and τ, until the upper sheet of transitions is encountered. The transition will, in general, be first-order, and the value of σ will undergo a jump at the transition, but the value of τ will not. Two phases are now in coexistence. Each phase is characterized by definite lipid and cholesterol concentrations in each leaf, and definite lipid order parameters in each leaf. The cholesterol concentrations in the outer and inner leaves of the coexisting phases differ by

$$\begin{aligned}\delta\phi_o &= \delta\sigma\cos\theta,\\ &= 2\left(\frac{-d_2}{2d_4}\right)^{1/2}\cos\theta,\end{aligned} \tag{14}$$

and

$$\begin{aligned}\delta\phi_i &= \delta\sigma\sin\theta,\\ &= 2\left(\frac{-d_2}{2d_4}\right)^{1/2}\sin\theta.\end{aligned} \tag{15}$$

In the case when the coupling between the two leaves is weak, this expression for $\delta\phi_i$ reduces to

$$\delta\phi_i \approx \delta\phi_o\frac{\Omega\Gamma_i\Gamma_o}{2DQ}. \tag{16}$$

Again, this equation is clear: because the outer leaf undergoes a transition, ϕ_o undergoes a jump. This concentration difference interacts with the lipid order parameter with strength Γ_o. These lipids in the outer leaf are coupled to those in the inner leaf with a strength Ω, and these lipids in the inner leaf interact with the cholesterol concentration in the inner leaf with a strength Γ_i. In the case of weak coupling of the two leaves, Eq. 16 shows that the concentration difference in the inner leaf is linearly related to the strength, Ω, of this coupling, so that a reduction in the coupling between leaves results in a concomitant reduction in the inner leaf concentration difference.

As a consequence of the interaction of the cholesterol concentrations with the lipid order and of the coupling of the leaves with one another, the lipid order in the two leaves of the coexisting phases differ, according to Eqs. 6 and 7, by

$$\begin{aligned}\delta\psi_o &= \frac{2b_i\Gamma_o}{D}\delta\phi_o + \frac{\Omega\Gamma_i}{D}\delta\phi_i,\\ \delta\psi_i &= \frac{\Omega\Gamma_o}{D}\delta\phi_o + \frac{2b_o\Gamma_i}{D}\delta\phi_i.\end{aligned} \tag{17}$$

Of course, the larger value of the lipid order parameter is associated with the phase with the larger cholesterol concentration. When the coupling between leaves is weak, these equations become

$$\begin{aligned}\delta\psi_o &\approx \frac{2b_i\Gamma_o}{D}\delta\phi_o,\\ \delta\psi_i &\approx \frac{\Omega}{2b_i}\delta\psi_o,\\ &\approx \frac{\Omega\Gamma_o}{D}\delta\phi_o.\end{aligned} \tag{18}$$

Again, this last equation reiterates our central point that a compositional ordering in the outer leaf is reflected in the chain ordering of the inner leaf due to the interaction, Γ_o, between composition and chain order in the outer leaf, and coupling, Ω, between chain order in the two leaves. A reduction in the strength of either of these causes a concomitant reduction in the chain ordering of the inner leaf when the outer leaf orders compositionally.

A second transition occurs at a lower temperature when the lower sheet depicted in Fig. 1 is encountered. We expect that the first transition occurs above physiological temperatures, but that the second one occurs below them. This expectation is based upon the fact that the transition temperatures of the weakly coupled system are not very different from that of the uncoupled system, and the experimental in vitro evidence that leaves with compositions of the outer leaf are phase-separated (1,2,22–24), while those with compositions of the inner leaf are not (25). If the uncoupled inner leaf exhibited no phase transition at all, then the coupled system would not exhibit a second phase transition. Nonetheless, for completeness we assume that the uncoupled inner leaf would undergo a phase separation at some relevant temperature, and discuss the further consequences for the coupled system of the second phase transition. Figs. 2–5 show the behavior of the four order parameters, that of concentration in the outer and inner leaves, ϕ_o and ϕ_i, and of the chain order in the outer and inner leaves, ψ_o and ψ_i.

