

A theory of the chain melting phase transition of aqueous phospholipid dispersions

(lipids/statistical thermodynamics)

RUSSELL E. JACOBS, BRUCE HUDSON, AND HANS C. ANDERSEN

Department of Chemistry, Stanford University, Stanford, California 94305

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ABSTRACT A model for the chain melting phase transition in dilute aqueous phospholipid bilayer dispersions is presented. This model includes interactions between head groups, between hydrocarbon chains, and within the chains. The head groups are modeled as hard disks which are constrained to lie on a two-dimensional surface separating the aqueous and hydrocarbon regions. The chain statistics problem is treated in an approximate manner using an approach motivated by scaled particle theory to describe the inter-chain steric repulsions in a mathematically tractable way. In this approach the whole system interacts with any given chain through an average lateral pressure which is proportional to the hard disk pressure. Following Nagle, we assume that the steric repulsions between chains and between head groups and the *trans-gauche* rotation energies are the dominant interactions in determining the transition and we describe the effect of the other interactions with a mean field approximation. Using the known transition temperature of a series of 1,2-diacyl phosphatidyl cholines to adjust two parameters in the theory, the model gives enthalpy and area changes that are in quite reasonable agreement with experiment. Moreover, the curvature observed in the plot of the transition temperature against acyl chain length is reproduced.

Aqueous dispersions of phospholipid bilayers exhibit characteristic changes in spin label (1) and fluorescent probe parameters (2, 3), x-ray diffraction spacings (4, 5), and thermal properties (6, 7) over a very small temperature range, indicating the existence of some type of phase transition. The present study is concerned with a specific model for the main transition observed in aqueous dispersions of pure phospholipids. We shall assume that the transition involves disordering of the hydrocarbon chains. This order-disorder model is well known and has been used by other workers in the field (8-12). In our approach we consider how the interactions between phospholipid head groups, interactions between hydrocarbon chains, and the conformational energy of the chains affect the thermodynamic properties of a bilayer. The contribution of each of these interactions to the partition function of a bilayer is approximated in a convenient mathematical way, and the resulting partition function predicts that a first order transition involving a cooperative disordering of the hydrocarbon chains is possible. We calculate transition temperatures, enthalpy changes, and area changes for the phase transition and compare them with experimental values for the phosphatidyl cholines. Excellent agreement is found with only two adjustable parameters. In this paper we describe the model, our statistical mechanical method for treating it, and the numerical results. A comparison with the work of other investigators is also made.

METHOD

The physical picture of a bilayer that we use is the following. The head groups of the phospholipid molecules are

constrained to lie approximately on a two-dimensional surface at an interface between the aqueous medium and a region of space containing the hydrocarbon chains. Each head group has one or more hydrocarbon chains attached to it. (In this paper we will only consider the case of two chains per head group.) The chains extend away from the aqueous medium, and two such parallel layers with their hydrocarbon regions in contact form a bilayer. The important type of interactions or energies are: the interactions of the head groups with water, the interactions between head groups, the interactions between hydrocarbon chains, and the intramolecular energy associated with chain conformation. The interactions of the head groups with each other are of two types: the excluded volume repulsions and the longer range dispersion and dipolar forces. The interactions between hydrocarbon chains are also of two types: excluded volume repulsion and longer range attraction. The intramolecular energy is of two types: the energy associated with rotation about single bonds and the interactions between different segments of the chain as a result of chain flexibility, which may allow these segments to approach each other. This is basically the same physical picture as is used by Nagle (8), Scott (9), Marčelja (10), Marsh (11), and McCammon and Deutch (12).

The statistical mechanical model we use is similar to that of Marčelja. We consider only one of the layers in the bilayer. We assume that the only coordinates needed to specify the state of a molecule are the two-dimensional position, r , of its head group, the orientation angle, θ , of its head group in the two-dimensional plane, and a set of labels, α_j , $j = 1, \dots, m$, for each chain of the molecule, where m is the number of bonds on a chain that can exist in both *gauche* and *trans* configurations. We let $\alpha_j = +1, 0$, or -1 if the j th bond on the chain is *gauche* +, *trans*, or *gauche* -. (The collection of m labels for a chain will be denoted by α .) The interaction energy of a collection of N such molecules is represented in the following form:

$$H = H_{\text{head}}(r^N, \theta^N) + H_{\text{chain}}(\alpha^{2N}; r^N, \theta^N) + \sum_{i=1}^{2N} H_{\text{conf}}(\alpha^{(i)}) \quad [1]$$

The first term on the right represents the interactions among the head groups. These interactions are, however, effective interactions since they include some effect of the aqueous medium due to hydration and hydrophobic interactions. The second term is the interaction between chains. Its parametric dependence on r^N and θ^N is indicated explicitly. The third term is the conformational energy of the chains. In this equation we have restricted ourselves to the case of two chains per head group. Thus, for N molecules there are $2N$

sets of chain coordinates α . The two chains are assumed to be equivalent.

