Protein–lipid interactions in bilayer membranes: A lattice model

(intrinsic molecules/phase transitions/boundary layers)

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ABSTRACT A lattice model has been developed to study the effects of intrinsic membrane proteins upon the thermodynamic properties of a lipid bilayer membrane. We assume that only nearest-neighbor van der Waals and steric interactions are important and that the polar group interactions can be represented by effective pressure–area terms. Phase diagrams, the temperature T_0 , which locates the gel–fluid melting, the transition enthalpy, and correlations were calculated by mean field and cluster approximations. Average lipid chain areas and chain areas when the lipid is in a given protein environment were obtained. Proteins that have a “smooth” homogeneous surface (“cholesterol-like”) and those that have inhomogeneous surfaces or that bind lipids specifically were considered. We find that T_0 can vary depending upon the interactions and that another peak can appear upon the shoulder of the main peak which reflects the melting of a eutectic mixture. The transition enthalpy decreases generally, as was found before, but when a second peak appears departures from this behavior reflect aspects of the eutectic mixture. We find that proteins have significant nonzero probabilities for being adjacent to one another so that no unbroken “annulus” of lipid necessarily exists around a protein. If T_0 does not increase much, or decreases, with increasing c , then lipids adjacent to a protein cannot all be all-*trans* on the time scale (10^{-7} sec) of our system. Around a protein the lipid correlation depth is about one lipid layer, and this increases with c . Possible consequences of ignoring changes in polar group interactions due to clustering of proteins are discussed.

The nature of the protein–lipid interaction and its consequences in biomembranes is being studied in many laboratories (1–5). In this paper we examine some of the effects of cholesterol-like or protein molecules upon lipid bilayer organization and thermal properties. We shall be concerned with phase diagrams, transition enthalpies, the lipid hydrocarbon chain order, and correlations which are reflected in measurements made via DSC (differential scanning calorimetry), electron spin resonance, deuterium magnetic resonance, fluorescent probes, and enzyme activity. We envisage the situation of a molecule with no specific interaction between it and the lipids of the bilayer, or situations in which different parts of the hydrophobic surface of a protein may interact differently with lipid chains. We also consider cases in which a protein has several lipid molecules specifically attached to its circumference for a time that is long compared to times characterizing lipid diffusion, rotation, or *trans*–*gauche* transitions. We shall use a lattice model of the lipid bilayer to examine these effects. Throughout we shall assume that the polar head group interactions are unaffected by varying the concentration of intrinsic molecules, but we shall remark upon some consequences if this does not hold.

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THEORY

A pure lipid system, with M carbon nuclei per chain, freezes onto a triangular lattice with the chains in their all-*trans* state. Above the transition temperature T_c , identified here as the peak position in DSC curves, the chains take on both positional and conformational disorder (6). For temperatures sufficiently below T_c , each chain projects upon the plane of the bilayer an effective area A_0 characteristic of the all-*trans* state which is taken to possess internal energy = 0. Above T_c the chains can be in any of a great number of excited “*gauche*” states, with various internal energies and effective areas (7). In addition, the lipid molecules possess energies due to diffusion and rotation. This system was modeled (8) by assuming that (i) each lipid chain occupies the site of a triangular lattice both in the gel and in the fluid phase; (ii) a lipid chain can be in one of two states: an all-*trans* (ground) state of energy 0, area A_0 , and degeneracy 1, and an excited state of energy E_e , area A_e , and degeneracy D^M which depends upon M ; and (iii) rotational and diffusional states can be included in E_e and D^M but are otherwise ignored. The replacement of all the excited states by one effective excited state implies that the time scale is long enough for the lipid chain to sample a sufficient number of its excited states. If the time for *trans*–*gauche* transition is about 10^{-9} to 10^{-10} sec, then we are concerned with times of about 10^{-6} to 10^{-7} sec. We assume that only van der Waals and steric interactions between nearest-neighbor pairs of chains need be considered and we denote them by: (i) $-J_0^M$, the all-*trans*–all-*trans* interaction energy, (ii) $-J_0^M J_2^M$, the all-*trans*–excited state interaction, and (iii) $-J_0^M J_3^M$, the excited state–excited state interaction. The Hamiltonian operator for this system is

$$H_L = \frac{-J_0^M}{2} \sum_{\langle ij \rangle} (G_i G_j + 2J_2 G_i E_j + J_3 E_i E_j) + \sum_i (\Pi A_0 G_i + (\Pi A_e + E_e) E_i). \quad [1]$$