The behavior of the concentration-order parameter of the outer leaf, ϕ_o, is shown in Fig. 2. We have assumed that ϕ_o is positive in the one-phase, high-temperature region, which corresponds to the system being cholesterol-rich. On encountering the upper transition, coexistence appears between the cholesterol-rich phase, $\phi_o > 0$, and a cholesterol-poor phase, $\phi_o < 0$. As the temperature is decreased the magnitude of the order parameters increases. When the lower transition is

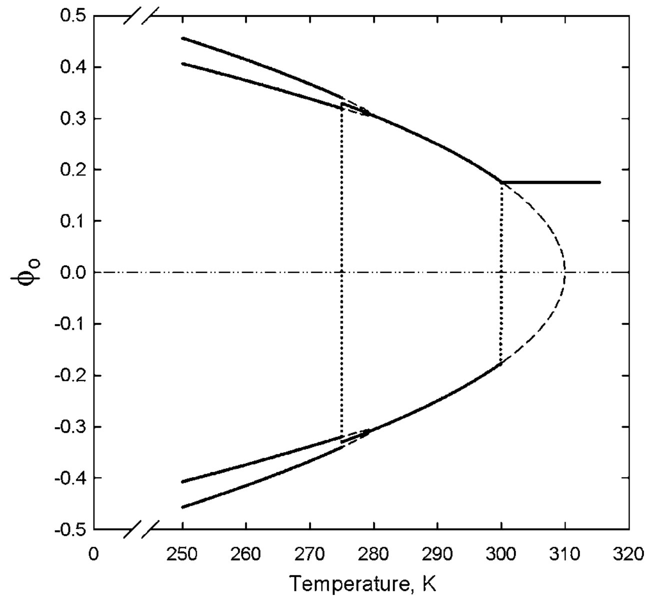

FIGURE 2 Behavior of the concentration order parameter of the outer leaf, ϕ_o. At the upper transition, which is encountered at $T = 300$ K, there is phase separation between a cholesterol-rich phase, $\phi_o = 0.2$, and a cholesterol-poor phase, $\phi_o = -0.2$. The magnitude of the order parameter increases with decreasing temperature. When the lower transition is encountered at ~275 K, the order parameter undergoes another, but much smaller, jump. There are now four phases characterized by four different values of ϕ_o. Projections of the phase boundaries on the T, ϕ_o plane are shown as dashed lines. Coexisting values of ϕ_o are connected by dotted lines.

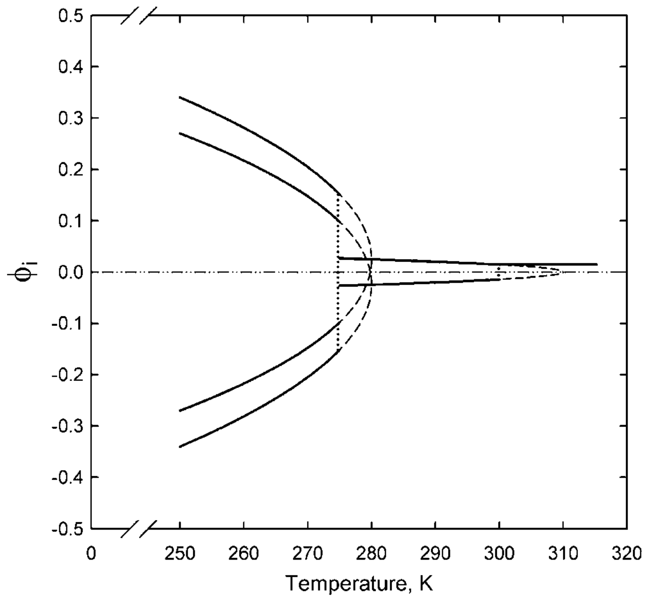

FIGURE 3 Behavior of the concentration order parameter of the inner leaf, ϕ_i. At the upper transition, which is encountered at $T = 300$ K, there is a small jump of the order parameter and phase separation between a phase which is somewhat cholesterol-rich, $\phi_i \approx 0.03$, and one which is somewhat cholesterol-poor, $\phi_i \approx -0.03$. The magnitude of the order parameter increases slightly with decreasing temperature. When the lower transition is encountered at ~275 K, the order parameter undergoes another, but much larger, jump. There are now four phases characterized by four different values of ϕ_i. Projections of the phase boundaries on the T, ϕ_i plane are shown as dashed lines. Coexisting values of ϕ_i are connected by dotted lines.

encountered, the cholesterol-rich phase splits into two, with one being even richer, the other being somewhat less cholesterol-rich. Similarly, the cholesterol-poor phase splits into two, with one being even more cholesterol-poor, the other being somewhat less so.