To proceed further, it is necessary to approximate the various terms in Eq. 1 to obtain expressions that make the calculation of a partition function possible. How one does this depends on a decision about which interactions are predominant in determining the nature of the phase transition. Nagle (8) assumed that the important features were the excluded volume interactions between chains and the energies associated with the formation of *gauche* bonds. The intermolecular attractions were regarded as important only for providing an overall cohesive energy and a ΔE for the transition, and were approximated in a simple mean field way. With a two-dimensional lattice model, in which the excluded volume problem could be solved exactly, Nagle showed that the excluded volume interactions and *gauche* energies alone were capable of giving a chain melting phase transition. Marčelja (10), on the other hand, assumed that the important interaction for determining the transition is an *attractive* interaction between chains which is stronger in an ordered phase than in a disordered phase. In his calculation, the strength of the interaction was proportional to an order parameter, whose value was calculated self-consistently. Although Marčelja's assumptions are often used in theories of liquid crystals, we believe Nagle's idea of the importance of excluded volume interactions is fundamentally more correct for the bilayer phase transition. Nagle's assumptions can be regarded as a generalization of the van der Waals idea for nonpolar fluids, namely, that the short-range repulsive forces of the molecules determine the structure and that the attractions merely provide some cohesion (13). This idea gives a qualitatively correct account of the existence of a liquid-gas transition. It also accounts for the liquid-solid transition in simple liquids (14). Moreover, it forms the basis for the recent perturbation theories of simple liquids (15) which have been shown to be quantitatively correct as descriptions of thermodynamic and structural properties.

We assume, therefore, that all the attractive parts of H_{head} and H_{chain} , as well as the longer range repulsions (if any) in H_{head} can be approximated in a mean field form. We also assume that the only important contribution to H_{conf} is an energy of ϵ for each *gauche* bond. Thus we have the following approximate form for the energy

$$H = H_{\text{head}}^{\text{exv}}(\mathbf{r}^N, \theta^N) + H_{\text{chain}}^{\text{exv}}(\alpha^{2N}; \mathbf{r}^N, \theta^N) - N(A_0/A)\Delta H_{\text{sub}}(2n + \delta) + \sum_{i=1}^{2N} \sum_{j=1}^m |\alpha_j^{(i)}| \epsilon. \quad [2]$$

The superscripts on the first two terms on the right indicate that only the short range excluded volume part of the interactions is included in these terms. The third term is the mean field attraction. The fourth term is the sum of the *gauche* rotation energies. Here ΔH_{sub} is the heat of sublimation per CH_2 group of long chain hydrocarbon. The term containing $2n$ is the interchain attraction. Here n is the number of CH_2 groups per chain; hence $2n$ is the number per molecule. Also, A is the area of the system of N molecules, and A_0 is the corresponding close-packed area. The parameter δ is a dimensionless number characterizing the strength of the long range attractions (or repulsions) between the head groups.

We simplify the problem further by assuming that the excluded volume problem of the packing of head groups in

their two-dimensional layer is the same as that of the packing of hard disks in two dimensions. (The head groups are in fact somewhat elliptical in shape, but assuming them to be circular is probably a good approximation in calculating the partition function.)