Here the effect of the polar group interactions has been represented by a pressure–area term (9). G_i and E_i are ground (all-*trans*) state and excited state operators, respectively, for site i , Π is the effective lateral pressure, and $\langle ij \rangle$ denotes a sum over nearest neighbors. By using known or calculable values for all the parameters except J_0^M , we can fit the transition temperature to its experimental value and so determine J_0^M .

For a lipid bilayer with an intrinsic membrane molecule, the internal states of which may be ignored and which has approximately the same area as a lipid chain and does not specifically bind lipids, our model can be extended by assuming that each site can be occupied by either the molecule or a lipid

Abbreviation: DSC, differential scanning calorimetry.

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chain. The appropriate interactions between chains and the molecule are then introduced so that we add to Eq. 1 the following term, to obtain the total Hamiltonian, H ,

$$H_P = \frac{-J_0^M}{2} \sum_{\langle ij \rangle} (2J_1 G_i P_j + 2J_4 E_i P_j + J_P P_i P_j) + \Pi A_P \sum_i P_i, \quad [2]$$

$$H = H_L + H_P.$$

Here P_i is the projection operator for the molecule of area A_P at site i , and J_1 , J_4 , and J_P are the G - P , E - P , and P - P interactions. With reasonable values for J_1 , J_4 , and J_P , calculations upon the effect of varying concentrations of cholesterol in dimyristoyllecithin were made (8) where the limitations of the model were discussed. This is called *Model I*. The "cholesterol-like" case is characterized by choosing $|J_4| \ll |J_1|$, $J_c \approx J_1$ and $J_1 \approx 0.4$, and corresponds to a case where the hydrophobic surface of the molecule is sufficiently "smooth" so that it prefers to have rigid chains or another like molecule next to it, but in the fluid phase it mixes well with excited chains. Another case has $J_4 \approx -J_1$ so that homogeneous mixing does not occur in the fluid phase.

The dynamics of a system described by Eq. 1 or 2 have not been solved exactly. We, therefore, define a trial mean field Hamiltonian H_0 , linear in the projection operators, for which we can solve the dynamics exactly. The free energy per site of the original system is given by

$$F = F_0 + \frac{1}{N} \langle H - H_0 \rangle_0 + (x \ln x + (1-x) \ln(1-x)) / \beta,$$

$$F_0 = \frac{-1}{N\beta} \ln Z_0, \langle \dots \rangle_0 = \text{Tr}(e^{-\beta H_0} \dots) / Z_0, \quad [3]$$

$$Z_0 = \text{Tr}(e^{-\beta H_0}), \beta = 1/k_B T,$$

where k_B is Boltzmann's constant and Nx is the number of lattice sites occupied by intrinsic molecules, with N being the total number of sites. The unknown parameters that appear in H_0 are chosen by minimizing F with respect to them. The concentration of intrinsic molecules is $c = 2x/(1+x)$. The temperature at which the specific heat C_P has a maximum will be identified as the position of the maximum of the DSC curve and will be called $T_0(c)$, written simply as T_0 , with $T_0(0) = T_c$. The transition enthalpy is the change in enthalpy in some temperature range across $T_0(c)$, given by $\Delta H_{(\tau_1, \tau_2)} = H(T_0 + \tau_1) - H(T_0 - \tau_2)$, where $H(T)$ is the enthalpy at temperature T . When the peak is narrow, we choose $\tau_2 = \tau_1 = \tau$ and so define $\Delta H_{(\tau)} = \Delta H_{(\tau, \tau)}$. Phase diagrams were calculated as follows: For a given temperature, the free energies of two systems with concentration c_1 and c_2 ($c_2 \geq c_1$) of intrinsic molecules, were calculated. The total free energy of the combined system was taken as the sum of the two free energies (i.e., interactions between the two phases were ignored). c_1 and c_2 were then varied, keeping the total concentration, c , constant until a minimum value of the total free energy was found. The values of c_1 and c_2 , confirmed to be independent of c , for this value of total free energy were taken as the concentrations in the two phases.

