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A THEORETICAL DESCRIPTION OF PHASE DIAGRAMS FOR NONIDEAL LIPID MIXTURES

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Summary

A theoretical description of phase diagrams for nonideal lipid mixtures is presented. The phase diagrams in this model are constructed by a quasi-chemical approach for the calculations of enthalpies of the regular solutions and by van der Waals attractive energy of lipids which described the degree of nonideality in the solid and fluid phases. The results of theoretical calculations of phase diagrams for dimyristoyl phosphatidylcholine/dipalmitoyl phosphatidylcholine, dimyristoyl phosphatidylcholine/distearoyl phosphatidylcholine, and dipalmitoyl phosphatidylcholine/distearoyl phosphatidylcholine mixtures are in good agreement with experimental data.

Introduction

Within the past few years there has been a large number of theoretical models for phase phenomena in pure lipid bilayers [1—15]. Recently, theoretical work in this field has progressed from basic attempts to understand the structural transitions in pure lipid bilayers to the study of lipid mixtures [9,16—20]. Phase diagrams representing lateral phase separations in binary mixtures of lipids and in the biological membranes have been described as two-dimensional equilibria between the fluid and solid phases [21]. It was shown by Mabrey and Sturtevant [22] that the phase diagrams of binary mixtures of lipids obtained calorimetrically did not agree with that theoretical curves calculated for ideal mixtures. The disagreement in phase diagrams of binary lipid mixtures between experimental data and theoretical calculation for ideal mix-

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Abbreviations: DPPC, dipalmitoyl phosphatidylcholine; DMPC, dimyristoyl phosphatidylcholine; DSPC, distearoyl phosphatidylcholine; DLPC, dilauroyl phosphatidylcholine.

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tures suggests that nonideal mixing interactions between lipids are involved. The calculation of binary lipid mixtures by assuming nonideal mixing in the solid and fluid phases has recently proposed by Lee [16,17] in which he used regular solutions or althermal solutions to calculate the excess Gibbs free energy. Using one parameter to describe the nonideal mixing of the lipids, the Lee model can reproduce the experimental phase diagrams for binary mixtures of lipids. However, these two parameters whereby one describes the degree of nonideality in the solid phase and the other describes the degree of nonideality in the fluid phase can be reduced to only one parameter by introducing van der Waals attractive energy. The purpose of this paper is to present a theoretical study of phase diagrams for nonideal lipid mixtures by using one freely adjustable parameter to describe the degree of nonideality in both the solid and fluid phases. The phase diagrams in this model are constructed by a quasi-chemical approach for the calculations of enthalpies of the regular solutions and by van der Walls attractive energy of lipids which describes the degree of nonideality in the solid and fluid phases. It seems that the model gives a simple analysis of phase diagrams for binary mixtures of lipids by using one parameter, van der Waals energy constant, to describe nonideal mixing of the lipids. In this paper we study the binary lipid mixtures of DMPC/DPPC, DMPC/DSPC and DPPC/DSPC. These lipids have the same head group but differ in chain length.

Method

From the condition of equilibrium between the fluid and solid phases, the equations for calculating phase diagrams of nonideal lipid mixtures are given by [16,17]

$$\ln\frac{X_A^s}{X_A^f}=\frac{\Delta H_A^m}{R}\left(\frac{1}{T}-\frac{1}{T_A^m}\right)+\frac{1}{RT}\left(W^f(1-X_A^f)^2-W^s(1-X_A^s)^2\right) \qquad (1)$$

$$\ln\frac{(1-X_A^s)}{(1-X_A^f)}=\frac{\Delta H_B^m}{R}\left(\frac{1}{T}-\frac{1}{T_B^m}\right)+\frac{1}{RT}\left(W^f X_A^{f2}-W^s X_B^{s2}\right) \qquad (2)$$

where X_A^s and X_A^f are the molar fractions of component A in the solid and fluid phases, respectively; ΔH_A^m and ΔH_B^m are the heats of transition of the pure lipids and T_A^m, T_B^m are their absolute transition temperatures; W^s and W^f are two parameters which describe the nonideality of mixing in the solid and fluid phases, respectively. These two nonlinear equations with two unknowns, X_A^s and X_A^f, can be solved for various temperatures by adjusting two parameters W^s and W^f. However, these two parameters can be reduced to only one by introducing van der Waals attractive energy between lipid molecules in this study.