The most difficult part of the problem is a description of the excluded volume interaction between chains. To quantify the steric hindrance a chain feels to bending, we use an approach motivated by scaled particle theory (16). We assume that a bend (i.e., a *gauche* rotation) in any given chain increases the effective cross-sectional area of that chain, and this increase in area must take place against an average pressure P due to the presence of the other chains. Therefore, work must be done in order for a bend to form in the *all trans* chain (i.e., for the chain to change states). This work is the product of the pressure and the additional area the molecule occupies as a result of the change in conformation. This area increase depends only on the coordinates α for the chain, and is assumed to be independent of the position and orientation of the head group. Thus we approximate

$$H_{\text{chain}}^{\text{exv}} = \sum_{i=1}^{2N} P \Delta A(\alpha^{(i)}), \quad [3]$$

where the sum extends over all chains, $\alpha^{(i)}$ is the set of conformational labels for chain i , ΔA is the increase in effective cross-sectional areas of a chain with coordinates $\alpha^{(i)}$ relative to an *all trans* chain, and P is the effective lateral pressure on the chain due to the steric repulsions of the other chains. To evaluate P , we note that after a chain bends, its additional area, ΔA , is no longer available to the rest of the system. Specifically, this limits the freedom of the hard disk head groups and thereby changes their free energy. This free energy change per unit area change is simply proportional to the hard disk pressure. As a first approximation, we set the pressure in Eq. 3 equal to the hard disk pressure, P_{HD} . The applicability of such a simple approximation is borne out by the results obtained, although refinement through a self-consistent calculation might give better results and would be conceptually interesting. (This could be done by using $P = P_{\text{HD}}$ as a first approximation and then calculating $P' = kT(d \ln Q' / dA)$, where $Q' = Q_{\text{HD}} Q_{\text{CHAIN}}$, and Q_{CHAIN} is the partition function for the chains. Then P' would be used to calculate the next approximation for Q_{CHAIN} , and the process could be iterated to self-consistency.)

With these approximations, the partition function factors into the following form:

$$Q(N, A, T) = Q_{\text{HD}}(N, A, T) [q_{\text{CHAIN}}(T; P_{\text{HD}})]^{2N} \times \exp[-N(A_0/A)\Delta H_{\text{sub}}(2n + \delta)/kT] \quad [4]$$

where q_{CHAIN} is the partition function of a chain and is given by

$$q_{\text{CHAIN}}(T; P_{\text{HD}}) = \sum_{\alpha} \exp\left(-\left[\epsilon \sum_{i=1}^m |\alpha_i| + P_{\text{HD}} \Delta A(\alpha)\right]/kT\right). \quad [5]$$

In the first sum, each of the $m\alpha_i$'s is to be summed over each of its three possible values, thus summing over all rota-

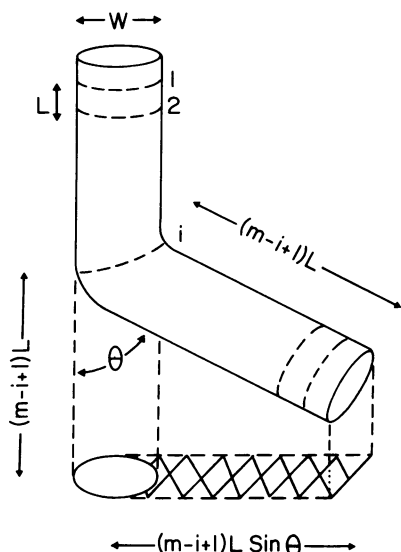


FIG. 1. A schematic representation of a bend at carbon i in an acyl chain where L is the vertical distance between CH_2 groups, W the diameter of the chain, and θ the angle between the bent and vertical portions. The projected cross-hatched region is the maximum additional area the chain occupies after bending.

tional isomeric states of a chain with m rotatable bonds.

To simplify the calculation of q_{CHAIN} , we assume that $\Delta A(\alpha)$ is dependent only on the distance of the first *gauche* bond from the head group (see Fig. 1). Additional *gauche* bonds below this initial one may increase or decrease the effective area, but we assume that on the average these additional *gauche* rotations have no effect on the area. From Fig. 1, we see that if the i th bond from the head group is the first one to have a *gauche* rotation, then

$$\Delta A \propto wL(m - i + 1)\sin\theta$$

where L is the length, measured along the axis of a *trans* chain, of a CH_2 group and w is the width of the chain. Thus we let

$$\Delta A = \gamma(m - i + 1)A_0/N \quad [6]$$

and let γ be an adjustable parameter which we expect to be independent of chain length, of the nature of the head group, and of i , the location of the first *gauche* bond. Using typical bond lengths and angles we calculate a maximum value for γ to be on the order of one-tenth the closest packed area per molecule. Substituting Eq. 6 into Eq. 5 gives

$$q_{\text{CHAIN}} = 1 + \sum_{i=1}^m (1 + 2 \exp[-\beta\epsilon])^{m-i} \times 2 \exp\{-\beta[\epsilon + P_{\text{HD}}A_0\gamma(m - i + 1)/N]\} \quad [7]$$

where $\beta = 1/kT$.