We also calculated the probability of finding a rigid lipid at site l , or the average area of a lipid at site l , if there are intrinsic molecules at sites $i, j, \dots, k$. These are

$$[P_i P_j \dots P_k G_l] = \langle P_i P_j \dots P_k G_l \rangle / \langle P_i P_j \dots P_k L_l \rangle,$$

$$[P_i P_j \dots P_k A_{Ll}] = \langle P_i P_j \dots P_k A_{Ll} \rangle / \langle P_i P_j \dots P_k L_l \rangle, \quad [4]$$

$$A_{Ll} = A_0 G_l + A_e E_l, L_l = G_l + E_l,$$

in which L_l is the lipid chain projection operator and A_{Ll} is the

FIG. 1. Model I with a "cholesterol-like" molecule ($J_1 = 0.4$, $J_4 = 0.005$, $J_P^S = 0.7$) in dimyristoyllecithin. (A) C_3 approximation (panel C) was used to calculate the average area of a chain (open circle) located at site 1 (in i-iii) or site 2 (in iv and v) when there is one protein at site 2 (ii) or site 3 (iii), two proteins at sites 1 and 3 and a lipid at site 4 (iv), or three proteins at sites 1, 3, and 4 (v). i gives the average area of a chain at site 1 with no restriction as to the occupancy of sites 2, 3, and 4. (B) Chain area as a function of temperature for $c = 0.5$. The average chain area (i) and v refer to the corresponding correlations of A; the short and long dashed curves refer to ii and iii, respectively, of A. (C) Clusters used in the C_2 (a) and C_3 (b) approximations. Each vertex can be occupied by any of the unit entities of models I-III.

lipid area operator, here the lipid chain order parameter, for site i .

To calculate nontrivial correlations in (8) we made use of cluster approximations, called here C_2 and C_3 , in which the trial Hamiltonian, H_0 , was not the mean field form but contained the exact bilinear terms describing the interactions between sites of the same cluster with all other interactions linearized. The clusters are shown in Fig. 1C. In a phase with concentration c' of intrinsic molecules, the average areas of chains in given configurations are obtained from Fig. 2C or 1B for $c = c'$.

By considering the relative strengths of interaction of two adjacent chains in various states, we estimated that $J_2 = 0.1$ and $J_3 = 0.04$. $A_0 = 18.1 \text{ Å}^2$ for reasons given in ref. 8. It was argued that the pure lipid phase transition is triggered by the appearance of states of energy $\approx 0.3 \times 10^{-13}$ ergs (11) and that the melted chain area $\approx 30 \text{ Å}^2$. These values were assigned to E_e and A_e . The degeneracy of the low-lying states for dimyristoyllecithin chains was estimated to be between 80 and 100, and we

FIG. 2. Model I with a "cholesterol-like" molecule ($J_1 = 0.4$, $J_4 = 0.005$, $J_P^S = 0.607$) in dipalmitoyllecithin. (A) Phase diagram. Shorter dashes show the locus of $T_0(c)$; longer dashes show how the phase diagram would change if many-body effects were included, which tend to squeeze the molecule from lipid-rich regions. (B) Transition enthalpy, ΔH_{16} , against concentration. Circled points are the data of Estep et al. (10). (C) Average area of a lipid chain is shown by a (305 K, gel-like region) and b (325 K, fluid-like region). Average area of a lipid chain adjacent to a protein is shown by c (305 K) and d (325 K).

chose $D^{14} = 87$. We took $\Pi = 30$ dynes/cm. $J_0^{14} = 0.403 \times 10^{-13}$ ergs was chosen so that $T_c^{14} = 294.8$ K. Since the degeneracy of the low-lying lipid chain states is proportional to $(M - 1)$, it follows that $D^{16} = 100$. Further, Salem (12) has shown that J_0^M is proportional to $(M - 1)$ so that $J_0^{16} = 0.465 \times 10^{-13}$ ergs. This gives $T_c^{16} = 313.8$ K, in satisfactory agreement with experiment.

