

A THEORETICAL STUDY OF LIPID-PROTEIN INTERACTIONS IN BILAYERS

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ABSTRACT We present a theoretical study of the effect of different types of lipid-protein interactions on the thermodynamic properties of protein-containing lipid bilayers. The basis of this work is a theoretical model for pure lipid bilayer phase transitions developed earlier by Scott. Simple assumptions on the nature of the lipid conformations near a protein strongly affect the predicted properties of the model. Here we consider (a) random protein-lipid contacts, (b) enhanced contact between protein and lipid with a number of gauche bonds, and (c) enhanced contact between protein and all-*trans* but tilted lipid chains. Comparison of predicted results with experimental data seems to favor point *c* above but, by itself point *c* does not work well at larger protein concentrations. The results are discussed in the light of spectroscopic data, lipid-protein (plus annular lipid) miscibility, and interprotein forces.

INTRODUCTION

Molecular interactions in reconstituted bilayers containing both phospholipid and protein are of considerable interest in studies of structure-function relationships in membranes. While the detailed nature of these interactions must depend upon the specific structures and conformations of the molecules in question, experiments using mainly phosphatidylcholines reconstituted with a variety of membrane proteins (1-6) suggest several general features. (a) There is an annulus of lipid surrounding each protein in which the lipid states differ from those in the protein-free region of the bilayer. The annular lipid exchanges with the unperturbed lipid at a rate that is fast on a nuclear magnetic resonance (NMR) timescale, but slow on an electron spin resonance (ESR) timescale (2-7). Spectroscopic data indicate that the annular lipid is disordered conformationally. There are two ways in which this disordering may occur. Lipid chains may have large numbers of gauche rotations as they fit against a rough protein surface. Or, equally consistent with the data, the chains may be in nearly all-*trans* conformations, but tilted with respect to the bilayer normal (7). In either case the implication is that there is a preferred subclass of lipid conformations for the annular lipid that maximizes the Van der Waals contacts between the molecules. (b) The presence of small amounts of protein leaves the lipid-phase transition temperature unchanged while the enthalpy change, at the transition, decreases with increasing protein concentration. Above a

certain protein concentration, the transition does not occur. The lower or pretransition in phosphatidylcholines disappears at extremely small protein concentrations (1,8). (c) Freeze-fracture studies of protein-containing bilayers quenched from temperatures above the lipid-phase transition show particles (presumably membrane proteins) distributed randomly throughout the lipid bilayer. Bilayers quenched from temperatures below the phase transition temperature show regions containing high concentrations of protein (plus some lipid) and regions containing no protein (1, 9).

Although we shall concentrate on the general properties listed above, note that highly specific interactions between lipids and proteins are also common. In the case of Ca^{2+} ATPase, while the protein binds many different types of lipids equally well, the activity of this protein varies strongly with the chain length of the lipid surrounding it (10, 11). Supplementing the experimental work, a number of theoretical studies have appeared recently. Owicki and McConnell (12, 13) have developed a Landau theory for the decay of order in a lipid bilayer as a function of distance from the embedded protein, and this theory has been further extended by Jähnig (14). In the Landau type theories, intermolecular interactions are not directly involved; the calculations begin at a thermodynamic level. Obviously a deeper understanding of the system is obtained if one starts at a molecular level, but this is difficult to do because of the complex nature of the interactions at this level. The first quantitative attempt in this direction is due to Marčelja, (15). Using his statistical model of the bilayer (16), Marčelja constrained lipids in close contact with protein to be more highly spatially and rotamerically

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ordered than the unperturbed fluid lipid. The calculation predicted an attractive lipid mediated interprotein force, capable of producing protein aggregation, but the initial assumptions regarding the order of the annular lipid are not consistent with the more recent spectroscopic data. Scott and Cheng (17) considered the effect of proteins using a hard-disk model for lipid bilayers by inserting large inert disks, which represent proteins, into the system. The insert disks greatly increased the area per molecule, thereby increasing the Van der Waals energy, so that the larger lateral pressures were introduced phenomenologically to stabilize the transition temperatures. This led to a critical point in the pressure-protein concentration plane similar to that discussed by Jähnig (14), but for which no firm experimental evidence exists. An important point in all the theories that predict phase transitions (12–14, 17) is that the insertion of proteins produces changes in the transition temperatures (except for one special case in the Landau Theory). This result, seemingly straightforward, is in fact contrary to the experimental data listed earlier.

