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PHOSPHATIDYLCHOLINE BILAYERS

A THEORETICAL MODEL WHICH DESCRIBES THE MAIN AND THE LOWER TRANSITIONS

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Summary

We present a theoretical model which describes both the main and the lower phase transition in phosphatidylcholine bilayers. The main transition involves a melting of the hydrocarbon chains while the lower transition is seen as a nematic to isotropic transition involving entire lipid molecules (which are rod shaped when projected onto the bilayer plane). This latter transition is consistent with experimental data which suggest the presence of long-axis rotation for temperatures below the main melting transition. The model is extended to mixtures of phosphatidylcholines and compared with experimental data.

Introduction

The main hydrocarbon chain-melting phase transition in phospholipid bilayers has been extensively modeled by a wide variety of theoretical techniques [1]. However, the weaker transition found several degrees below the main transition in some phospholipids (most notably the phosphatidylcholines) has not received the same degree of attention [2]. Experimental evidence has shown that this lower transition has the following characteristics:

(i) It is broader in temperature and has an enthalpy change approx. 5-times smaller than the main transition [3]. The enthalpy change does not appear to be strongly dependent upon hydrocarbon chain length, but for longer chains, the lower transition occurs closer in temperature to the main transition [3].

(ii) The lower phase transition coincides with the appearance of the $P_{\beta'}$, or 'ripple' phase [4—6].

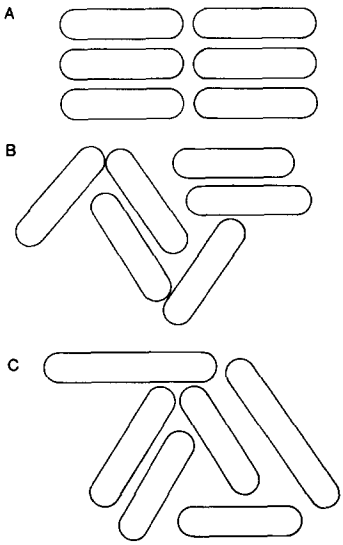
(iii) Saturation-transfer ESR experiments using nitroxide labels suggest that

the lower phase transition signals the onset of long-axis rotation [7], although NMR studies with perdeuterated chains [8] suggest that this rotation also occurs at temperature below the lower transition. In both experiments the data suggest a rather sudden onset of long-axis rotation at temperatures below the main lipid phase change, perhaps at a point close to the recently discovered 'subtransition' in dipalmitoylphosphatidylcholine [9].

The purpose of this paper is to present a theoretical model which describes both a hydrocarbon chain-melting transition and a weaker transition from a rigid, anisotropic phase to a phase with long-axis rotational freedom. The location of the weaker transition relative to the main phase transition depends very sensitively upon the hard core shape of the molecule, as we shall show. Our model is similar in structure to a model presented earlier [10] in which we described the lipids in a bilayer by treating their projections onto the bilayer plane as circles with varying radii. The radius of the circle was determined by the isomeric state of the molecule in question, and in this way hydrocarbon chain statistics were introduced. The hard core properties of the system were then calculated using Scaled Particle Theory for a mixture of hard disks of varying radius. The model gives a description of the main phase transition in phosphatidylcholines which agrees well with experiment. One disadvantage is that this model presumes that the lipids are cylindrical in overall shape, since their shadows in the bilayer plane are circles. Thus, long-axis rotation can always occur. In order to more accurately study these systems, it is natural to consider the anisotropic hard core interactions among molecules. To do this we consider the lipid molecules to cast elongated, rather than circular shadows on a plane parallel to the bilayer plane. The precise dimensions of the elongated shadows will again depend upon the isometric state of the molecule, and so we can include chain statistics in the same fashion as mentioned [10] using a mixture of rods of varying size. Then, highly disordered molecules cast longer rod-shaped shadows than do molecules with ordered chains. In a earlier examination of planar systems of hard rods, we found that Scaled Particle Theory plus an attractive van der Waals interaction often (but not always) led to two-phase changes [11]. One involved the 'freezing' of the rod from a state in which all rod conformations and orientations are allowed into a closely packed state in which all rods cast the smallest allowed shadow (corresponding to an all *trans* lipid chain conformation) but with random orientation. The other transition involved a change from a state in which all orientations of the rods in the plane were allowed, to a state in which the rods were nearly all parallel in orientation.