Since the product $J_0^M J_P^M$ must be independent of M , we chose $J_P^{14} = 0.7$ and $J_P^{16} = 0.607$. Because a "cholesterol-like" intrinsic molecule prefers to have rigid chains next to it but does not interact as strongly with them as a pair of rigid chains interact, we chose $J_1 = 0.4$ and $J_4 = 0.005$, as was used in ref. 8. Other calculations with model I were done with cholesterol in dimyristoyllecithin (Fig. 1) and a small molecule in dimyristoyllecithin (Fig. 3).

When the intrinsic molecule has an area larger than a lipid chain we cannot assume that such a molecule is surrounded by not more than six lipid chains. It is, however, convenient to retain our lattice model so that we now assume that each site of a triangular lattice can be occupied by either one protein or several lipid chains. We thus ignore a few lipid configurations. **Model II** has each lipid site occupied by a lipid molecule which possesses three states: $GG = \mathcal{G}$ (energy $-J_0^M$, area $2A_0$, degeneracy 1), GE or $EG = \mathcal{E}_1$ (energy $-J_0^M J_2 + E_e$, area $A_0 + A_e$, degeneracy $2D^M$), or $EE = \mathcal{E}_2$ (energy $-J_0^M J_3 + 2E_e$, area $2A_e$, degeneracy $(D^M)^2$). **Model III** has three chains, arranged on an equilateral triangle, per lipid site, possessing states: $\mathcal{G}$ (energy $-3J_0^M$, area $3A_0$, degeneracy 1), $\mathcal{E}_1$ [energy $-J_0^M(1 + 2J_2) + E_e$, area $2A_0 + A_e$, degeneracy $3D^M$], $\mathcal{E}_2$ [energy $-J_0^M(2J_2 + J_3) + 2E_e$, area $A_0 + 2A_e$, degeneracy $3(D^M)^2$], or $\mathcal{E}_3$ [energy $-J_0^M J_3 + 3E_e$, area $3A_3$, degeneracy $(D^M)^3$].

In each case we assume that the interaction between a lipid site and another lipid site is the average interaction obtained by permuting the various chain states at the respective sites, but that the interaction between a lipid site and a protein site is the total interaction. This merely gives us the possibility of more lipid chains around each protein. Thus, in model III, the $\mathcal{G}$ - $\mathcal{E}_3$ interaction is $-2J_0^M J_2$, and the $\mathcal{E}_1$ -protein interaction is $-J_0^M(2J_1 + J_4)$.

When a protein specifically binds lipids, we assume that they are sufficiently tightly bound for times longer than lipid diffusion, rotation, or *trans-gauche* transition times. The orientation of this protein-lipid complex is important because the interaction with a neighboring site depends upon whether a bound lipid or a "bare" protein is adjacent to that site. We assume that each protein has three binding sites symmetrically arranged around it and that it can bind lipids either in an all-*trans* or an excited state (Fig. 4A). The Hamiltonian for this case in model II is:

FIG. 3. Model I with a small molecule ($J_1 = 0.35$, $J_4 = -0.05$, $J_P^{14} = 0.7$) in dimyristoyllecithin. (A) Phase diagram. Dashed lines show the loci of the two peaks in the specific heat. (B) Transition enthalpy against concentration. Schematic plot of the specific heat as a function of temperature is shown for some values of c . The position of the pure lipid peak is indicated.

