

Theory of the intermediate rippled phase of phospholipid bilayers

(phase transitions/membranes/modulated structures/two-dimensional systems)

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ABSTRACT A mathematical model of the periodic, rippled $P_{\beta'}$ phase of phosphatidylcholine is described. In this model the $P_{\beta'}$ phase shows a periodic variation in membrane properties corresponding to a lateral periodic variation between fluid-like and crystal-like states. The model is based on a curvature-dependent Landau–de Gennes free-energy functional. Our proposed form of the curvature-dependent free energy remedies a subtle mathematical flaw in an earlier model having similar physical content [Falkovitz, M. F., Seul, M., Frisch, H. L. & McConnell, H. M. (1982) *Proc. Natl. Acad. Sci. USA* 79, 3918–3921].

There have been numerous experimental and theoretical studies of the rippled ($P_{\beta'}$) phase of phosphatidylcholine bilayers. Leading references to relevant literature can be found in a recent communication by Parsegian (1). See also Nagle (2). This thermodynamic phase is observed over moderate temperature ranges (10–30°C) for a number of phosphatidylcholines, and is of special interest because it shows long-range, long-wavelength (≈ 200 – 300 Å) periodic structures (1–3). At higher temperatures, the rippled phase undergoes a first-order phase transition to a fluid, flat membrane (L_{α} phase). At lower temperatures, the rippled phase undergoes a first-order phase transition to a crystalline, flat phase, L_{β} . The rippled phase is thus a thermodynamically stable phase intermediate between the solid L_{β} phase and the fluid L_{α} phase.

In previous work it has been suggested that this periodic structure of the $P_{\beta'}$ phase reflects a periodic variation between a solid-like and a fluid-like structure together in a single thermodynamic phase (4). As pointed out to one of us (H.M.M.) by G. Kirk and S. M. Gruner and by J. Nagle and S. M. Bhattacharjee, the model previously employed to describe this intermediate phase contains a mathematical flaw. A model of the $P_{\beta'}$ phase proposed by Doniach has a similar difficulty (5). In both cases a low-amplitude, high-spatial-frequency “sawtooth” solution is mathematically acceptable but physically unreasonable. Such unrealistic solutions can, of course, be eliminated by including higher-order derivatives in the free energy function. The purpose of the present paper is to provide such an improved Landau–de Gennes free energy expression that yields a rippled intermediate thermodynamic phase with physical properties similar in essential respects to those that have been suggested earlier (4).

LANDAU–DE GENNES FREE ENERGY

We employ the following expression for the free energy (per unit area) of bilayer membrane

$$G = G_0 + G_1, \quad [1]$$

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where

$$G_0 = \frac{1}{2}(\bar{T}t^2 - 2t^3 + t^4) \quad [2]$$

$$G_1 = \frac{1}{2}(t'')^2 + \alpha t t'' \quad [3]$$

Here, t is an order parameter that describes the membrane thickness, specifically,

$$t = (d - d_f)/(d_s - d_f), \quad [4]$$

where d is the thickness of the membrane at the rescaled dimensionless temperature \bar{T} , and d_f and d_s are the thicknesses of the fluid and solid membrane phases, respectively, at $\bar{T} = 1$. This thickness parameter is numerically nearly equal to the lipid area order parameter that has been discussed elsewhere (4, 6, 7).

The function $G_0(t)$ is the free energy per unit area of a membrane with uniform thickness. G_0 has two minima: $t = 0$ corresponding to a thinner fluid phase, and $t = [3 + (9 - 8\bar{T})^{1/2}]/4$ corresponding to a thicker solid phase. The transition between these two phases, as predicted by G_0 alone, takes place at $\bar{T} = 1$ and is first order. The quantity G_1 is a curvature-dependent correction to G_0 . In this expression, primes denote differentiation with respect to position x . We consider only one-dimensional spatial variations of $t(x)$; and, for present purposes, we shall ignore the difference between curvature and t'' . With $\alpha > 0$, G_1 is the simplest mathematical expression coupling membrane thickness to membrane curvature. This term favors negative curvature ($t'' < 0$) for $t > 0$, positive curvature for $t < 0$, and a flat surface for $t = 0$. A more physically intuitive interpretation of G_1 will be presented in *Discussion*.

Our problem is to determine the variation of thickness with position $t = t(x)$ such that the following expression is a minimum.

$$G\{t\} = \frac{1}{L} \int_0^L G dx. \quad [5]$$

Here L is a suitably large distance in the plane of the membrane. As described above, there are two local minima with $t = \text{constant}$. As a first approximation, we seek a third local minimum using the following trial function.

$$t(x) = A + B \cos kx. \quad [6]$$

Numerically, we find that, for $\alpha > 0.335$ and for a limited α -dependent temperature range, the rippled phase provides an absolute minimum. For this phase, $k^2 = \alpha$, and the amplitude of the ripple is given by

$$A = \frac{1}{2} + \{\text{sgn}(r)\} \{(3|q|)^{1/2} \sin[\frac{1}{3} \tan^{-1}(q^3/r^2 + 1)^{1/2}] - (|q|)^{1/2} \cos[\frac{1}{3} \tan^{-1}(q^3/r^2 + 1)^{1/2}]\} \quad [7]$$

$$B = (\frac{1}{3}[(\alpha^2 - \bar{T})/2 + 3A - 3A^2])^{1/2} \quad [8]$$

$$q = (2\bar{T} - 4\alpha^2 - 3)/60$$

$$r = (\bar{T} - 1)/40.$$

The membrane thickness $t(x)$ calculated by using the trial function Eq. 6 is shown in Fig. 1 for $\alpha = 0.4$.

