Estimation of Lateral Species Separation from Phase Transitions in Nonideal Two-Dimensional Lipid Mixtures†

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ABSTRACT: The two-dimensional statistical mechanical equations necessary to analyze the phase diagram of a nonideal lipid mixture are derived. The Prigogine approximation of the combinatorial term is used in the maximum term of the partition function which contains two parameters that describe the degree of nonideality in the liquid crystal and gel phases. These parameters are used to calculate the mole fraction of like-like and like-unlike lipid interfaces which describe the lateral species separation of like lipids in each phase. Data for several temperatures and compositions of the DMPC/DPPC, DPPC/DSPC, and DMPC/DSPC mixtures are analyzed.

The membrane phospholipids undergo a thermally induced thermodynamically reversible order-disorder transition from a solidlike gel (g) to a liquid-crystalline (l) state which occurs at a characteristic transition temperature (Hinz and Sturtevant, 1972; Shimshick and McConnell, 1973). Many biological properties of the membrane, such as enzyme function, ion transport, antibody stimulated cell lysis, and cell fusion, depend both on the physical state of the membrane lipids and on the lipid composition of local regions of the membrane. In a mixture of A and B lipids each of which undergoes a phase transition characterized by a transition temperature (T_A or T_B) and a transition enthalpy (ΔH_A and ΔH_B), the phase transitions of the mixed system can be described by a phase diagram. For an ideal mixture it is possible to obtain expressions for f_A (the mole fraction of A in the whole system), X_A (the mole fraction of A in the liquid-crystalline state), and Y_A (the mole fraction of A in the α-gel state) as a function of T and the thermodynamic parameters characterizing the phase transition (Selz, 1934; Von Dreele et al., 1971). With these equations ideal phase diagrams can be calculated. These theoretical diagrams for ideal mixtures do not fit the experimental data indicating the presence of nonideal interactions between the lipids. In the present paper, we will develop a theory which can be used to fit the phase diagram. We use two independent equations which are functions of ΔH_A, T_A, ΔH_B, T_B, X_A, X_B, Y_A, and Y_B and two parameters ν_g and ν_l which describe the degree of nonideality present in the gel and liquid-crystal phases, respectively. When values of ΔH_A, T_A, ΔH_B, and T_B taken from pure lipid data and experimental values of X_A, X_B, Y_A, and Y_B are used, a unique pair of parameters ν_g and ν_l can be found which will minimize both equations. The two independent equations are obtained in the following way: first, write down the partition function for each phase using expressions for the nonideal combinatorial term and nonideal free energy (Prigogine et al., 1952; Hill, 1956); second, obtain the chemical potential of each species in each phase; third, obtain the difference in chemical potentials for a species which exists in two different phases at the same temperature; and fourth, use the Gibbs-Helmholtz equation to relate the change in the free energy of pure A at some temperature T to that at T_A. The A species and the B species each give a separate equation. The function which is minimized is the sum of the squares of the value of each equation and the variables are the two nonideality parameters, ν_g and ν_l. The phase diagrams for binary mixtures of dimyristoyllecithin (DMPC), dipalmitoyllecithin (DPPC), and disteroyllecithin (DSPC)[1] are fitted in this manner and the ν_g and ν_l values obtained are converted to mole fractions of AA, AB, and BB interfaces which can be used to describe the degree of lateral species separation and the clustering together of like lipids in the membrane surface.