$$\begin{aligned}
 H = & \frac{-J_0^M}{2} \sum_{i\delta} [X_{\mathcal{G}\mathcal{G}} \mathcal{G}_i \mathcal{G}_{i+\delta} + 2X_{\mathcal{G}\mathcal{E}_1} \mathcal{G}_i \mathcal{E}_{1i+\delta} \\
 & + 2X_{\mathcal{G}\mathcal{E}_2} \mathcal{G}_i \mathcal{E}_{2i+\delta} + X_{\mathcal{E}_1\mathcal{E}_1} \mathcal{E}_{1i} \mathcal{E}_{1i+\delta} \\
 & + 2X_{\mathcal{E}_1\mathcal{E}_2} \mathcal{E}_{1i} \mathcal{E}_{2i+\delta} + X_{\mathcal{E}_2\mathcal{E}_2} \mathcal{E}_{2i} \mathcal{E}_{2i+\delta}] \\
 & - J_0^M \sum_{m=\pm} \sum_{\delta>0} [(X_{\mathcal{G}\alpha P_{mi}} + X_{\mathcal{G}P_{-mi}}) \mathcal{G}_{i+m\delta} \\
 & + (X_{\mathcal{E}_1\alpha P_{mi}} + X_{\mathcal{E}_1 P_{-mi}}) \mathcal{E}_{1i+m\delta} \\
 & + (X_{\mathcal{E}_2\alpha P_{mi}} + X_{\mathcal{E}_2 P_{-mi}}) \mathcal{E}_{2i+m\delta}] \\
 & - \frac{J_0^M}{2} \sum_{m=\pm} \left[\sum_{\delta} X_{\alpha P_{mi}} P_{mi+\delta} + \sum_{\delta>0} \right. \\
 & \left. \times (X_{\alpha\alpha P_{mi} P_{-mi+\delta}} + X_{PP_{mi} P_{-mi-\delta}}) \right] \\
 & + \sum_i [(\Pi A_{\mathcal{G}} + E_{\mathcal{G}}) \mathcal{G}_i + (\Pi A_{\mathcal{E}_1} + E_{\mathcal{E}_1}) \mathcal{E}_{1i} \\
 & + (\Pi A_{\mathcal{E}_2} + E_{\mathcal{E}_2}) \mathcal{E}_{2i} + \sum_{m=\pm} \Pi A_P P_{mi}], \quad [5]
 \end{aligned}$$

$$\begin{aligned}
 X_{\mathcal{G}\mathcal{G}} &= 5/3, \quad X_{\mathcal{G}\mathcal{E}_1} = 5(1 + J_2)/6, \quad X_{\mathcal{G}\mathcal{E}_2} = 5J_2/3, \\
 X_{\mathcal{E}_1\mathcal{E}_1} &= (2 + 5J_2 + 3J_3)/6, \quad X_{\mathcal{E}_1\mathcal{E}_2} = 5(J_2 + J_3)/6, \\
 X_{\mathcal{E}_2\mathcal{E}_2} &= 5J_3/3, \quad X_{\mathcal{G}P} = 2J_1, \\
 X_{\mathcal{E}_1P} &= J_1 + J_4, \quad X_{\mathcal{E}_2P} = 2J_4, \quad X_{PP} = J_P^M.
 \end{aligned}$$

Here δ runs over the set of nearest neighbors $\pm 1, \pm 2, \pm 3$ in Fig. 4A and $\delta > 0$ runs over 1, 2, and 3. P_{ni} is a projection operator for a protein of orientation n at site i of the lattice with $n = \pm$, and $\mathcal{G}_i$, $\mathcal{E}_{1i}$ and $\mathcal{E}_{2i}$ are projection operators of lipid molecules for site i . α ($= \mathcal{G}$ or $\mathcal{E}_2$) denotes in what state the lipid is bound to the protein. The Hamiltonian for model III will look like [5] except that the state $\mathcal{E}_3$ must be included and the interactions must be changed. When the proteins have no specific binding sites or an otherwise inhomogeneous surface, then there will be only one protein operator since protein orientation is now irrelevant. When the protein does not have a binding site but,

FIG. 4. (A) Orientation and interactions when a protein has three lipid binding sites. (a) 1, 2, 3 (-1, -2, -3) represent the binding sites when the protein is in the + (-) orientation. (b and c) Protein-lipid complex in a + (b) and - (c) orientation. A solid oval lipid molecule is shown interacting with a (strongly) bound lipid molecule; a dashed oval lipid is interacting with a "bare" protein. (d-f) Adjacent proteins in the + and - orientations. The only interactions here are those between two bound lipids (d), between a bound lipid and a "bare" protein (e), and between two "bare" proteins (f). (B) Model II with a larger protein in dimyristoyllecithin; case ii (a): lipid bound in the all-*trans* state. Phase diagram. Dashed line is the locus of $T_0(c)$. (C) Areas of unbound lipid chains for the case of B. (a) Average area of such a chain; (b and c) areas of chains when they are adjacent to a protein in a particular orientation, as indicated in the upper right corner. The lower triplet is for $T = 290$ K (gel-like phase), the upper for $T = 320$ K (fluid-like phase).