The van der Waals dispersion forces at small distance have been recognized as a contributing to the stability of many biological structures [23]. It is clear that dispersion forces are important in maintaining lipid-bilayer chains in the cell membranes. The attractive energy between two long parallel hydrocarbon chains has been calculated by Salem [23]. Actually, one has a variety of choices for the van der Waals attractive energy [1]. In this paper we use

$$E = -\frac{C}{A^{5/2}} \tag{3}$$

where A is the molecular area and C is a constant related to van der Waals energy.

To describe the quasi-chemical approximation for the calculation of the enthalpies of regular solutions, we consider a mixture of molecules of two types, A and B, and assume that both types pack in the same way so that they have a common value of the first coordinate number Z. For a two-dimensional lipid system, $Z = 6$ in a hexagonal packing. A mixture of the two kinds of molecule can also pack in the same way as each of a single component, since we are concered with the mixtures of two very similar lipids. In the quasi-chemical approximation [24] it is assumed that the average potential energy can be obtained by assigning the nearest-neighbor interactions to each type of molecular pair in the system. Then the excess free energy can be written as [24]

$$\begin{aligned} G &= Z(U_{AB} - (U_{AA} + U_{BB})/2)\, X_A X_B \\ &= W X_A X_B \end{aligned} \tag{4}$$

where W indicates the degree of nonideality mixing and U_{AA}, U_{BB}, and U_{AB} are the A-A, B-B, and A-B nearest-neighbor energies, respectively. We now assume that the presence of nonideal interaction between unlike lipids is only the van der Waals attractive interaction of hydrocarbon chains, since we have considered a system in which the two components have a identical head group but different CH_2 chains. In this case, the A-A, B-B, and A-B pair energies can be converted to van der Waals attractive energies as

$$E_{AA} = ZU_{AA}\,, \qquad E_{BB} = ZU_{BB}\,, \qquad \text{and } E_{AB} = ZU_{AB} \tag{5}$$

where E_{AA}, E_{BB} and E_{AB} are van der Waals attractive energies of A-A, B-B, and A-B pairs, respectively.

The parameter W interpreted as A-A, B-B, and A-B pair energies can be used to describe the information about the nonideal mixing of the lipids, and is readily expressed in terms of van der Waals energy. From Eqns. 3, 4 and 5, the parameter W can be written as

$$W = -\left(\frac{C_{AB}}{A^{5/2}} - \left(\frac{C_{AA}}{A^{5/2}} + \frac{C_{BB}}{A^{5/2}}\right)/2\right) \tag{6}$$

where C_{AB}, C_{AA} and C_{BB} are van der Waals energy constants of E_{AB}, E_{AA} and E_{BB}, respectively. In this model, we first determine the like-pair energy constants, C_{AA} and C_{BB}, in Eqn. 6, and then we find the unlike-pair energy constant, C_{AB}, by fitting procedure.

From density measurement studies [25], the change in van der Waals energy at the main transition for DMPC was 4.1 kcal/mol. Using this finding, the equation of change in van der Waals energy at the phase transition in this model for DMPC is given by

$$-\frac{C_{14}}{A_f^{5/2}} - \left(-\frac{C_{14}}{A_s^{5/2}}\right) = 4.1 \text{ kcal/mol} \tag{7}$$

where A_f and A_s denote the cross-sectional area in the fluid and solid phases, respectively, and C_{14} is the energy constant for DMPC. The experimental data also showed that the area changes across the transition for DMPC, DPPC and DSPC are of the order 20—35% [26]. Therefore, it is reasonable to choose the cross-sectional area $A_s = 48$ Å^2 per molecule in the solid phase and $A_f = 60$ Å^2 per molecule in the fluid phase near the transition temperature. Then the energy constant C_{14} determined from Eqn. 7 is 153 067 kcal · Å^5 · mol^{-1}. Similarly, we calculate the energy constants $C_{16} = 205\,334$ kcal · Å^5 · mol^{-1} and $C_{18} = 272\,535$ kcal · Å^5 · mol^{-1} for DPPC and DSPC, respectively. The results of our calculations for van der Waals energy constants and the van der Waals energies in the fluid phase E_f and in the solid phase E_s for DMPC, DPPC and DSPC are listed in Table I.