Description of the model

The method developed by Scott [11] has now been applied to dimyristoyl-, dipalmitoyl-, and distearoylphosphatidylcholine bilayers. We have classified the hydrocarbon chains in exactly the same manner as Scott and Cheng [10], with the same statistical weights per class and the rod area for a given class is also the same. This classification scheme is used again because the number of classes is neither so small that the system is oversimplified, nor too large for convenient numerical analysis. In this work, in contrast to the circles used by Scott and



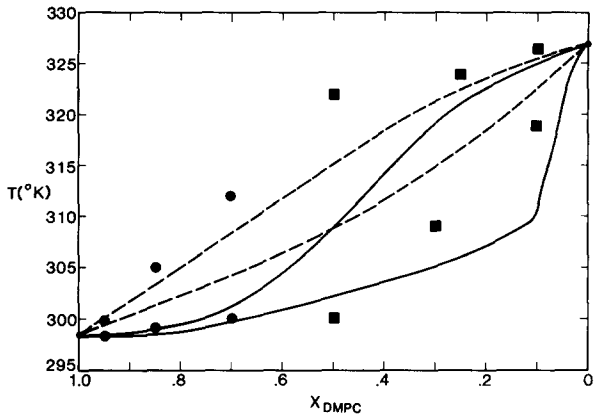


Fig. 1. Schematic illustration of the types of ordering possible in the model. A. All rods parallel and having the shortest possible dimensions. B. Rods rotationally disordered, but still having shortest lengths possible. C. Rods rotationally disordered and exhibiting isomeric disorder as longer rod states become occupied.

Fig. 2. Phase diagram for DML/DSL mixtures. ------, ideal mixing case, in which the van der Waals energy is given by Eqn. 2. ———, non-ideal solid phase mixing, in which the fluid phase van der Waals energy is given by Eqn. 2, and the solid phase is described by Eqns. 2 plus 3. ○, experimental data of Nagle and Wilkinson [11]; □, data of Mabrey and Sturtevant [3]. DML, dimyristoylphosphatidylcholine. DSL, distearoylphosphatidylcholine.

Cheng [10] and to the rectangular rods used by Scott [11], we use circularly capped rods (the addition of the circular cap actually makes the computation simpler than the square-ended rods). The additional degrees of freedom in these models are then the rod length, or the cap diameter and the rod orientation. As we shall show, the length variable is critical in locating the rotational transition. As mentioned [11] we restrict orientations to multiples of 60°, for numerical purposes. In addition to using the same areas as previously [10], we also impose a lateral pressure of 50 dynes/cm [10]. Although this value for the applied pressure is not agreed upon by all workers in the field, it worked well previously [10] so we use it here. Lower pressures lead to transitions in which the higher temperature state resembles a gaseous monolayer rather than a confined bilayer. Fig. 1 illustrates the capped rods and the three states which this model exhibits.

If we let α_k denote the fraction of the molecules in state k, where k denotes a particular rod size and orientation, the Gibbs free energy of the system can be written as

$$G/Nk_BT = \sum_i \alpha_i(\ln \alpha_i - \ln \omega_i) + \ln\left[\rho\Big/\left(1 - \rho \sum_i \alpha_i A_i\right)\right]$$
$$+ \left[\tfrac{1}{2}\rho\Big/\left(1 - \rho \sum_i \alpha_i A_i\right)\right] \sum_{k,l} \alpha_k \alpha_l (\pi d_k d_l/2 + l_k d_l + l_l d_k + l_k l_l |\sin(\theta_{kl})|)$$
$$- C/(A - 38)^{1.5} + \pi A \qquad (1)$$

TABLE I

PROPERTIES OF THE THEORETICAL MODELS

Experimental values for enthalpy changes are given in parentheses. Data for ΔH_l and ΔH_m are from Ref. 3.