Partition Function

When the liquid crystal and gel phase are both present in a mixture of A and B lipids, we can write eq 1 and 2 to describe the equilibria present.

$$A_g \rightleftarrows A_l \quad (1)$$

$$B_g \rightleftarrows B_l \quad (2)$$

The following terms will be used in the partition function for each phase, Q: N_A is the total number of moles of A lipids; N_{Al} is the number of moles of A lipids in the l state; N_{Ag} is the number of moles of A lipids in the g state ($N_A = N_{Al} + N_{Ag}$); N is the number of moles of all lipids present ($N = N_A + N_B$); N_l is the number of moles of lipids in the l state ($N_l = N_{Al} + N_{Bl}$); Y_A is the mole fraction of A lipids in the g state ($Y_A = N_{Ag}/N_g$); X_A is the mole fraction of A lipids in the l state ($X_A = N_{Al}/N_l$); and f_A is the mole fraction of A lipids in the system ($f_A = N_A/N$). Since the two states exist as two physically distinct phases, the partition function for each phase, Q, is written separately for the l and g phases. The expressions for an ideal mixture of A and B species are shown in eq 3 and 4 where q is the partition function for one kind of lipid in one phase.

$$Q_l = q_{Al}^{N_{Al}} q_{Bl}^{N_{Bl}} \frac{N_l!}{N_{Al}!N_{Bl}!} \quad (3)$$

$$Q_g = q_{Ag}^{N_{Ag}} q_{Bg}^{N_{Bg}} \frac{N_g!}{N_{Ag}!N_{Bg}!} \quad (4)$$

For a real mixture, the partition functions shown in eq 3 and 4 must be modified in two places: first, in the combinatorial term to use the proper number of configurations of the system and second in the q term to include the nonideal free energy of interaction. In two- and three-dimensional systems where the molecules can freely change places with each other, the nonideal free energy and the number of configurations are

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[1] Abbreviations used: DMPC, dimyristoyllecithin; DPPC, dipalmitoyllecithin; DSPC, disteroyllecithin.

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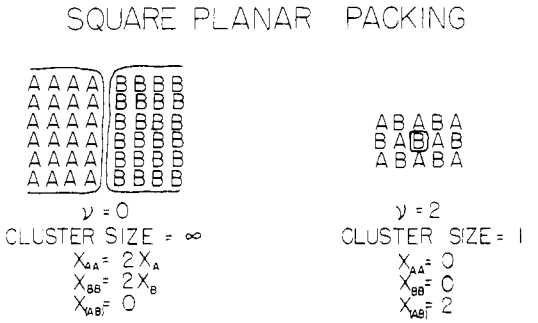

FIGURE 1: A schematic representation of the arrangement of lipids in the membrane surface for nonideal interaction energies. $\nu = 0$ corresponds to an infinitely large repulsive A–B interaction energy leading to a cluster size of infinity, while $\nu = 2$ corresponds to an infinitely large attractive interaction energy leading to a cluster size of unity.

related. Qualitatively the nature of the changes in the system can be understood by considering three cases. In case 1 where there are nonideal repulsive forces between the molecules, the excess repulsive forces present at the AB interface cause the molecules to rearrange to minimize the number of AB interfaces thereby minimizing the pairwise energy in the system. In the extreme of an infinitely large repulsive energy, the system will separate into a region of pure A and a region of pure B with only one AB contact point or line. There is then only one configuration of the system. In case 2 where there are no nonideal forces between the molecules, there are no excess repulsive or attractive forces at the AB interface so the molecules arrange randomly and all configurations have the same energy. The number of configurations of the system is $N!/N_A!N_B!$. In case 3, where there are no nonideal attractive forces between the molecules, the molecules rearrange to maximize the number of AB interfaces. In the extreme of an infinitely large attractive energy, the system will arrange in a large checkerboard of regularly alternating A and B molecules (see Figure 1).

A system in which each molecule has Z neighboring molecules will have $Z/2$ unique neighboring pairs per molecule. For a one-dimensional system, $Z = 2$. For a two-dimensional system $Z = 4$ in a square planar packing and $Z = 6$ in a hexagonal packing. The system of N moles will have $ZN/2$ mol of pairs of neighboring interfaces. The number and mole fraction of AA, BB, and (AB) pairs in the system as a function of Z, N_A, N_B, and $N_{(AB)}$ are given in eq 5.