From Eqn. 6, the parameters W^f and W^s for DMPC/DPPC mixtures can be written as

$$W^f = -\left(\frac{C_m}{A_f^{5/2}} - \left(\frac{C_{14}}{A_f^{5/2}} + \frac{C_{16}}{A_f^{5/2}}\right)/2\right) \tag{8}$$

$$W^s = -\left(\frac{C_m}{A_s^{5/2}} - \left(\frac{C_{14}}{A_s^{5/2}} + \frac{C_{16}}{A_s^{5/2}}\right)/2\right) \tag{9}$$

where the parameter C_m is interpreted as an unlike-pair interaction energy constant. Substituting W^f and W^s into Eqns. 1 and 2, we rewrite these two equations as functions of X^s_A, X^f_A and C_m. Then Eqns. 1 and 2 can be solved simultaneously to obtain two unknowns, X^s_A and X^f_A, by adjusting parameter C_m. Similarly, we obtain the equations for calculating phase diagrams of DPPC/DSPC, and DMPC/DSPC mixtures.

Eqns. 1 and 2 can be solved by a numerical method, in this case by the Newton-Raphson technique. The fitting procedure used in this paper is to find the value of the parameter C_m in Eqns. 1 and 2 for which our theoretically calculated phase diagrams agree as closely as possible with the experimental phase diagrams. The van der Waals energy constants after the fitting procedure are 167 489, 195 512, and 220 250 kcal · Å^5 · mol^{-1} for DMPC/DPPC, DPPC/DSPC and DMPC/DSPC respectively. Due to lack of data for determining excess enthalpies of mixing in lipid-bilayer systems [22], it is not possible to compare the experimental data with the C_m value obtained by the fitting procedure used

TABLE I

EXPERIMENTAL AND CALCULATED PHASE TRANSITION PROPERTIES OF PHOSPHATIDYLCHOLINES

Lipid	Acyl chain length	Transition temperature (K)	ΔH (kcal/mol)	ΔU (kcal/mol)	$-E$(solid) (kcal/mol)	$-E$(fluid) (kcal/mol)	C (kcal · Å^5 · mol^{-1})
DMPC	C_{14}	297 *	5.4 *	4.1 **	9.6	5.5	153 067
DPPC	C_{16}	314 *	8.7 *	5.5 **	12.9	7.4	205 334
DSPC	C_{18}	328 *	10.6 *	7.3 **	17.0	9.8	272 535

* Ref. 22.

** Ref. 25.

in this paper. However, the C_m value found in this model can be used to obtain the values of W^s and W^f, the effective van der Waals attractive energies describing the degree of nonideality in the solid and fluid phases.

From Eqn. 8 or 9 the parameter C_m can be expressed as

$$C_m = \tfrac{1}{2}(C_{14} + C_{16}) - W^f(A_f)^{5/2} \tag{10}$$

or

$$C_m = \tfrac{1}{2}(C_{14} + C_{16}) - W^s(A_s)^{5/2} \tag{11}$$

Hence, knowing of C_m is equivalent to obtaining W, which describes the degree of nonideality of the lipids in the fluid and solid phases. I shall explain results in terms of molecular interactions in the next section.

Results and Discussion

The results of theoretical calculations for the unlike pair attractive energy constants C_m, the unlike pair attractive energies in the solid phase E_{AB}(solid) and in the fluid phase E_{AB}(fluid), and the degree of nonideality in the solid phase W^s and in the fluid phase W^f are listed in Table II. The deviation of solution from ideal mixing, the parameter W, depends on the relative magnitudes of E_{AB}, E_{AA}, and E_{BB} as described in Eqns. 4 and 5. If the interaction energy between unlike molecules, E_{AB}, is equal to the average of like molecules, $(E_{AA} + E_{BB})/2$, then $W = 0$ and mixing is ideal. In this case, the resulting Eqns. 1 and 2 for calculating phase diagrams become that for ideal mixing. Examining the Tables I and II, we find that the values of E_{AB} are greater than the average of E_{AA} and E_{BB}. That is, two like molecules attract more strongly than two unlike molecules, since van der Waals attractive forces are negative. This indicates that the nonideal mixtures of DMPC/DPPC, DMPC/DSPC, and DPPC/DSPC represent positive deviation from ideality, and DMPC/DSPC has a larger degree of nonideality. These results can also be seen from the fifth and sixth columns in Table II, since W^s and W^f are positive. From the data listed in Table II, the degree of nonideality in the solid phase W^s are found to be larger than those in the fluid phase W^f. This is consistent with the results obtained by Lee [17] and Dreele [18].