The behavior of the concentration-order parameter of the inner leaf, ϕ_i, is shown in Fig. 3. At the higher temperature transition, the concentration of the inner leaf undergoes a small jump. It should be recalled that, were there no coupling between leaves at all, the inner leaf would not undergo any phase separation at this temperature. So the existence of the jump at the upper transition temperature is a direct consequence of the interleaf coupling. When the lower transition is encountered, near the temperature at which the inner leaf would undergo a transition even were it uncoupled from the outer leaf, ϕ_i undergoes a large jump. At the lower transition, there is now four-phase coexistence.

Fig. 4 shows the behavior of the chain-order parameter of the outer leaf. At the higher temperature transition, it undergoes a large jump with the cholesterol-rich phase being characterized by a positive value of ψ_o, and the cholesterol-poor phase being characterized by a negative value. At the lower transition, the chain-order parameter of the outer leaf undergoes a small jump.

Of most interest is the behavior of the chain-order parameter of the inner leaf, the leaf that, were it not coupled to the outer one, would display no discontinuities at all at the higher transition temperature. One sees from Fig. 5 that the chain-order parameter of the inner leaf does undergo a jump at the upper transition, one that is entirely due to its coupling to the outer leaf. The chain order of the inner leaf of the liquid-ordered, cholesterol-rich, phase is increased to $\psi_i \approx 0.05$ in our example, and decreased to $\psi_i \approx -0.05$ in the liquid-disordered, cholesterol-poor, phase. This illustrates our main point that the phase transition, which is driven primarily by the concentration in the outer leaf, is nonetheless reflected in the chain order of the inner leaf due to the interleaf coupling. At the lower transition, the chain-order parameter of the inner leaf undergoes a large jump, as expected.

In contrast to the situation above in which we have assumed that the concentrations of the two leaves differ considerably, the behavior of all order parameters in artificial bilayers in which the leaves are of identical composition is rather different. In this case, the coefficients are $a_i = a_o$, $b_i = b_o$, etc., and one finds from the expressions in Eq. 11 that the mixing angle $\theta = \pi/4$. The behavior of the system is as follows.

When the temperature is lowered at a given value of $\phi_o = \phi_i$, a value which depends upon the particular initial system, one first encounters a first-order transition into two phases, one cholesterol-rich, the other cholesterol-poor, as shown in Fig. 6. The value of σ undergoes a jump, but the value of τ does not.