$$N_{AA} = \frac{Z}{2}N_A - N_{AB} = \frac{Z}{2}N_A - \frac{1}{2}N_{(AB)}$$

$$N_{BB} = \frac{Z}{2}N_B - N_{BA} = \frac{Z}{2}N_B - \frac{1}{2}N_{(AB)}$$

$$X_{AA} = \frac{Z}{2}(X_A - \nu X_A X_B) \qquad (5)$$

$$X_{BB} = \frac{Z}{2}(X_B - \nu X_A X_B)$$

$$X_{(AB)} = Z\nu X_A X_B$$

The quantities Z, N_A, and N_B are known from the model and the A/B ratio being studied. The term N_{AB} refers to A–B pairs, the term N_{BA} refers to B–A pairs, and the term $N_{(AB)}$ is the sum of the A–B and B–A pairs; hence, $N_{(AB)}/2 = N_{AB} = N_{BA}$. The N_{AB} term is given by $Z\nu N_A X_B/2$ where ν is unity for a random mixture and other than unity for a nonideal mixture. The $Z/2$ factor is included which gives the number of unique pairs which one A molecule will have for each model used. With these definitions, the sum of the mole fraction of lipid pairs (ΣX_{ij}) is two for the square planar model and three for the hexagonal model.

The mathematical problem involved in the statistical mechanical computation of the thermodynamic properties of nonideal mixtures is obtaining an appropriate expression for the combinatorial term. For an ideal mixture ($\nu = 1$), the number of distinct configurations of the system which have the proper number of AA, BB, and (AB) pairs for an ideal system is $N!/N_A!N_B!$ or 2^N for a system with equal amounts of A and B. For the extreme cases of $\nu = 0$ and $\nu = 2$, the combinatorial term is 1^N or unity. The Nth root of the combinatorial term designated γ is a constant and varies from 1 to 2 to 1 as ν varies from 0 to 1 to 2. The nature of the functional relation between ν and γ has been determined exactly by Onsager (1944) for the square planar model. The solution is not in closed form but involves converging series. Several approximations of the solution have been made. The one which we shall use by Prigogine, Mathot-Sarolea, and van Hove (1952) is exact at $\nu = 0$, 1, and 2 and close to the correct value over the rest of the range. This approximation can be extended to the $Z = 6$ case. The approximation has a significant advantage over the Onsager solution in that it is in a form which can be differentiated so closed expressions for the thermodynamic parameters can be obtained from the partition function. The explicit expressions used for the combinatorial term are given in eq 6 where a large N is assumed.

$$\gamma^N = \left[1 + \alpha\left(\frac{g^{*1/N}}{\beta} - 1\right)\right]^N \qquad (6)$$

where

$$\beta = X_A^{-ZX_A/2}X_B^{-ZX_B/2}$$

$$\alpha = (\beta^{2/Z} - 1)/(\beta - 1)$$

$$g^* = \left(\frac{Z}{2}N\right)!/((N_{(AB)}/2)!^2(N_{AA})!(N_{BB})!)$$

The second modification of the partition function which is made is to alter the conformational partition function for each species, i.e., the q values, to include the nonideal interaction energy. The molar energy of a phase can be written as the sum of the energies for all of the pairwise interactions.

$$E = \frac{2}{Z}[N_{(AB)}E_{AB} + N_{AA}E_{AA} + N_{BB}E_{BB}]$$

$$= \frac{1}{Z}[N_{(AB)}E_{non} + N_A E_{AA} + N_B E_{BB}] \qquad (7)$$

where

$$E_{non} = 2E_{AB} - E_{AA} - E_{BB}$$

On combining the modified combinatorial term and the energy term, we obtain the partition function for a system having fixed N_A and N_B which is given in eq 8.

$$\Xi = q_A^{N_A}q_B^{N_B}\sum\gamma^N \exp\frac{1}{RT}(-N_A E_{AA} - N_B E_{BB} - N_{(AB)}E_{non}/Z \qquad (8)$$

We will use a maximum term approximation and therefore the partition function we will use is shown in eq 9.