The results of phase diagrams calculated for binary mixtures of DMPC/DPPC, DMPC/DSPC and DPPC/DSPC using nonideal mixing are shown in Figs. 1, 2, and 3. The solid lines in these figures are the theoretical results calculated from Eqns. 1 and 2 and dashed lines represent the phase diagrams calculated

TABLE II

CALCULATED PHASE TRANSITION PROPERTIES OF PHOSPHATIDYLCHOLINE MIXTURES

Lipid mixtures	C_m (kcal · Å^5 · mol^{-1})	$-E_{AB}$(solid) (kcal/mol)	$-E_{AB}$(fluid) (kcal/mol)	W(solid) (kcal/mol)	W(fluid) (kcal/mol)
DMPC/DPPC	167 489	10.5	6.0	0.73	0.42
DPPC/DSPC	195 512	12.2	7.0	1.08	0.62
DMPC/DSPC	220 250	13.8	7.9	1.17	0.67

for an ideal mixture. Phase diagrams for binary mixtures of lipids the chain lengths of which differ by two or four carbons have been measured by calorimetric [22,27], ESR [21], and fluorescence spectroscopy [28,29] techniques. These methods give qualitatively the same phase diagrams. In Figs. 1, 2 and 3, the circles represent the calorimetric data of Mabrey and Sturtevant [22], and the squares represent the ESR data of Shimshick and McConnell [21]. From these figures, we find that the theoretical calculations of phase diagrams for DMPC/DPPC, DMPC/DSPC and DPPC/DSPC mixtures are in good agreement with experimental data. This indicates that the phase diagrams calculated for nonideal mixing in this model are closer to experimental phase diagrams than that calculated for ideal mixing.

In Fig. 2 for DMPC/DSPC mixtures, the theoretical calculation exhibits the same nearly horizontal solidus curve as is observed experimentally. For this system, there is disagreement as to whether the two components are solid-phase immiscible or not. Some experimenters have shown a horizontal solidus curve up to at least a 50 : 50 molar mixture which indicates a solid-phase immiscibility [21,27,29,30], whereas other experimenters have reported a solid curve with a non-zero slope [22,31,32]. Recently, dilatometric study of lipid mixtures performed by Wilkinson and Nagle [33] has shown that there is no solid-solid immiscibility in DMPC/DSPC mixtures. To find the slope of solidus curve for DMPC/DSPC mixtures in this model, we calculate dT/dX^s_A in Eqns. 1 and 2. The results of calculated slopes in the model are in reasonable agreement with experimental data for solid curve with non-zero slope in the mole fraction

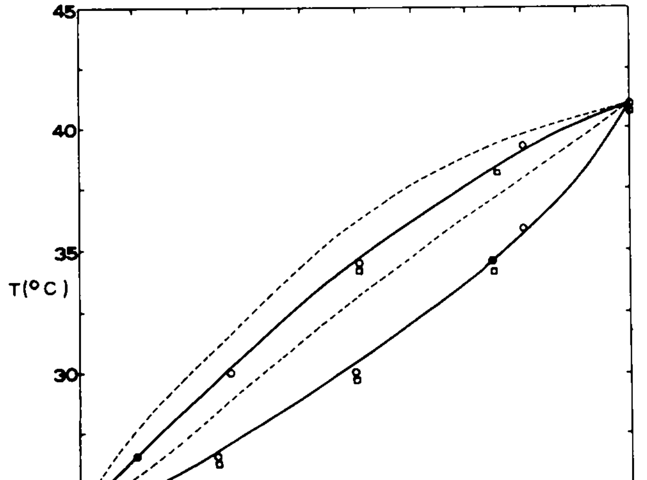

Fig. 1. Temperature-composition phase diagram for DMPC/DPPC mixtures with nonideal mixing. The solid lines are the theoretical results calculated from Eqns. 1 and 2, dashed lines represent phase diagram calculated for ideal mixing, the circles represent the calorimetric data of Mabrey and Sturtevant [22], and the squares represent the ESR data of Shimshick and McConnell [21].