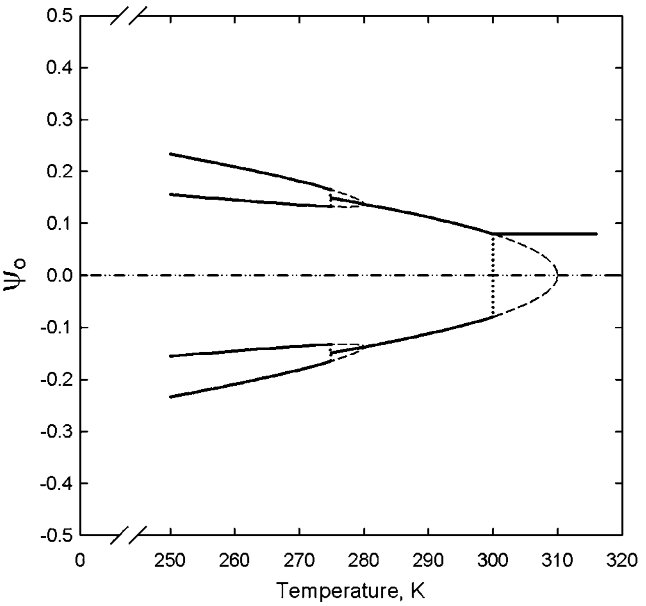

FIGURE 4 Behavior of the chain order parameter of the outer leaf, ψ_o. At the upper transition, which is encountered at $T = 300$ K, there is phase separation between a liquid-ordered, cholesterol-rich phase, $\psi_o \approx 0.08$, and a liquid-disordered, cholesterol-poor phase, $\psi_o \approx -0.08$. The magnitude of the order parameter increases with decreasing temperature. When the lower transition is encountered at ~275 K, the order parameter undergoes another, but much smaller, jump. There are now four phases characterized by four different values of ψ_o. Projections of the phase boundaries on the T, ψ_o plane are shown as dashed lines. Coexisting values of ψ_o are connected by dotted lines.

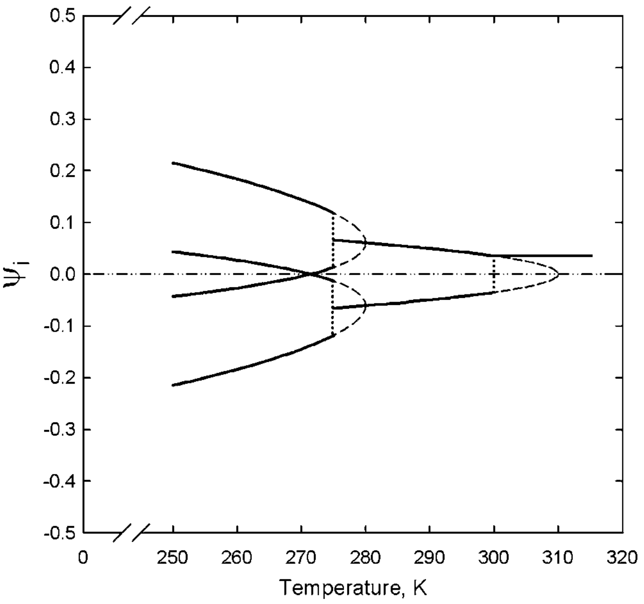

FIGURE 5 Behavior of the chain order parameter of the inner leaf, ψ_i. At the upper transition, which is encountered at $T = 300$ K, there is phase separation between a liquid-ordered, cholesterol-rich phase, $\psi_i \approx 0.05$, and a liquid-disordered, cholesterol-poor phase, $\psi_i \approx -0.05$. When the lower transition is encountered at ~275 K, the order parameter undergoes another, much larger, jump. There are now four phases characterized by four different values of ψ_i. Projections of the phase boundaries on the T, ψ_i plane are shown as dashed lines. Coexisting values of ψ_i are connected by dotted lines.

Thus from the expressions in Eq. 10, the concentrations in each leaf undergo equal discontinuities, and the chain-order parameters in each leaf also undergo equal and abrupt changes. In the two coexisting phases, there is no distinction between the inner and outer leaves. However, as the temperature is lowered further, the second transition is encountered. The value of τ becomes nonzero and the concentrations and lipid order parameters are different in the two leaves.

At temperatures below the second transition, there are four phases that can coexist. In one, the outer leaf that had been cholesterol-rich becomes even richer, while the inner leaf, which had been cholesterol-rich, becomes less rich. In a second phase, the roles of the inner and outer leaves are simply reversed. In the third phase, the outer leaf that had been cholesterol-poor becomes even poorer, whereas the inner leaf, which had been cholesterol-poor, becomes less so. In the fourth phase, the roles of inner and outer leaves are simply reversed. In experiment, the concentrations of the two leaves are rarely controlled. As a consequence, at coexistence, one will most likely observe all four phases. This will appear, primarily, as the coexistence of cholesterol-rich and cholesterol-poor phases. However, closer inspection should reveal that the cholesterol-rich regions have a bimodal distribution, consisting of those somewhat more enriched, and those somewhat less enriched in cholesterol. Similarly, the cholesterol-poor regions should be characterized by a bimodal distribution. If the bimodal nature of the distribution cannot be resolved experimentally, the second transition should make itself evident at least in a broadening of the distribution of concentration differences in both cholesterol-rich and -poor regions.