FIG. 5. Larger protein in dimyristoyllecithin; case ii (a), lipid bound in the all-*trans* state. (A) Transition enthalpy against concentration. (B) $T = 320$ K. (a) Probability of finding a protein at any given site. (b-e) Conditional probabilities defined as: If any one of the proteins in the pairs b, c, d, or e are oriented as shown (upper), what is the probability that the other protein will occupy the chosen adjacent site with the chosen orientation?

instead, an inhomogeneous surface that is not necessarily "smooth" so that one part of the protein interacts with lipids differently from another part of it, we can still use [5].

Our comments about phase diagrams, T_0 , ΔH , and correlations made in the previous section hold here also. Again we assume that the addition of proteins to a bilayer does not affect the pressure-area terms in [5].

We studied two cases, both involving proteins in dimyristoyllecithin: in both we chose $J_1 = 0.3$, $J_4 = 0.005$, and $J_P^{14} = 0.7$, with $J_0^{14} = 0.406 \times 10^{-13}$ ergs so that $T_c = 296.2$ K (instead of 294.8 K as before).

Case i: No specific binding of lipid to protein. Model III was used so that each protein could interact with up to 18 chains with $A_P = 120 \text{ \AA}^2$. The interactions chosen correspond to a "smooth"-surfaced "cholesterol-like" protein.

Case ii: Specific binding of lipid to protein. Model II was used, and we considered situations where (a) the bound lipid is in the all-*trans* state (Figs. 4 and 5) and (b) the bound lipid is in some of its excited states, $\mathcal{C}_2$. In both cases the mean field and the C_3 cluster approximations were used and gave consistent results.

Note that we have not considered any effect of c upon the effective pressure II.

DISCUSSION AND CONCLUSIONS

(i) **No Specific Binding of Lipid to Protein.** Fig. 2 shows results for the "cholesterol-like" case and dipalmitoyllecithin of model I. Phase separation occurs in the gel-like region below the melting point of pure lipid ($T_c = 313.8$ K). Thus, at $T = 310$ K, if $0.15 < c < 0.25$, the lipid-rich phase is gel-like with an average chain area of $19.7 \text{ \AA}^2$, giving rise to a sharp x-ray line; the other phase, in which the cholesterol concentration is 0.25, is fluid-like with an average chain area of $27.9 \text{ \AA}^2$, which would exhibit a diffuse x-ray line. As c increases, the gel (fluid) phase becomes more fluid (rigid) on the average, as shown by the average areas of Fig. 2C. Lipid chains adjacent to a molecule are more (less) fluid in the gel (fluid) region than are chains on the average. This, of course, is the reason for the decrease in transition enthalpy as c increases. Fig. 2B shows the transition enthalpy $\Delta H_{(1)} = H(T_0 + 1) - H(T_0 - 1)$, which has the characteristic shape predicted in ref 7. The range of 1 K on either side of T_0 includes most of the enthalpy, and the experimental peak is possibly broadened due to finite-size effects. The results of Estep *et al.* (10) are shown circled, with ours adjusted to agree with theirs at $c = 0$. Five correlations (Fig. 1A) were calculated, and Fig. 1B shows the effect of "trapping" upon the ordering of a dimyristoyllecithin chain. A lipid partially sur-

rounded by molecules is considerably more fluid (rigid) in the gel-like (fluid-like) phase than the average. As long as the intrinsic molecule has a polar group that does not perturb the bilayer unduly (such as cholesterol), this conclusion is justified.