$$Q = q_A^{N_A}q_B^{N_B}\gamma^N \exp\frac{1}{RT}(-N_A E_{AA} - N_B E_{BB} - N_{(AB)}E_{non}/Z) \qquad (9)$$

A relation between the nonideality parameter ν used in the combinatorial term and the nonideal interaction energy can

be obtained by taking advantage of the fact that we are using the maximum term approximation. This means that $0 = \partial Q/\partial N_{AB} = \partial \ln Q/\partial N_{AB}$. When this derivative is taken, we obtain the expression shown in eq 10 in which E_{non} is a function of N_A, N_B, T, Z, and μ

$$\frac{E_{non}}{ZRT} = \ln(X_{AA}^{1/2}X_{BB}^{1/2}/(X_{(AB)}/2))/\left(1+\beta\left(\frac{1}{\alpha}-1\right)\Big/ g^{*1/N}\right) \quad (10)$$

Therefore, selection of ν determines both the combinatorial term and the nonideal interaction energy. This is true in both phases and independent values of ν (that is ν_g and ν_l) can be expected to exist for each phase.

Functional Equations Defining the Nonideality of a Lipid Mixture

The chemical potential of the A lipids in a mixture which is in the gel state can be obtained by taking the derivative of the partition function defined in the previous section in the usual manner.

$$u_{Ag} = -RT\frac{\partial \ln Q_g}{\partial N_{Ag}} \quad (11)$$

This derivative comes in three parts as shown in eq 12.

$$u_{Ag} = -RT\left[\frac{\partial \ln \gamma^N}{\partial N_{Ag}} + \frac{\partial(N_A(\ln q_A - E_{AA}/RT) + N_B(\ln q_B - E_{BB}/RT))}{\partial N_{Ag}} + \frac{\partial(-N_{(AB)}E_{non}/ZRT)}{\partial N_{Ag}}\right] \quad (12)$$

The first term in eq 12 is shown in eq 13 and it reduces to $+RT \ln Y_A$ for an ideal mixture.

$$\frac{\partial \ln \gamma^N}{\partial N_{Ag}} = + \frac{N}{\gamma}\left\{\frac{\alpha g^{*1/N}}{\beta}\left(\frac{\partial \ln 1/\beta}{\partial N_A} + \frac{\partial \ln g^{*1/N}}{\partial N_A} + \frac{\partial \ln \alpha}{\partial N_A}\right) - \alpha\frac{\partial \ln \alpha}{\partial N_A}\right\} \quad (13)$$

where

$$\frac{\partial \ln(1/\beta)}{\partial N_{Ag}} = \frac{Z}{2N}\ln Y_A$$

$$\frac{\partial \ln g^{*1/N}}{\partial N_{Ag}} = \frac{1}{NY_A}\left[\ln g^{*1/N} + \frac{ZY_B}{2}\ln(Y_B - \nu Y_A Y_B)\right]$$

$$\frac{\partial \ln \alpha}{\partial N_{Ag}} = \frac{1}{N}\left(\frac{Z}{2}\ln Y_A\right)\left[\frac{\beta}{\beta-1} - \frac{2\beta^{2/Z}}{Z(\beta^{2/Z}-1)}\right]$$

The second term in eq 12 combines the molecular partition function for A molecules, $q_A^{N_A}$, with the interaction potential energy for A-A interactions, E_{AA}. When the derivative with respect to N_{Ag} is taken the result is the chemical potential of pure A lipid in the gel phase which has near neighbor interactions, i.e., u_{Ag}'. The third term in eq 12 reduces to $\nu^g E_{non}{}^g Y_B$ when the derivative is taken. It becomes zero for an ideal mixture. An expression like eq 12 exists for u_{Al}, u_{Bg}, and u_{Bl}. At temperatures where the two phases coexist, $u_{Al} = u_{Ag}$ or $u_{Al} - u_{Ag} = 0$ producing eq 14.