Just below the second transition temperature, the sum of the magnitudes of the composition order parameters, $|\phi_i| + |\phi_o|$ is small, while that of the difference, $|\phi_i| - |\phi_o|$, is even smaller. Were there no coupling between leaves, this difference would be identically zero, and would remain so even as the magnitude of the sum of order parameters would increase with decreasing temperature. There would be only two possible, equal and opposite, values of the order parameters ϕ_i and ϕ_o. In the regime of weak coupling between leaves depicted in Fig. 6, these two possible values, large in magnitude, are still clearly seen at low temperatures. However, due to the coupling between leaves, the difference in the magnitude of the compositional order parameters also eventually increases as the temperature decreases, just as the sum of the orientational order parameters does. As a consequence, in one phase the magnitude of ϕ_i becomes large, whereas ϕ_o remains small. In a second phase, the roles of inner and outer leaves are reversed. In phases three and four, the signs of the order parameters are simply reversed. Again, this behavior is shown in Fig. 6. The behavior of the chain-order parameters as a function of temperature is shown in Fig. 7.

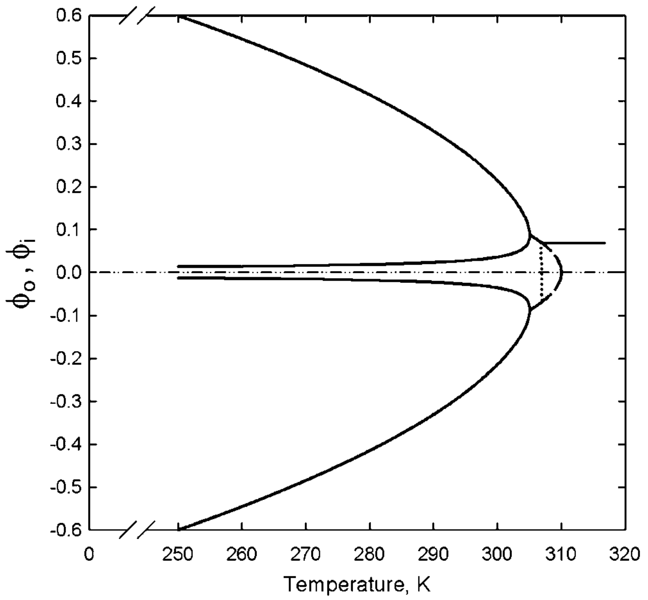

FIGURE 6 Behavior of the concentration order parameters, ϕ_o and ϕ_i, in a bilayer with leaves of equal composition. At the upper transition, the concentrations of the cholesterol-rich and cholesterol-poor phases are the same in each of the two leaves. At the lower transition, which is a continuous one, this symmetry is broken, and the concentrations in the two leaves are different.

Note that at the lower transition, neither the concentration nor chain order parameters undergo a jump, but instead, change continuously. This is because the thermodynamic trajectory of the system passes through the critical point of the lower transition. This can be seen from Eq. 10 and the fact that $\phi_o = \phi_i$ in each of the two coexisting phases as the second transition is approached from above. This implies that τ is zero just above the transition, i.e., it takes its critical value. Therefore, we would expect critical fluctuations between the two leaves to occur at this second transition.