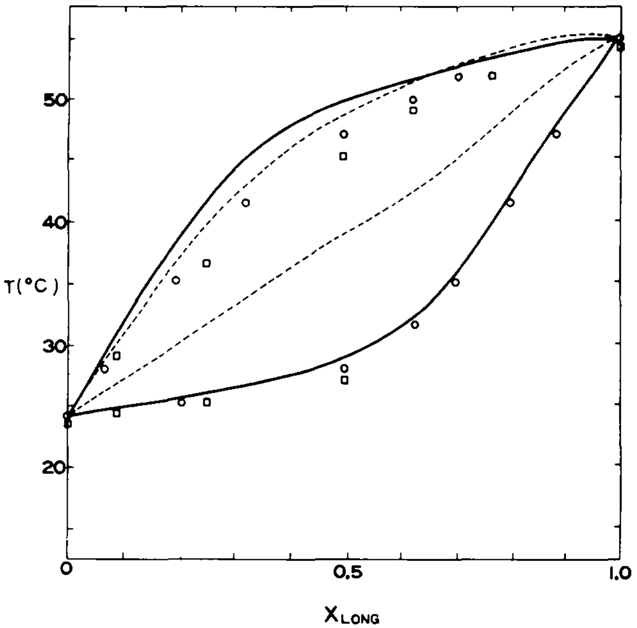

Fig. 2. Temperature vs. composition phase diagram for DMPC/DSPC mixtures with nonideal mixing. The solid lines are the theoretical results calculated from Eqns. 1 and 2, dashed lines represent phase diagrams calculated for ideal mixing, the circles represent the calorimetric data of Mabrey and Sturtevant [22], and the squares represent the ESR data of Shimshick and McConnell [21].

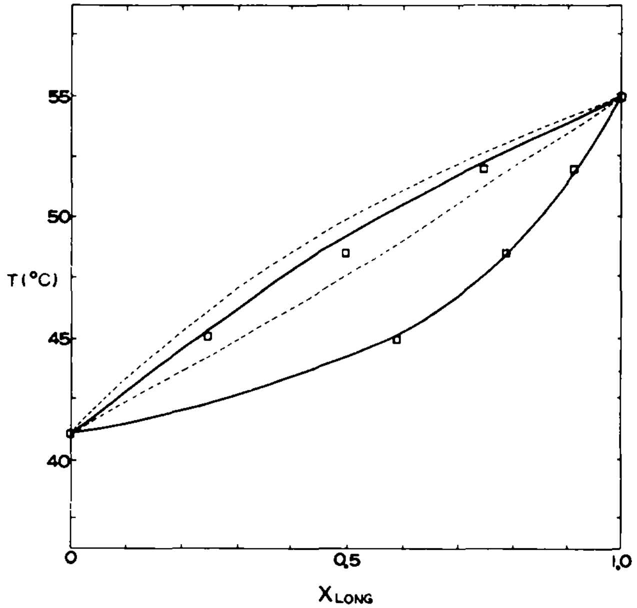

Fig. 3. Temperature vs. composition phase diagram for DPPC/DSPC mixtures with nonideal mixing. The solid lines are the theoretical results calculated from Eqns. 1 and 2, dashed lines represent phase diagram calculated for ideal mixing, and the squares represent the ESR data of Shimshick and McConnell [21].

range 0—0.3 obtained by Wilkinson and Nagle [33].

For DMPC/DSPC mixtures, although the model describes well the phase diagram, the theoretical fluidus curve is still higher than the experimental points, as shown in Fig. 2. The reason why the model cannot reproduce completely the experimental fluidus curve is that we have not considered the other possible contributions to the energy of nonideal mixing interactions between unlike lipids as the mixtures of lipids differing in fatty acyl chain length by more than four carbons. In general, the nature of nonideal mixing interactions between unlike lipids is complex and subtle. If the head groups are identical, then the most important interactions between unlike lipids not treated here are those arising from the difference in chain length, and the effect that mismatches in chain length may have on chain packing [20]. The DMPC/DSPC mixture is considerably further removed from ideal than are the systems of DMPC/DPPC and DPPC/DSPC, as described previously. For mixtures of lipids differing in chain length by more than four carbons such as DMPC/DSPC and DSPC/DLPC, the chain mismatching interactions may keep two unlike lipids further apart. However, such an interaction is difficult to include explicity in our simple model.

Using van der Waals attractive energy to describe the degree of nonideality in the solid and fluid phases, a simple theoretical model for binary lipid mixtures phase diagrams has been presented. The most important of the calculations of lipid mixtures in this model is that in which van der Waals attractive energy is applied directly to describe the nonideal lipid mixtures. An advantage of the method described in this study of the van der Waals attractive energy is that only one free parameter is used to adjust both the solid and fluid phases, while the Lee [16,17] and Dreele [18] models needed to use two adjustable parameters to describe the degree of nonideality in their models.

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