The method we have used to calculate the effective area of a molecule incorporates the view that a *gauche* bond near the head group results in a larger area increase than one near the chain tail. Since the area increase results in unfavorably steric interactions, there will be a higher probability for *gauche* bonds near the chain tails than near the head group.

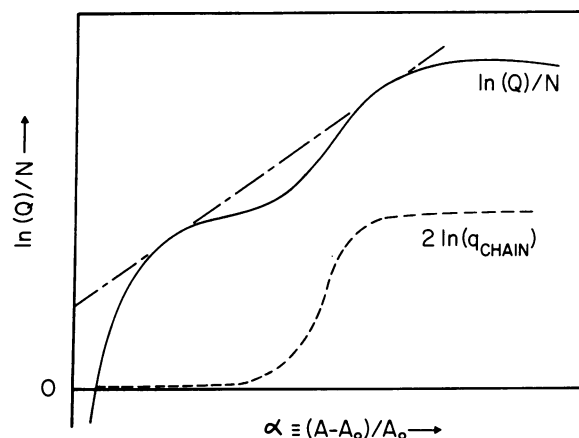


FIG. 2. A schematic representation of the functions $2\ln q_{\text{CHAIN}}$ and $\ln Q/N$ with the double tangent indicated. The states of the system corresponding to the points of tangency necessarily have the same Gibbs free energy and pressure. The rationale for such a double tangent construction is given in standard texts [for example, Huang, K. (1967) *Statistical Mechanics* (John Wiley and Sons, New York), Section 2.3].

Molecular dynamics calculations give us a partition function (Q_{HD}) and pressure (P_{HD}) for the hard disk system. The high density expansion for hard disks (which we have used exclusively) is of the form (17-19):

$$Q_{\text{HD}} = (\alpha^2/\rho_0)^N \exp[N(.06 + 0.1 \alpha + 0.385 \alpha^2 + 0.243 \alpha^3 \dots)] \quad [8]$$

$$P_{\text{HD}}A/NkT = 2/\alpha + 1.90 + 0.67 \alpha + 1.5 \alpha^2 + \dots \quad [9]$$

where $\alpha = (A - A_0)/A_0$. The expansion in α was truncated to 3rd order for Q_{HD} and to 2nd order for $P_{\text{HD}}A/NkT$ as given.

Eqs. 4-9 are the basic equations of the present theory. Although many simplifications and approximations have been made to obtain them, they retain the essential physical picture of Nagle, that the steric interactions between chains and the *gauche-trans* bond rotation energy are the important features that determine the phase transition.

The theory has five parameters: ϵ , ΔH_{sub} , γ , δ , and the hard disk diameter of the head group. The first, namely, the energy of a *gauche* bond relative to a *trans* bond, is known to be about 500 cal (2.09 kJ) per mole for hydrocarbon chains (20, 21), and we adopt that value. The second, ΔH_{sub} , is taken as 1.84 kcal (7.70 kJ)/mole of CH_2 groups, in accordance with experimental determinations (22). The last parameter, the hard disk diameter, need not be known explicitly, because we use reduced area units in our calculations. The third and fourth, γ and δ , are determined by comparison of theory with experiment. It should be noted that γ is independent of the nature of the head group and the length of the chain, and δ depends only on the identity of the head group.

The partition function in Eq. 4 predicts that, for certain values of the parameters γ and δ , a first order phase transition exists for this system. Schematic representations of $(\ln Q)/N$ against α , the dimensionless area parameter, and $2 \ln(q_{\text{CHAIN}})$ are shown in Fig. 2. The tangent points of the double tangent in Fig. 2 correspond to coexisting phases in a first order phase transition. Thus the difference in the ab-