In this paper we present a direct, molecular approach to the question of protein-lipid interactions. The objective of our work is to determine the theoretical consequences of several different types of lipid-protein interactions. Based upon spectroscopic data we evaluate several different types of preferred lipid conformations for the annular lipid. Inserting these preferences into a statistical mechanical calculation, we determine the predicted thermodynamic properties of the system, specifically the location and nature of the lipid-phase transition. This prediction is compared directly with the thermal data and the validity of the initial hypothetical lipid-protein interaction is thereby assessed. The calculations are based upon a theoretical model (18, 19) for lipid bilayers that accurately describes both phase transitions in phosphatidylcholines, as well as the single transition in phosphatidylethanolamines. The theory considers the rod-shaped projection of phospholipid molecules into the bilayer plane. Different length to width ratios for the shadows correspond to different acyl chain conformations, and these are classified and assigned statistical weights to reduce the very large number of states to a realistic, but manageable subset. The system is then analyzed using the Scaled Particle Theory (SPT), a method known to be very accurate for hard rods of the type we consider (20). Details are given in reference 18. The theory provides both a hydrocarbon-chain melting transition (the main transition) and, for suitable rod dimensions, a molecular axial rotational transition (the pretransition). At the main transition the number of gauche isomers, portrayed as elongated rods, increases; the layer expands from 45 Å²/mol to ~65 Å²/mol, and the enthalpy of transition is ~9 kcal/mol for dipalmitoylphosphatidylcholine (DPPC) with $T_m = 41^\circ\text{C}$. The pretransition, which occurs at 35°C for DPPC, is first order with an enthalpy of ~0.5 kcal/mol. At this temperature there is an increase in area per molecule from ~44 Å²/mol to ~45 Å²/mol, and the system

changes from an oriented layer to a rotationally isotropic layer, signifying long-axis rotation (21). The model is two-dimensional and so it cannot describe the ripples that also occur between the pretransition and main transition. These are described by a complementary mechanism involving asymmetric head-group packing in competition with vertical displacement of molecules out of the bilayer plane (22).

In this work proteins are considered large objects of circular cross section. The circular shape assumption is unlikely to affect results as long as the protein considered is much larger in cross section than is the phospholipid. In addition, the use of a smooth surface (rather than a molecularly rough shape) will not be a serious compromise. As shown subsequently one can, in the SPT, allow for some surface roughness in the calculations. In the following section we describe the types of calculations performed. The final two sections contain results and our conclusions, respectively.

THEORETICAL MODEL

As described above, lipid and protein molecules are depicted by their projections onto the bilayer plane. The resultant shadows experience each other via the excluded areas they present and via long-range attractive Van der Waals (VDW) interaction. The lipid shadows are rodlike in shape. The protein is represented by a circle of radius 15 Å that is roughly the size of a single ATPase as estimated from the molecular weight of the protein. Its excluded area interaction with lipid is treated as that of a smooth hard disk interacting with rods of various lengths. However, later we do allow the surface of the protein disk to become uneven.

The contribution of the hard-core packing of the rods and disks to the free energy is calculated using SPT. In addition we must include the attractive Van der Waals energy. The original model (17), modifying a suggestion by Nagle (23), treats the Van der Waals interaction energy thus

$$E_{VDW} = \frac{-C}{(A - A_0)^{3/2}}, \quad (1)$$

where A is the thermodynamically averaged area for molecule, C a phenomenological constant, and A_0 (kept equal to 38 Å² for reasons described in [17]) a means of softening the excessively hard-cores of the lipid molecules. Originally, A represented the area per lipid molecule. One naturally expects the protein to contribute to the Van der Waals energy of the system, however, so Eq. 1 must be modified. Here we suppose that the protein contributes in exactly the same manner as if the region occupied by protein were actually occupied by lipid chains. To do this, we replace the overall average area per molecule A , in Eq. 2, by the area per lipid molecule, $A_L = (A - X_p A_p)/X_L$:

$$E_{VDW} = \frac{-C}{\left(\frac{A - X_p A_p}{X_L} - A_0\right)^{1.5}}. \quad (2)$$

In Eq 2 C , A , and A_0 are as defined in Eq. 1, and A_p is the area of a single protein molecule, and X_L and X_p are the lipid and protein concentrations, respectively. Eq. 2 reduces to Eq. 1 when $X_L = 1$. Eq. 2 provides an estimate of the van der Waals energy of the system under the assumption that the protein-lipid and protein-protein contributions are roughly of the same strength as the lipid-lipid contributions.