$$0 = \Delta u_A' - RT\frac{\partial \ln \gamma^N}{\partial N_{Al}} + RT\frac{\partial \ln \gamma^N}{\partial N_{Ag}} + \nu^l X_B E_{non}{}^l - \nu^g Y_B E_{non}{}^g \quad (14)$$

where $\Delta u_A' = u_{Al}' - u_{Ag}' = \Delta H_A(T_A - T)/T_A$ from the Gibbs–Helmholtz equation, and γ^N is given by eq 6. A second functional equation can be obtained from $u_{Bl} - u_{Bg} = 0$ and this is shown in eq 15.

$$0 = \Delta u_B' - RT\frac{\partial \ln \gamma^N}{\partial N_{Bl}} + RT\frac{\partial \ln \gamma^N}{\partial N_{Bg}} + \nu^l X_A E_{non}{}^l - \nu^g Y_A E_{non}{}^g \quad (15)$$

These two equations are functions of two unknowns, ν_g and ν_l, and therefore the equations can be solved simultaneously to obtain the unknowns. Analytic expressions for ν^g and ν^l do not exist so a two-dimensional grid search over ν^l and ν^g was conducted to locate the lowest value of the function f given by eq 16.

$$f = (u_{Al} - u_{Ag})^2 + (u_{Bl} - u_{Bg})^2 = (\text{eq } 14)^2 + (\text{eq } 15)^2 \quad (16)$$

Each set of X_A, Y_A, and T values was fitted independently since there is no a priori reason to assume that the degree of nonideality is independent of the composition.

Lateral Species Separation in DMPC/DPPC, DPPC/DSPC, and DMPC/DSPC Mixtures

The lipids DMPC, DPPC, and DSPC are a homologous series of phospholipids having saturated hydrocarbon alkyl chains differing in chain length by two CH_2 groups. The DMPC, DPPC, and DSPC have 14, 16, and 18 carbon chains, respectively. The phase diagrams of mixtures of these lipids and their transition temperatures have been obtained by Shimshick and McConnell, while the transition enthalpies have been measured by Hinz and Sturtevant. A comparison of the experimentally determined phase diagrams and the theoretically calculated diagrams for ideal mixtures (Figure 2) indicates that some degree of nonideal mixing is present in all three mixtures. The X_A, Y_A, and T values from these diagrams were indexed as described in the previous section and fitted using two adjustable parameters, ν_g and ν_l. The ν_g and ν_l values obtained from fitting the data are shown on the phase diagrams in Figure 2 and as ν vs. composition in Figure 3. As can be seen especially in the DMPC/DSPC mixture, large deviations in the experimental data from the ideal curves lead to large deviations of ν from unity. The nonideality parameter ν is composition independent for ideal mixtures so variations in ν from unity as a function of composition for the mixtures under study reflect real changes in the magnitude of the nonideal interaction energy. The mole fractions of like-like and like-unlike lipid interfaces were calculated from ν using eq 5 and the results for three mixtures being studied are shown in Figure 4 along with the results for an ideal mixture. A phospholipid mixture having ideal energy interactions will show a variation in X_{ii} with composition due simply to probability considerations. The value of X_{AA} will increase monotonically with an increase in X_A while the value of X_{AB} will increase to a maximum at $X_A = 0.5$ and then decrease to 0 as X_A increases. If the nonideal interaction energy between A-B pairs is repulsive ($0 < \nu < 1$), then X_{AB} will be smaller and X_{AA} will be larger than the values for the ideal case (Figure 5). Conversely, for nonideal attractive interaction energies ($1 < \nu < 2$), the X_{AB} will be larger and the X_{AA} will be smaller than in the ideal mixture. Thus, by comparing the values for X_{ii} calculated on the DMPC/DPPC, DPPC/DSPC, and DMPC/DSPC and shown in Figure 4 to the schematics in Figure 5, we can de-