To find the exact periodic local minima of Eq. 5, we have solved numerically the Euler-Lagrange equation corresponding to Eq. 5,

$$\bar{T}t - 3t^2 + 2t^3 + 2\alpha t'' + t''' = 0. \quad [9]$$

Two numerical techniques have been used, a shooting scheme and an implicit relaxation scheme. Both methods give identical results, which are surprisingly close to those obtained with the trial function Eq. 6. A comparison of the exact $t(x)$ with the trial $t(x)$ is given in Fig. 1. Plots of the free energy vs. temperature are given in Fig. 2 for various values of the coupling constant α . Note that the range of temperature in which there is rippling increases with the coupling constant. Results obtained with the trial function and exact function are the same to within 10%.

Plots of ripple amplitude vs. temperature are shown in Fig. 3. It will be seen that increasing the coupling parameter α increases the ripple amplitude. There is also a temperature dependence of this amplitude, such that the amplitude always decreases with increasing temperature.

DISCUSSION

The molecular origin of the curvature-dependent free energy G_1 can be made plausible, as follows. Rewrite G_1 in the form

$$G_1 = \frac{1}{2}(t'' + \alpha t)^2 - \frac{1}{2}\alpha^2 t^2 \quad [10]$$

and note that the last term can be combined with G_0 by redefining \bar{T} to be $\bar{T} - \alpha^2$. Thus, G_1 is effectively a nonnegative energy that favors $t'' = -\alpha t$. In the crystalline L_β phase, where $t \approx 1$, the hydrocarbon chains of the phospholipids are fully extended (8, 9), and the projected area of each choline head group can, depending on the assumed configuration, exceed that of the two chains. Thus there may be a tendency of the headgroup region to "splay" ($t'' \approx -\alpha < 0$). [Similar effects are shape-determining factors for detergent micelles (10).] In the fluid phase of the bilayer, where $t \approx 0$, the projected area of the hydrocarbon chains is larger and, in the limit of a thin membrane, must exceed the projected area of

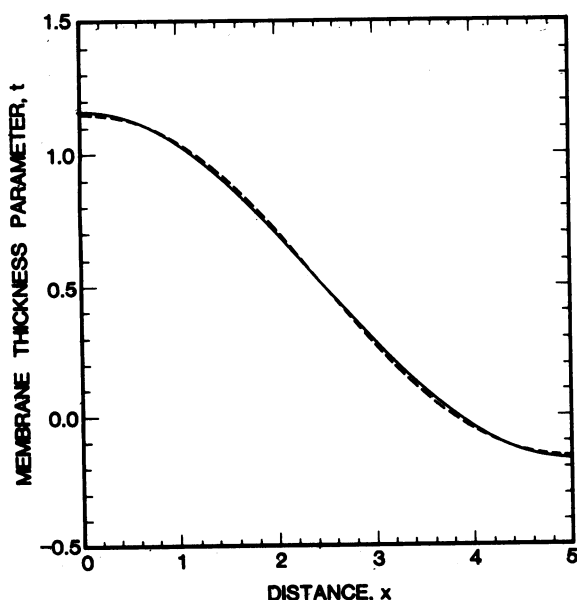


FIG. 1. Membrane thickness parameters $t(x)$. The two periodic variations shown correspond to the $A + B \cos kx$ trial function (---) and the exact solution to Euler-Lagrange Eq. 9 (—).

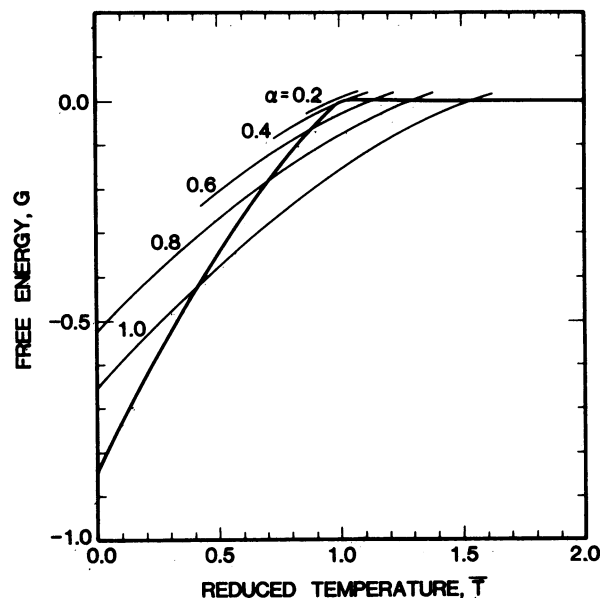


FIG. 2. Calculated free energy G per unit area of membrane. The heavy line to the left of the figure (lower temperatures) gives the calculated free energy of the flat solid bilayer phase, and the heavy line to the right of the figure gives the free energy of the flat fluid bilayer phase. The thin lines corresponding to different values of the coupling parameter give the free energy of the rippled intermediate phase.