The construction of the Gibbs Free Energy of the system follows exactly as in reference 17 with the Van der Waals energy given by Eq. 2

and with extra excluded area terms associated with protein-lipid interactions added to the SPT expression. These extra areas have the form

$$A_{ex}^i = \pi(r + R)^2 + (r + R) a_i, \quad (3)$$

where r is the lipid-cap radius (the same for all lipid states), R is the protein radius, and a_i is the length of the rod associated with lipid state, i . Protein-protein repulsive interactions must also be considered. The excluded area function used in SPT to this end is

$$A_{ex}^{pp} = 4\pi R^2. \quad (4)$$

The use of SPT for mixtures of objects of varying shape has been described in detail elsewhere (18, 24, 25). The resulting expression for the Gibbs Free Energy is

$$\begin{aligned} G/RT = & \sum_i (\alpha_i \ln \alpha_i - \alpha_i \ln W_i) - \ln \left(\frac{\rho}{1 - \rho \sum_i \alpha_i A_i} \right) \\ & + \frac{1}{2} \rho \sum_{k,p} \alpha_k \alpha_p a_{kp} \frac{1}{\left(1 - \rho \sum_i \alpha_i A_i \right)} \\ & - \frac{C}{\left(\frac{A - X_p A_p}{X_L} - 38 \right)^{1.5}} + \pi A, \quad (5) \end{aligned}$$

where we use the notation of reference 18, the sums run over all the states of the molecules in the system. α_i is the fractional population of state, i ($\alpha_{\text{protein}} = X_p$), W_i its statistical weight, $W_{\text{protein}} = 1$, and A_i its hard-core area. $A = 1/\rho$ (without subscript) is the thermodynamic average area per molecule. The quantity, a_{kj} , is calculated from SPT and has the form (18)

$$a_{kj} = \pi b_k b_j + b_k a_j + b_j a_k + a_k a_j \sin \theta_{kj}, \quad (6)$$

if k and j both denote lipid states of length a_k , a_j , and cap radii, b_k and b_j , respectively, as given in reference 18¹;

$$a_{kp} = 2\pi R b_k + R a_k \quad (7)$$

if k is a lipid state, and p is the protein state with protein radius, R . For two-protein interactions, we have a contribution

$$a_{pp} = 2R^2 \pi. \quad (8)$$

As in previous work, the procedure is to minimize the Gibbs Free Energy with respect to the α_i and A , at fixed T , and π and X_L ($X_p = 1 - X_L$). Phase transitions occur when the minimization routine yields two or more distinct solutions. In this case the correct state is the solution for which Eq. 5 is an absolute minimum, and under some circumstances this minimum changes from, say, an ordered to disordered phase, which signals a first-order phase transition. Using this approach we have examined lipid-protein interactions under a variety of circumstances. We present our results in the following section.

RESULTS

Nonspecific Lipid-Protein Interactions

The simplest model for lipid-protein interactions that we consider is a straight forward calculation of the phase properties as a function of protein concentration using the free energy of a random assembly of molecules as

¹In reference 18 the values of b_k should for DPPC be 5.27 for all k and $C = 1.39 \times 10^5 \text{ kcal}/\text{\AA}^3$.

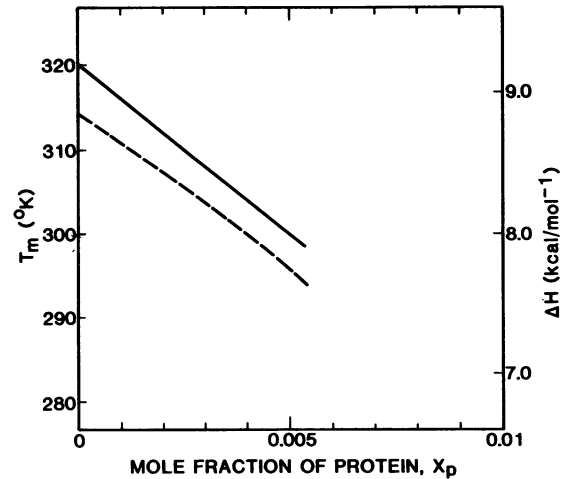


FIGURE 1 Plots of main-phase transition temperature, T_m (—), and enthalpy change, ΔH (---) vs. protein concentration for the case of random lipid-protein contacts.