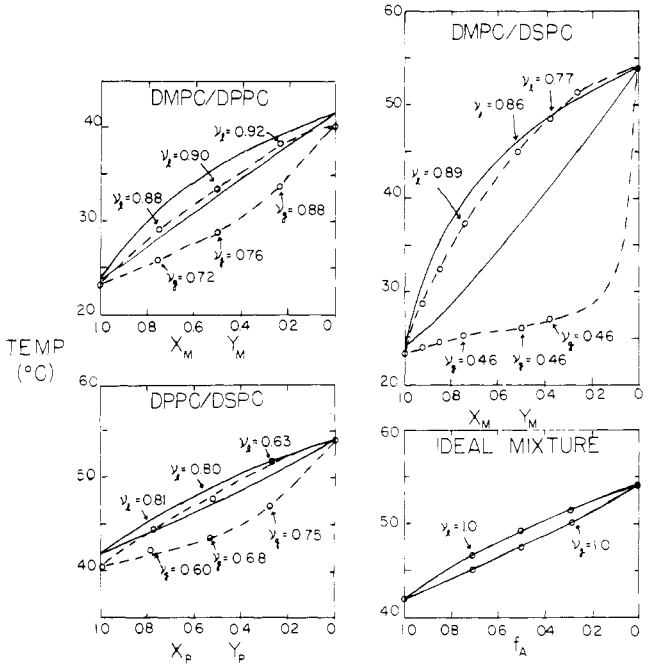

FIGURE 2: The phase diagrams for DMPC/DPPC, DPPC/DSPC, DMPC/DSPC, and an ideal lipid mixture. The theoretical curves (—) for the phase diagram if ideal mixing of the two components occurred are shown along with the experimental data (○). The ν_g and ν_l values calculated from the theory in the present paper with $Z = 4$ are also shown for various compositions of each phase.

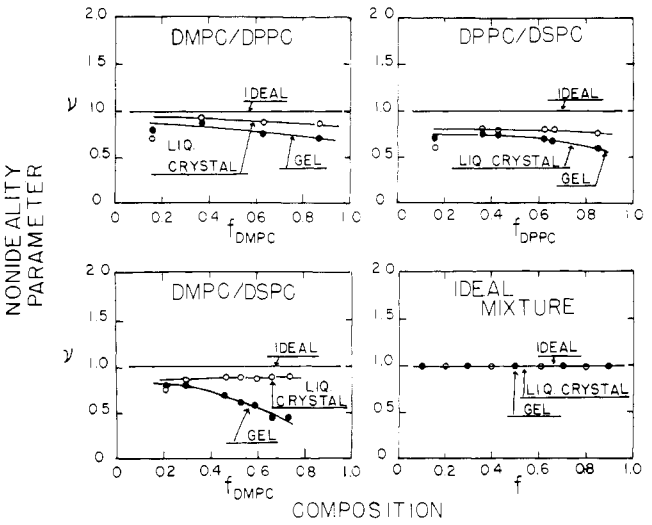

FIGURE 3: The nonideality parameters for the gel and liquid crystalline phases obtained by fitting the multilayer data for DMPC/DPPC, DPPC/DSPC, and DMPC/DSPC mixtures and also for an ideal lipid mixture are shown as a function of composition. The filled symbols correspond to the gel phase (ν_g) and the open symbols correspond to the liquid crystalline phase (ν_l).