Lipid	States					Lower transition		Main transition	
	Average number of *gauche* rotations	Cap diameter (Å)	Length (Å)	Weight	C ($\times 10^5$)	T_l (K)	ΔH_l (kcal/mol)	T_m (K)	ΔH_m (kcal/mol)
Dimyristoylphosphatidylcholine	0	5.18	3.81	1					
	2	5.18	4.41	20					
	3.75	5.18	5.12	192					
	5.84	5.18	5.96	676	1.24	287	0.35 (1.0)	297	6.0 (6.3)
	4.75	5.18	6.96	192					
	4.00	5.18	8.18	20					
Dipalmitoylphosphatidylcholine	0	5.25	3.60	1					
	2	5.25	4.20	24					
	3.79	5.25	4.89	280					
	5.90	5.25	5.71	1328	1.38	307	0.39 (1.8)	314	9.0 (8.4)
	4.78	5.25	6.70	3288					
	4.84	5.25	8.90	256					
	4.50	5.25	9.40	24					
Distearoylphosphatidylcholine	0	5.30	3.54	1					
	2	5.30	4.13	28					
	3.81	5.30	4.82	384					
	5.97	5.30	5.63	5240	1.51	324	0.35 (1.8)	327	10.8 (10.6)
	7.83	5.30	6.61	9456					
	5.43	5.30	7.81	1116					
	4.58	5.30	9.31	728					
	5.00	5.30	11.23	28					

In Eqn. 1, ω_i is the statistical weight assigned to state i, ρ is the particle density, A_i, d_i and l_i are the area, cap diameter and length of state i, respectively, θ_{kl} is the orientation angle between states i and j, and k_BT is Boltzmann's constant times the absolute temperature. The numerical values assigned these quantities are given in Table I. A is the average molecular area, π is the lateral pressure (50 dynes/cm in this work) and C is the effective van der Waals constant [10—11]. As mentioned above, the only values allowed for θ_{kl} are multiples of 60°. This is done to keep the number of variables at a managable level, and does not qualitatively affect the results.

Results and Discussion

The Gibbs free energy must be minimized with respect to all unconstrained variables, that is, the occupation numbers (α_k) and the average area per molecule A. Details of the computational procedure are as given [10—11], so here we turn directly to the results, which we present in Table I, the weights and areas per state conform exactly to those previously used [10]. The state energies are equal to those used previously [10], and are not temperature dependent. Inclusion of temperature in the calculation of the energies for dimyristoylphosphatidylcholine shifts the model transition temperature by approx. 2°C, equivalent to changing the van der Waals constant by approx. 1%. For simplicity we have not included this small temperature dependence in the calculations. The procedure in all calculations is identical to that used previously [10]. The parameter C is adjusted so that the main transition occurs at the temperature found by Nagle and Wilkinson [12]. The cap diameter is then varied until the lower transition occurs at the value reported [12]. Table I shows that the calculated enthalpy changes at the upper transitions for the three systems are close to the values reported by Mabrey and Sturtevant [3] (but are larger than the recently reported results of Chen et al. [9]). The enthalpy changes at the lower transition are uniformly lower than the experimental values. A further check of the model can be made by calculating the change in van der Waals energy at the main transition. The calculated values are 4.1, 6.0, and 7.3 kcal/mol for dimyristoylphosphatidylcholine, dipalmitoylposphatidylcholine, and distearoylphosphatidylcholine, respectively. Experimental values calculated from data [12] are 4.1, 5.5, and 7.3 kcal/mol for dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine, and distearoylphosphatidylcholine, respectively, so that agreement is very good.

Several aspects of this model are worthy of discussion. Firstly, it is important to note that the lower transition in this model is a purely hard core phenomenon involving the chain and glycerol regions and perhaps the head group. The transition is a natural occurrence in a system of hard rods, requiring no external parameters or functions representing head group interactions. Our manipulations of the cap diameters served only to locate the lower transition, relative to the main transition. The fact that the cap diameters became larger for longer chains is due to the fact that the lower transition occurs nearer the main transition in these systems. The results suggest that distearoylphosphatidylcholine has, effectively, a more nearly circular shadow in the bilayer plane than does dipalmitoylphosphatidylcholine or dimyristoylphosphatidyl-

choline. If the shadow were circular, then no lower transition could occur. The difference in shape may be due to the more drastic effect of even a slight amount of isomeric disorder in the longer chains, compared to the shorter chains, at temperatures below T_m.