described in the previous section. In this case the contact between the protein disks and the lipid rods is random and nonspecific. In Fig. 1 we plot the main transition temperature and the enthalpy change vs. protein concentration. For the system considered, the sharp decrease in T_m is not surprising. The relatively large proteins will, regardless of their exact shape, act as a strong perturbant to lipid packing to the ordered phase. Even though the predominant interaction is of short-range, one cannot presume the resulting perturbations due to the proteins are of short-range (25), especially near a phase transition. Fig. 1 also shows that the enthalpy change at the transition decreases rather slowly with increasing X_p . This is a result of the fact that, with increasing X_p , one finds larger values for the area per molecule, A , in the disordered phase so that, despite the proteins, most of the lipid is able to take part in the disordering process. By extrapolation $\Delta H = 0$ at a lipid per protein ratio of 52:1. The experimental value for ATPase is 42:1 for a full bilayer.

Lipid-Specific Protein Interactions

While the basic thermodynamic assumption of random lipid-protein contacts is the most straightforward, the trends shown in Fig. 1 are at odds with experiments (1, 8) in which T_m remains constant but ΔH decreases more strongly with increasing X_p . The discrepancies are not surprising in light of spectroscopic data (1–7) described earlier that suggest enhanced lipid-protein interactions for specific lipid conformations. The way we chose to approach this specificity in our calculations was to allow the protein to interact favorably with particular lipid states by having the proteins provide a reduced excluded area to the chosen states. This was done by establishing a number of new lipid states. The new states are identical in energy and statistical weight to certain states available for pure lipid, but the maximal occupancy of these states is bounded by the

amount of annular lipid allowed. For Ca^{2+} ATPase (of radius $\sim 15\text{\AA}$) the accepted number of annular lipid per protein is 30 (15 for each monolayer of a bilayer) (25). The theoretical work we have done proceeds as follows. If lipid state k is to be specifically favored for interaction with protein, then we first create a new state, k' , with the same energetic and statistical properties as state k , and we insist $\alpha_{k'} \leq 15 X_p$. Then we replace the excluded area between the protein and the state k' (Eq. 3) by an expression with a smaller value of the rod length $a_{k'}$ so that $a_{k'} < a_k$. The physical implication is that, for the state k' , the relative excluded area produced by the protein is less than would normally be expected for a free lipid in state k . This may be due to surface roughness, chain folding or tilting into and around protrusions at the protein, or perhaps another mechanism that allows closer contact between the protein and state k as compared to other states. Given the current interpretations of the experimental data that annular lipid is not ordered, the logical first choice for the protein-favored lipid states are the excited states in which several gauche conformers can coexist. In the earlier theoretical work we found the most highly populated gauche conformers were those in the third excitation class of reference 18. For the DPPC models there are three such states, each with an average of 3.79 gauche rotations per molecule, and a hard-rod shadow length of 4.13\AA . The three states are all conformationally and energetically identical, but have different orientations in the bilayer plane. The solid lines in Fig. 2 show the plot of T_m and ΔH vs. X_p , which we obtain when we favor these states. Comparison with Fig. 1 shows that the T_m 's drop more rapidly with X_p in Fig. 2, and ΔH does not drop as rapidly in Fig. 2 as in Fig. 1. Favoring a disordered lipid for close contact with the proteins strongly favors disordering the system entirely. An attempt to

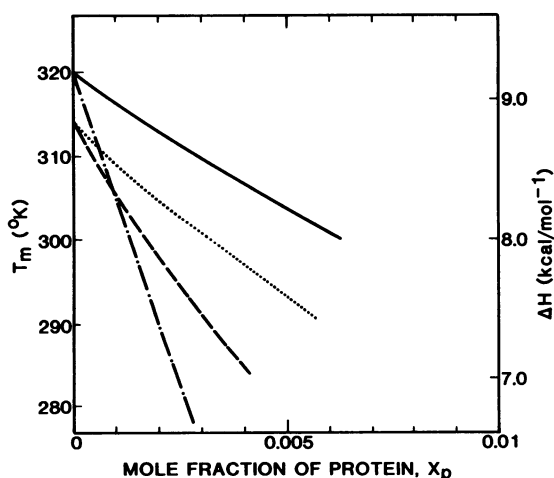


FIGURE 2 Plots of main-phase transition temperature, T_m (· · ·), and enthalpy change ΔH (—) vs. protein concentration for the case in which contact between protein and a chosen set of lipid exhibiting several gauche bonds was favored. Additional inclusion of a protein-lipid binding energy gave the dashed curve (—) for T_m vs. X_p , and the dash-dot curve (— · —) for ΔH vs. X_p .