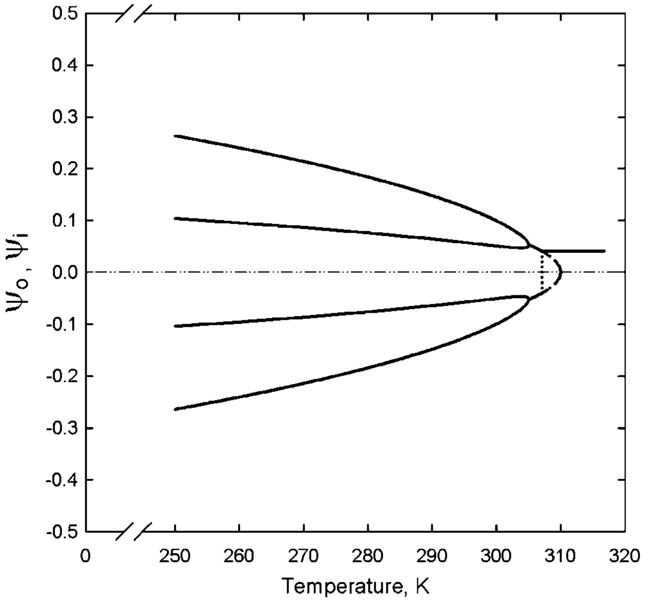

FIGURE 7 Behavior of the chain-order parameters, ψ_o and ψ_i, in a bilayer with leaves of equal composition. At the upper transition, the chain orders of the cholesterol-rich and cholesterol-poor phases are the same in each of the two leaves. At the lower transition, which is a continuous one, this symmetry is broken, and the chain orders in the two leaves are different.

For the plot of Figs. 6 and 7 we have taken $\Gamma/b = 0.75$, $\Omega/b = 0.75$, and $b/g = 40$ K as in the previous figures. However in contrast to that system, we take transition temperatures that are quite close to one another, as expected for two weakly coupled leaves of equal compositions: $d_2/2d_4 = (T - 310)/320$, $f_2/2f_4 = (T - 305.1)/320$.

DISCUSSION

We have considered a membrane of two leaves consisting of lipids and cholesterol. The composition of the outer one is such that, were both leaves of this composition, then phase separation would occur at physiological temperatures between a cholesterol-rich ordered liquid and a cholesterol-poor disordered one; e.g., its composition mimics that of the outer leaf of the plasma membrane (1,2,22–24). The composition of the inner leaf is such that, were both leaves of this composition, phase separation would not occur at physiological temperatures; e.g., its composition mimics that of the inner leaf of the plasma membrane (25). We have asked what occurs in a membrane of two such disparate leaves when they are coupled, either by interdigitation of the lipid tails and/or by rapid exchange of cholesterol (5–7). The result is that both leaves are expected to undergo phase separation at physiological temperatures into cholesterol-rich and cholesterol-poor liquids. The difference between the two cholesterol concentrations in the inner leaf in the two phases is, however, less than the difference in cholesterol concentrations in the outer leaf. This is because of the following chain of effects: the concentration difference in the outer leaflet interacts with the lipid order in the outer leaflet, with a strength Γ_o; this lipid order in the outer leaflet is coupled to the lipid order in the inner leaflet, with strength Ω; and this order in the inner leaflet interacts with the concentration difference in the inner leaf, with a strength Γ_i. This causal chain is clearly reflected in Eq. 16. Probably of greater importance is the result that the lipids in the inner leaf in the liquid-ordered region are indeed more ordered than they were before the transition. They are less ordered than the lipids in the outer leaf, but the ratio of ordering in the two leaves is only reduced by the single coupling strength Ω, as seen from Eq. 18. Because the lipids in the inner leaf will be better ordered in the liquid-ordered phase, the areal density in this phase will be greater than the areal density of lipids in the inner leaf of the liquid-disordered phase. This provides a mechanism by which the liquid-ordered phase can affect those proteins in the cytosol which sample the inner leaf of the plasma membrane by means of short acyl chains, because the partition coefficient of such chains is surely affected by the differing areal densities in the inner leaf of the liquid-ordered and liquid-disordered phases.

The advantage of the simple Landau theory is that it demonstrates quite generally that lipid ordering occurs in both leaves of the considered bilayer. Its disadvantage is that, without knowledge of the coefficients in the free energy (Eq. 5), the theory cannot state how large the ordering of the lipids in the inner leaf will be. Further, one does not know just how sensitive the partition coefficient of a protein's acyl chain is to the difference in ordering of liquid-ordered and liquid-disordered regions. Answers to these questions await a more microscopic theory.

Lastly, we have shown that the coupling of two identical leaves of an artificial bilayer should manifest itself in two distinct transitions. In the first of these, phase separation occurs between cholesterol-rich and -poor phases whose inner and outer leaf compositions are the same. At a second transition temperature, which is not expected to be too far below the first, the symmetry between inner and outer leaf compositions is broken. Furthermore, this second transition can be a second-order one, in the universality class of the two-dimensional Ising model, and the effect of critical fluctuations between leaves could be manifest in experiment.

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