Since this model does not consider head group-water interactions or cooperative chain tilting, no mechanism exists for the formation of the ripples seen between T_L and T_m [5,6]. Doniach [2] has suggested that a possible mechanism for the formation of a ripple phase involves a sudden change in the polarizability of the head group/water interface. A mechanism for this polarizability change may be the sudden rotational freedom available at the onset of the lower transition. The fact that ΔH_l for the model is substantially lower than the experimental values (Table I) suggest that additional degrees of freedom are activated at T_l although inclusion of more orientations in the model would raise ΔH_l somewhat. The fact that the lower transition is wider in temperature than the main transition means that perhaps several different changes in the system occur at or near T_l. It may also be possible that the newly discovered subtransition [9] is related to long-axis rotation. We discuss this briefly in Conclusion. It should be pointed out that our model does not exhibit the same symmetries as real systems in its phases below T_m. Such symmetries are extremely difficult to calculate in statistical mechanics. The phase change, though, could as easily occur in a hexagonal system as in the present model.

As a final application of the model we have calculated phase diagrams for mixtures of some of the systems whose properties are presented in Table I. The details of the calculations are very similar to those used previously [10], and are omitted here. The phase diagram for a mixture of distearoylphosphatidylcholine and dimyristoylphosphatidylcholine is presented in Fig. 2. This system is of particular interest because of the non-ideal nature of the mixing of the two components [3,13]. If this non-ideal mixing is due to molecular-packing differences in the two systems, our hard core theoretical model should be able to reproduce this, at least, at a qualitative level. However, the straightforward calculations, assuming the ideal form for the van der Waals energy of the mixture,

$$E = -(x_1 C_1 + x_2 C_2)/(A - 38)^{1.5} \tag{2}$$

where 1 and 2 refer to dimyristoylphosphatidylcholine and distearoylphosphatidylcholine, respectively, yields an ideal mixing phase diagram, shown as the dashed curve in Fig. 2. We conclude that the non-ideal nature of the dimyristoylphosphatidylcholine/distearoylphosphatidylcholine mixing has its origin in the van der Waals attractive interaction between the two types of molecules. Since the present model does not consider any detail of the attractive interactions between molecules, it cannot easily reproduce the non-ideal phase diagram. We have considered, in addition to Eqn. 1, a repulsive interaction having the form, added to the condensed phase only,

$$E_{mix} = 1/2 x_1 x_2 (C_1 + C_2)/(A - 38)^{2.5} \tag{3}$$

This represents a hypothesized correction to Eqn. 2 due to interactions between neighboring pairs of dimyristoylphosphatidylcholine and distearoylphosphatidylcholine molecules (when dealing with a restricted set of molecules;

no integrals over the bilayer are involved, and, thus, the power in the denominator of Eqn. 3 is 2.5, rather than 1.5 (see Ref. 10). As is evident in Fig. 2 the resulting diagram has some of the same qualitative features along the solid curve as do the experimental data. However, the fluidus curve differs considerably from the experimental data [3,13]. It appears that improvements to this result will require a much more detailed treatment of the van der Waals interactions between chains of different lengths.

Conclusion

In summary, the models presented here have two interesting features: (i) they provide a method for treating the hydrocarbon chain statistics, including intrachain and hard core interactions, systematically and accurately (although not exactly), and (ii) they provide a mechanism for the onset of the lower phase transition in the hard-rod nematic to isotropic transition in the plane of the bilayer. The T_l values for the models presented here are extremely sensitive to the size of the rods. Thus, small changes in effective rod size can lead to large changes in T_l value. If the long-axis rotational transition occurs at a temperature different from T_l, as suggested by Davis [8], then this suggests the cap diameters are different from those given in Table I. Davis' suggestion that long-axis rotational freedom begins rather suddenly near 20°C in dipalmitoylphosphatidylcholine is consistent with the discovery of a new phase transition at 17°C for this system [9], so that it may be that the rotationally free phase begins at this lower temperature. If so, then the present model still provides a natural description of the transition, while the pretransition may be explained via electrostatic mechanisms [2]. Finally, our calculations of phase diagrams suggest that proper theoretical understanding of the non-ideality of lipid mixtures will involve a detailed study of van der Waals forces between chain of different lengths.

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