the choline head groups. In this case the membrane surface will tend to be flat ($t'' \approx 0$) or perhaps to splay in the opposite direction (t'' positive) (analogous to an inverted micelle shape) (10). The idea that the shape of amphiphilic molecules affects membrane curvature is broadly recognized. Here we emphasize the fact that the "shape" of a chain-containing molecule changes at a chain melting phase transition.

The intermediate rippled phase predicted by our model occurs for temperatures near $\bar{T} = 1$, where neither the solid nor the liquid phase is overwhelmingly most favorable energetically. Under these conditions, the curvature effect described

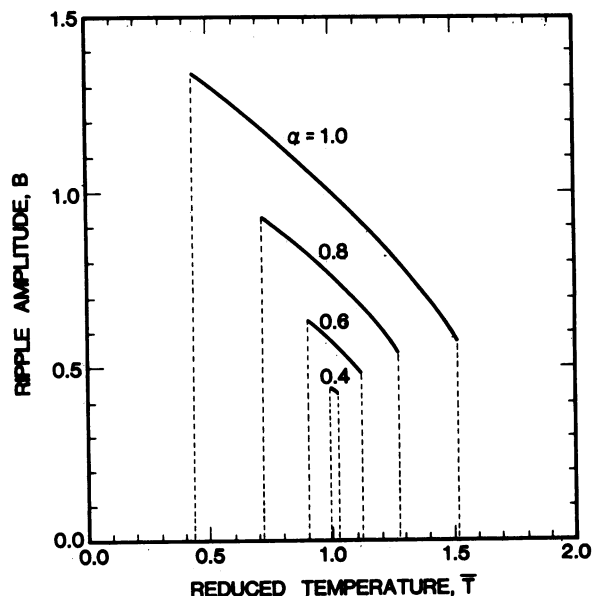


FIG. 3. Ripple amplitude as a function of the reduced temperature \bar{T} for different values of the coupling parameter α . The plots show that the ripple amplitude decreases with increasing temperature for a given value of the coupling parameter α .

above makes it energetically favorable for the surface of the membrane to curve inward in solid-like regions and, correspondingly, outward in liquid-like regions. The result is a periodic variation in membrane thickness and, presumably, a corresponding periodic variation in membrane "fluidity."

Some quantitative features of our model should not be taken literally. For example, as discussed previously, if the free energy function we have used for the bilayer is applied to a monolayer, and a 180° phase shift is made between the thickness variations on the two halves of a bilayer, then the periodic variation of membrane thickness is converted into a periodic corrugation, or undulation of a membrane of approximately constant thickness. There remains a lateral solid-fluid periodic variation, but this has opposite phase on the two sides of the bilayer. See figure 3 of ref. 4. The nearly pure cosine-like variation of membrane fluidity required for validity of the latter picture is consistent with our calculations but may be the result of our specially simple choice of the curvature energy G_1 .

In view of the observed 4.2-Å x-ray reflection (8, 9), there is no doubt that some of the hydrocarbon chains in the $P_{\beta'}$ phase are packed in crystalline arrays. The main question concerning the validity of the model is the degree to which some of the lipids in the $P_{\beta'}$ phase are in a fluid-like state. We would consider the model as not applicable to the $P_{\beta'}$ phase if all the hydrocarbon chains in this bilayer phase were *trans* and extended perpendicular to the bilayer plane, as is proposed in most models of the $P_{\beta'}$ phase (9, 11–15). At the present time neither the x-ray diffraction data nor the magnetic resonance data appear to us to prove or disprove our model. Raman spectroscopic determinations of the proportion of *gauche* vs. *trans* bonds may provide a critical test of the model. The available Raman data do indicate that *gauche* bonds are present in the $P_{\beta'}$ phase with about 1 *gauche* conformer per chain on the average (16–18). This could be interpreted as 2 *gauche* conformers on some chains, and no *gauche* conformers per chain on other chains. This is certainly the right order of magnitude expected for our model. For a recent review of relevant physical data on bilayers, see Marsh (19).

In conclusion, it is interesting to consider the temperature at which the phases P_{β} and L_{α} coexist. On the basis of the Gibbs phase rule one would not expect the fluid L_{α} to "invade" or mix with the putative fluid regions of the $P_{\beta'}$ phase. However, there is compelling evidence that a two-dimensional liquid mixture of cholesterol and phosphatidylcholine does invade the $P_{\beta'}$ phase, separating the crystalline 200-Å domains into parallel regions separated in a regular way by larger regions of the fluid mixture (20, 21). This interesting

phenomenon demonstrates that narrow 200-Å strips (ripples) of crystalline phospholipid can coexist with the fluid state of lipids.

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