We also considered a molecule in dimyristoyllecithin with a repulsive interaction between it and an excited chain (Fig. 3). Here $J_1 = 0.35$ and $J_4 = -0.05$ with $J_P^{14} = 0.7$. The phase diagram shows that T_0 decreases with increasing c and phase separation occurs in the fluid and in the gel phase. The phase separation observed by freeze fracture will depend upon the temperature at which the system is prepared and subsequently rapidly frozen. Note that there is a melting of a eutectic mixture with concentration $c' = 0.44$, at $T = 288.5$ K, 6.3 K below the melting of the pure lipid, if $0.2 \leq c \leq 0.83$. Such an effect may have been seen by Gómez-Fernández *et al.* (13). Fig. 3B shows how this manifests itself in the transition enthalpy where the associated specific heat (DSC) curves are drawn schematically with the position of the main transition indicated. A peak due to the formation of a melted cluster with $c' = 0.44$ appears on the shoulder of the main transition peak and gradually grows, eventually vanishing as c increases. A small hump on the ΔH vs. c curve appears. ΔH is still large even at $c = 0.6$, in contrast to the "cholesterol-like" case. In all cases the shape of the ΔH vs. c curve for small c is the characteristic one with decreasing slope as found in ref. 8. Curatolo *et al.* (14) have observed the appearance of a secondary peak on the high-temperature side of the main peak shoulder, and it is possible that their results and those of Gómez-Fernández *et al.* (13) are due to the formation of a melted eutectic mixture. In all these cases, increasing c makes the gel-like (fluid-like) phase more fluid (rigid).

The case of the large protein (model III) showed results similar to the cholesterol case. T_0 decreased somewhat with increasing c , the ΔH vs. c curve had the shape shown in Fig. 2B but with the high- c tail starting at $c \approx 0.15$, the gel (fluid) phase became more fluid (rigid) as c increased, and the phase diagram had the shape of Fig. 2A. Proteins of this kind, therefore, should behave like cholesterol as long as c is sufficiently small. This fact gives us confidence in expecting that large proteins with interactions similar to those of the system of Fig. 3 would display similar behavior.

Figs. 1-3 show that lipids can experience a variety of environments. Over time scales of the order of those with which we are concerned ($\approx 10^{-7}$ sec), these different environments might be manifested in different values of the lipid chain order parameter. Fig. 1B shows that the distance over which lipid near to a protein has its order parameter, its area, modified significantly from the average in the "cholesterol-like" case is about one lipid layer—i.e., this is the correlation depth of "boundary layer" lipid (see below). Note that it is a departure from the average at that c -value which itself is different from the pure lipid average. Our value of the correlation depth, λ , for $c = 0$ agrees with that of Owicki *et al.* (15). In general, at a given temperature, as c increases, so does λ , and for a given concentration λ increases as T approaches the temperature at which C_P peaks either from above or below. If further neighbor interactions are included, then λ could be larger.

(ii) **Protein Binds Lipids or Has an Inhomogeneous Surface.** For case (a) where the protein binds lipids, for times much longer than those characteristic of conformational changes or lipid diffusion or rotation, we assumed that a lipid adjacent to part of the protein that is "bare" interacts to a different extent. Since each protein binds three lipids, $c \leq 0.25$. Fig. 4B shows the phase diagram with a small two-phase region and T_0 shifting to higher values as c increases. The ΔH vs. c curve (Fig. 5A) displays a monotonic decrease with no appreciable en-

thalpy remaining for $c > 0.16$. The sharp transition vanishes at $c \approx 0.08$ though a broad peak persists to $c \approx 0.16$. Fig. 4C shows that the lipid adjacent to a bound lipid (curve *b*) is more rigid, both well above and well below T_0 , than the average (curve *a*), while that adjacent to a "bare" protein (curve *c*) is less (more) rigid in the gel-like (fluid-like) phase than the average. The gel (fluid) phase becomes more fluid (rigid) on the average as c increases. The formation of a melted eutectic mixture depends crucially on the extent to which the protein prefers to have rigid rather than melted chains adjacent to it. The results of Curatolo *et al.* (14) could indicate that the myelin apoprotein either has a sufficiently large value of J_1 or specifically binds lipids in their all-*trans* state (or both), and tends to repel excited lipids. In fact, this protein possesses covalently attached fatty acids (14). For case *ii* (*b*), where the lipid is bound in excited states, the effect of the lipid state degeneracy is to give the protein a degeneracy D_P . We took $D_P = 10$. The gel phase becomes more fluid, but the fluid phase may become either more rigid or even *more fluid*, depending upon the interactions, as c increases. In this case T_0 decreases as c increases (unpublished data). "Fluidity contours," connecting lipids that have the same chain areas, around the protein reflect the locations of the binding sites.