change the results by introducing a phenomenological binding energy of the form

$$E_B = -\epsilon \alpha_p \quad (9)$$

to the free energy, where α_p is the population of the protein-favored lipid states, has the effect, for reasonable value of ϵ , of substantially increasing the slopes in the T_m vs. X_p and ΔH vs. X_p curves as shown by the dashed lines in Fig. 2. Since experimental data point towards a flat T_m vs. X_p curve these results argue against favorable contacts between protein and conformationally disordered lipid.

We are therefore led to consider favoring the all-*trans* lipid for close contact with the protein and give the annular state the same dimension as the ordered lipid state and proceed as described above. In this case our mechanism of reducing excluded area between proteins and preferred lipid can be interpreted as all-*trans* lipid chains tilting away from the bilayer normal, to enhance contact with the protein surface. Such contacts are consistent with the spectroscopic data on annular lipid (7). The results of these calculations are shown in Fig. 3. Although T_m still decreases with X_p , the slope of the line is lowered appreciably; the transition disappears at a 1:35 protein to lipid ratio. We have limited the occupancy of the annular lipid states to a total of 45 molecules per protein molecule. (Each of three orientational states may contain up to 15 lipids per protein.) If this constraint is relaxed to allow as many as 135 lipids in the annular states, we obtain the dashed curve in Fig. 3 in which the T_m vs. X_p curve has nearly zero slope for small X_p . In this case the lipids closely associated with the protein presumably exist in more than one layer. Within the present model it takes about this

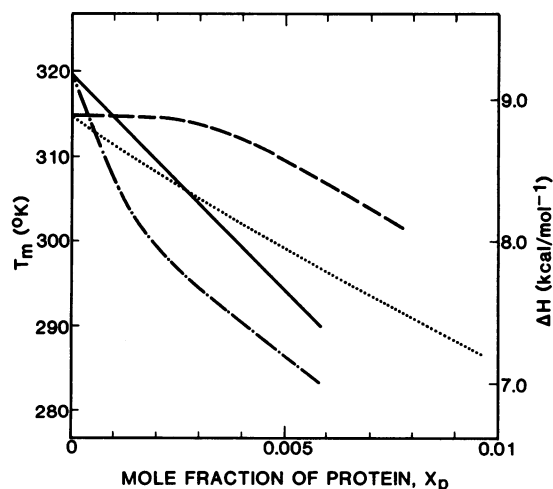


FIGURE 3 Plots of main-phase transition temperature, T_m , and the enthalpy change, ΔH , vs. protein concentration for the case in which contact between protein and lipid in all-*trans* conformation is favored. Two values of the maximum number of lipid molecules per protein allowed to participate in the favorable interaction are plotted. —, T_m vs. X_p for 135 lipids per protein; · · ·, T_m vs. X_p for 45 lipids per protein; —, ΔH vs. X_p for 45 lipids per protein; — · —, ΔH vs. X_p for 135 lipids per protein.

many ground-state lipids, closely associated with the proteins through reduced excluded area interactions, to produce a nonvarying transition temperature. Additionally, the ΔH at the transition now drops more rapidly as increasing numbers of the lipid are preferentially associated with the protein both above and below the phase transition. The transition disappears at a 1:100 protein to lipid ratio. Note that in the present model results are very sensitive to the value of the protein radius, but this sensitivity should be mainly reflected in the scale of the X_p -axis in Figs. 1–3 with X_p smaller for larger protein radius.

Pretransition

In all the studies discussed above we found that at very low protein concentration, below $\sim 10^{-3}$, the temperature at which the pretransition occurred dropped below 0°C. The protein strongly disrupts the long-axis molecular orientational ordering that occurs in this model at the lower transition.

DISCUSSION

We have used a statistical mechanical approach to examine the consequences of several different types of possible lipid-protein interactions in bilayers. The theoretical predictions are compared with experimental data and this provides a means for testing the feasibility of the various interactions. The results must, of course, be viewed in the light of the assumptions underlying the basic theoretical approach. In the present case the model does exaggerate the hard-core aspect of the molecules. It follows that perturbative effects of equally hard-core proteins may also be exaggerated somewhat.