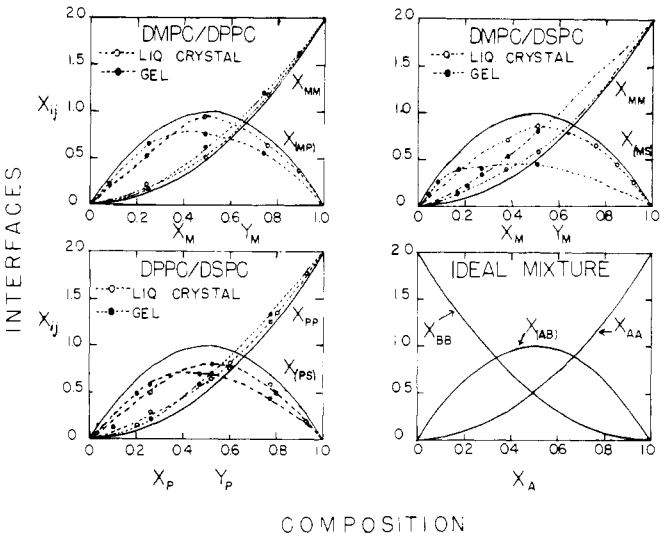

FIGURE 4: The mole fractions of like-like and like-unlike interfaces in the gel (●) and liquid crystal (○) phases as a function of composition. Values for DMPC/DPPC, DPPC/DSPC, and DMPC/DSPC multilamellar systems and also for an ideal mixture are shown. The abbreviations M = DMPC, P = DPPC, S = DSPC are used so X_{MM} is the mole fraction of DMPC-DMPC interfaces.

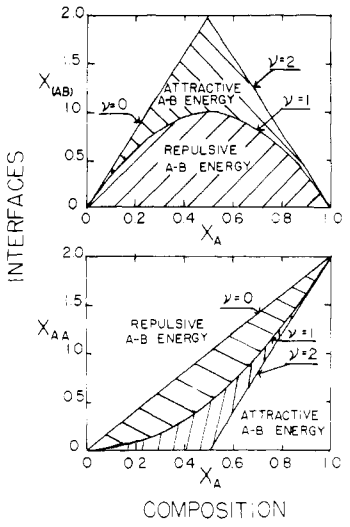

FIGURE 5: The mole fraction of like-like (X_{AA}) and like-unlike (X_{AB}) interfaces as a function of composition for a lipid mixture. The behavior for an ideal mixture ($\nu = 1$) is shown along with the regions in which the plot would occur for repulsive ($0 < \nu < 1$) and for attractive ($1 < \nu < 2$) A-B interaction energies.

termine whether the nonideal interactions are attractive or repulsive. Lipid mixtures having larger X_{AA} and smaller X_{AB} values than found for an ideal mixture show a tendency for like lipids to cluster together leading to increased lateral species separation. Conversely, mixtures having smaller X_{AA} and larger X_{AB} values than found for an ideal mixture show a tendency for the formation of like-unlike lipid pairs leading to reduced lateral species separation.

On examining Figures 2, 3, and 4, the following observations can be made. First, the value of ν differs in the gel and liquid crystal phases and the values in the two phases are not related. Thus, the nonideal interactions between two lipids are not an inherent property of the lipid, but depend on the way the lipids are packed in each phase. Second, the kind of nonideal interaction observed was repulsive in both phases in all three lipid mixtures. Third, in all three mixtures the gel phase is generally more nonideal than the lipid crystalline phase. Fourth, the degree of nonideality depends on the composition in the gel phase of the DMPC/DSPC mixture. Mixtures having small amounts of DMPC included in DSPC are nearly ideal; however, mixtures having small amounts of DSPC included in the DMPC are quite nonideal. Fifth, the nonideality parameter is not an additive property of a lipid or of a phase. Thus, the strong degree of nonideality observed in the gel phase of the DMPC/DSPC mixture is not a linear sum of the nonideality observed in the DMPC/DPPC and DPPC/DSPC mixtures.

Either the square planar or the hexagonal models can be used to fit the data. The alkyl chains of the lipids are arranged

in hexagonal close pack while the functional groups in the polar head group are not. Each model has some validity and the true representation of nature may be an average of the two. Since there are more near neighbors in the hexagonal than in the square planar model, the nonideal free energy is divided over more A–B interfaces so the ν values obtained on fitting the same data with both models will be smaller for the hexagonal than the square planar model.