Here, and in case (*i*) above, both the value and the slope of the transition enthalpy, ΔH , curve decreases with increasing concentration of intrinsic molecules when c is small, as predicted by Pink and Carroll (8) and shown in Figs. 2B, 3B, and 5A. There is also a high concentration tail. Such a result has now been seen by Gershfeld (16) for cholesterol in dioleoyl lecithin, by Chapman *et al.* (17) for gramicidin A in dipalmitoyl- and dimyristoyllecithin, and by Estep *et al.* (10) for cholesterol in dipalmitoyllecithin (Fig. 2B).

For the "cholesterol-like" case and for the case of bound rigid lipid considered here in model II, where a melted eutectic mixture does not appear, then the tail decreases quickly with increasing c and ΔH is small at high c (Figs. 2B and 5A). When a melted eutectic mixture forms as in the other case of model I, the high- c tail persists up to very large c values so that the magnitude of ΔH remains large over a great range of c and can (but not necessarily does) show a hump associated with the melted eutectic mixture (Fig. 3B).

There is a most probable distance for protein-protein separation in some models (9, 15). However, we find that there are non-negligible probabilities for proteins to be adjacent to one another—i.e., to be "touching" with no lipid between them. This is shown for case *ii* (lipids bound in their all-*trans* state) in Fig. 5B at $T = 320$ K, well into the fluid phase, where the conditional probability of finding a protein in a given orientation at a given site adjacent to one with a specified orientation can be greater than the probability of simply finding a protein at any site. Proteins not only have a non-negligible probability for being adjacent (*b* and *c*), but the fact that $(b + c + d + e)/4 > a$ shows that these proteins tend to cluster, and have a preference (*d* and *e*) for clustering in certain relative orientations. Thus, protein short-range order should be looked for, and the possibility of protein long-range order arises (unpublished data).

Similar results were obtained for all the other models. Our model thus offers no support for the concept of an unbroken annulus of lipid around a protein as suggested by Hesketh *et al.* (18). A "boundary-layer" lipid can be defined as one which has its order parameter sufficiently modified due to its proximity to an intrinsic molecule. In our picture the boundary layer of one protein can be penetrated by another protein, and the pair thus possesses a joint boundary layer.

Further, our calculation in case *ii*, where only three lipid molecules were bound in their all-*trans* state, showed that T_0

would increase significantly with c , and this increase will be all the greater if *all* chains immediately adjacent to a protein are constrained in their all-*trans* states on the time scale of 10^{-6} to 10^{-7} sec. Thus, for protein-lipid bilayers, wherein T_0 decreases, remains constant, or, in some cases, even increases, and where ΔH decreases (or looks like that of Fig. 3B), with increasing c , it is not possible for all the chains immediately adjacent to the protein to be all-*trans* on the time scale 10^{-6} to 10^{-7} sec. However, if a sufficiently small pool of "trapped" lipid (4, 5, 17) is sufficiently enclosed by a conglomeration of protein, then they might become significantly more fluid (rigid) than the average in the gel (fluid) regions (Fig. 1B).

In this regard, however, there are some important observations. We have assumed that the addition of intrinsic molecules does not affect the pressure-area term—i.e., it does not cause the effective pressure to change even locally. Such an assumption may be good either when c is small or when the polar group(s) of the molecule does not perturb the polar region. Thus, the assumption is probably good for cholesterol. When the intrinsic molecule has a polar section that perturbs the polar region, then for small c the hydrocarbon chain effects described here may dominate and the effect is linear in c . For large c , when protein clustering severely perturbs the polar region, one must consider the effect of local pressure changes upon chain dynamics, and this effect will be proportional to c^n ($n > 1$). This is a many-body effect which is not a combination of the two-body effects considered here. If, for example, protein clustering reduces the local pressure, then an initial decrease in fluidity ($\propto c$) could change to an increase in fluidity ($\propto c^n$, $n > 1$) (3).

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