Based upon our model one can rule out the hypothesis that the lipids in contact with protein are randomly selected from the bulk lipid, and exhibit a wide range of conformations. Models based on this assumption predict a T_m that declines rapidly with increasing X_p . Somewhat more surprisingly, our model also argues against a scheme whereby lipid with several gauche bonds per chain is favored for the annulus surrounding the protein. Models based on this assumption predict an even more rapidly falling T_m as X_p increases. Although an α -helix is rough on a small (atomic) scale, on the scale of ~ 10 Å the surface is fairly smooth, i.e., there are no large cavities into which chains or even whole molecules can disappear. We have further studied this problem in our model varying some of the lipid-protein excluded areas in a roughness parameter of atomic dimensions. The new parameter had very little effect on the properties of the models studied. The lipid-protein interaction mechanism that, in this model, gave the best results in comparison with experimental bindings, at least at small protein concentration, was that in which all-*trans* lipid is favored for the annular region. This result is consistent with phenomenological calculations by Pink et

al. (26), in which observed NMR spectral features were derived, assuming that, while lipid next to single proteins is highly ordered, lipid next to pairs and triplets of protein molecules is disordered. Also, studies of Ca^{2+} ATPase by fluorescence anisotropy have recently been interpreted as showing a layer of ordered lipid surrounded by a layer of highly disordered lipid at each protein (27). An alternative, reconciliation of the ordered vs. disordered annular lipid is that the molecules nearest the protein may be ordered conformationally but tilted with respect to the bilayer normal. Varying tilt angles for different phospholipids in contact with protein may allow for the observed binding behavior of lipids with Ca^{2+} ATPase (10, 11), in which there appeared to be little dependence upon the chain length. Although the model favoring all-*trans* lipid works best, one of its predictions is at variance with experimental findings, in that, large numbers of annular lipid are required, and at large X_p the transition temperature begins to drop. Allowing even larger numbers of annular lipid causes the transition temperature to rise somewhat before dropping rapidly. One way in which the theory could produce a flat T_m vs. X_p curve is if the size of the annular region increases as a function of X_p at a certain rate. This seems to us an unlikely circumstance.

Hesketh et al. (28) have suggested the notion of short-range perturbation of the lipid bilayer by incorporated protein, in which only a single annular shell of lipid is affected by a protein molecule. Within the framework of the calculations presented here such a concept is not likely. In a lipid bilayer with a random distribution of proteins co-operative effects due simply to packing lead, especially when close to the phase transition, to long-range correlations. Even if there is a specialized interaction involving, for example, the favoring of the ordered lipid state near a protein, our model requires that a rather large amount of lipid be allowed to associate closely with the protein (this may in part be an artifact of the infinitely hard-core interactions and the particular value of the protein radius used).

One of the assumptions of the theory presented here is that all molecules are homogeneously spatially distributed allowing no study of protein aggregation. If, in an otherwise pure lipid bilayer, proteins plus their annular lipid are completely immiscible with free lipid in both the fluid and the ordered phase, then the expected behavior of T_m and ΔH as functions of protein concentration is more or less what is observed; ΔH should decrease while T_m remains fixed with increasing x_p (29). While immiscibility of the protein-lipid complex in the ordered phase seems documented by freeze-fracture studies (1), there is no evidence for such behavior in the fluid phase. Given the present situation of immiscibility below T_m and miscibility above T_m , our theory results imply that a sort of hysteresis should be observed in the systems, with depressed T_m being obtained in cooling runs as compared with heating runs. These are apparently not observed.

Or, if protein-plus annular lipid is miscible in one lipid phase but not another, then one might expect the phase diagram of the mixture to resemble that of mixtures of, say, C_{14} and C_{18} lipids, which exhibit solid-phase immiscibility (29). While the existing scanning calorimetry data show no evidence for such phase properties (such as separate nearby peaks), more sensitive instruments and slower scan rates could provide more information on this question. Finally, in the present paper we do not consider interprotein forces. If, as experiments seem to suggest, the components are miscible in the fluid phase but not in the solid phase, then the existence of strong interprotein forces is implied. Such forces could presumably be electrostatic in nature or due to the lipid itself (15), and could depend in a complex manner on the protein concentration and the fluidity of the lipid. The existence of common properties for several lipid-protein assemblies suggests a nonspecific lipid-mediated interaction. The precise manner by which this interaction, if it exists, can produce the observed properties of the system is a subject of future work.

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