The value of ν which is obtained in this paper can be used to calculate the mole fractions of different kinds of near-neighbor pairs and also the magnitude of the pairwise nonideal interaction energy. Both of these are descriptive of the short range structure in the system. It is desirable to calculate a long-range correlation length and a value for the average cluster size of like lipids. For the two extremes of $\nu = 0$ and $\nu = 2$ where there is only one configuration of the system, the cluster sizes of like lipid are infinity and unity, respectively as shown in Figure 1. For the finite interaction energies and nonzero temperatures where numerous configurations of the system which are consistent with a given set of X_{AA}, X_{BB}, and X_{AB}, models can be used to relate ν to an average cluster size. These relations will be discussed in a future communication.

Discussion

The arrangement of lipids in a bimolecular leaflet was proposed in 1925 (Gorter and Grendel, 1925) and reemphasized 30 years later in 1956 (Danielli and Davson, 1956). Since that time numerous models which describe the lipid bilayer structure in more detail have appeared (Bretscher, 1973; McCammon and Deutch, 1975). The biological functioning of the membrane bound enzymes depends on both the physical state and composition of the lipids in the enzyme annulus. If local regions of one species of lipid in one state are large relative to the diameter of the enzyme, then the enzymatic activity will be characteristic of those lipids which are in the annulus and independent of the other lipids present in the membrane. Therefore, it is desirable to obtain detailed information on the sizes of the groups of lipids of the same species (cluster sizes) and the groups of lipids of the same phase (domain sizes).

The phospholipid multilayers can be well represented as a two-dimensional surface. In the present paper, a statistical mechanical model to treat a two-dimensional system is presented. The partition function for a binary lipid mixture is obtained using Prigogine's approximation of Onsager's (1944) combinatorial term and an additional free energy term for the nonideal interactions between the two kinds of lipids. A separate nonideality parameter for interactions between the two kinds of lipids is obtained for each phase present. The nonideal parameter is converted to mole fractions of like-like and like-unlike interfaces. In future communications the relation between the nonideality parameter and the average cluster size will be considered and also the average domain size for the gel and liquid crystalline phases will be calculated. When electron microscope techniques are refined to the point that the domain and cluster structure of the bilayer surface can be seen, comparison of the experimentally observed values to those predicted theoretically will serve as a test of the theory.

Note Added in Proof

This manuscript is part of a series of papers dealing with lipids. Other papers discuss: (1) pure lipids having higher order phase transitions (Von Dreele and Chan, 1978); (2) mixed lipids having higher order phase transitions (Von Dreele, 1978); (3) the influence of membrane superstructure on local lipid organization (Von Dreele and Chan, 1977).

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References

Bretscher, M. S. (1973), *Science 181,* 622.

Danielli, J. F., and Davson, H. (1956), *J. Cell Physiol. 5,* 495.

Gorter, E., and Grendel, F. (1925), *J. Exp. Med. 41,* 439.

Hill, T. L. (1956), Statistical Mechanics, New York, N.Y., McGraw-Hill, Chapter 6.

Hinz, H. J., and Sturtevant, J. M. (1972), *J. Biol. Chem. 247,* 6071.

McCammon, J. A., and Deutch, J. M. (1975), *J. Am. Chem. Soc. 97,* 6675.

Onsager, L. (1944), *Phys. Rev. 65,* 117.

Prigogine, I., Mathot-Sarolea, L., and van Hove, L. (1952), *Trans. Faraday Soc. 48,* 485.

Selz, H. (1934), *J. Am. Chem. Soc. 56,* 307.

Shimshick, E. J., and McConnell, H. M. (1973), *Biochemistry 12,* 2351.

Von Dreele, P. H. (1978), *J. Am. Chem. Soc.* (submitted for publication).

Von Dreele, P. H., and Chan, S. I. (1977) *Biochim. Biophys. Acta* (submitted for publication).

Von Dreele, P. H., and Chan, S. I. (1978) *Proc. Natl. Acad. Sci. U.S.A.* (submitted for publication).

Von Dreele, P. H., Poland, D., and Scheraga, H. A. (1971), *Macromolecules 4,* 396.