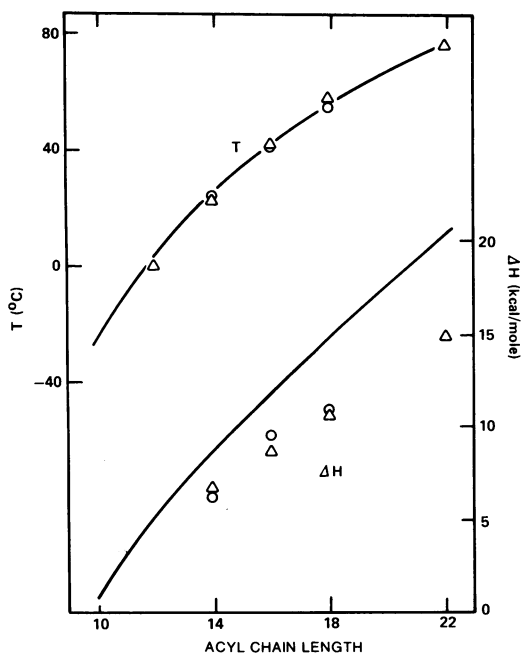


FIG. 3. Transition temperatures and enthalpy changes for phosphatidyl cholines. The solid lines are drawn through the values calculated for even chain lengths; triangles and circles are the experimental data of refs. 6 and 7, respectively.

scissas gives the area change for the transition and the slope of the common tangent is proportional to the pressure at which the transition occurs. Most experiments on bilayer systems are done at atmospheric pressure; hence the double tangent should have essentially zero slope. To determine the area parameter γ and the head group parameter δ for a given set of molecules which have the same head group but which differ in chain lengths, we choose some chain length, give some arbitrary value to δ , set T in Eqs. 4–9 equal to the known transition temperature for the molecule chosen, then vary γ until a horizontal double tangent is found. Using this γ and δ we find the temperature for which the transition occurs at zero pressure for all the other chain lengths. This gives a set of calculated transition temperatures for the series. This procedure is repeated with a different value for δ until the deviation between the set of known transition temperatures and the set of calculated temperatures is minimized. This gives the best values for γ and δ for the homologous series under consideration. The various thermodynamic properties of the coexisting phases can be calculated by taking the appropriate derivatives of $\ln Q$.

DISCUSSION

To complete the procedure described in *Method*, several transition temperatures for a homologous series of bilayer forming molecules are needed. Transition temperatures and enthalpies are known for five 1,2-diacylphosphatidyl cholines (6, 7). The experimental data are shown in Table 1, columns 2 and 4. The calculated transition temperatures, enthalpy changes, and area changes are shown in Table 1, columns 3, 5, and 6 (also see Fig. 3).

The calculated transition temperatures agree quite well with experiment, especially the dependence of the transition temperature on chain length. Choosing γ and δ by comparison with experiment in effect guarantees *only* that the calculated temperatures have the right magnitude and the

right slope or derivative with respect to chain length. The experimental data also show a distinct curvature, which is well fit by the theory. The enthalpy changes across the transitions are calculated to be somewhat larger than are found experimentally but of the correct magnitude. This agreement in magnitude and chain length dependence lends considerable credence to the model being examined here. The experimental area changes across the transition for these molecules are on the order of 20–35% (23, 24). The calculated area changes are of the same order of magnitude, though somewhat smaller for the shorter chains. No transition is found in calculations for the system with only eight carbons in its acyl chains using the parameters that best fit the longer chain data.

The optimized values of the parameters used in the calculation of Table 1 are $\gamma = 0.04784$ and $\delta = -6.4$. The first parameter, γ , represents the effective fractional area increase when the last bond of a chain becomes *gauche*. It is less than the estimated maximum value of 0.1. This parameter multiplies the pressure P and, therefore, its optimization also allows for the fact that the actual pressure may differ from the hard disk pressure. The parameter δ represents the effective head group interaction in units of the methylene interaction energy. Adjustment of this parameter has the effect of changing the rate of change of the transition temperature with chain length. A negative value of δ increases the importance of increasing the chain length. For instance, if $\delta = 0$ and γ is adjusted to fit the 22-carbon chain temperature ($\gamma = 0.03030$), then the 14-carbon chain transition is calculated to occur at 42° instead of 24° as observed. Little is known about the orientation or the conformation of the choline head group, but if the choline dipoles are approximately perpendicular to the bilayer surface, the dipole-dipole repulsions can be expected to be the dominant contribution to the longer range head group interactions which we have described in a mean field approximation. The -6.4 value found for δ indicates that the head groups in this set of calculations do have a repulsive interaction and that it is about the same magnitude as the attractive interactions of six methylene groups. The specific character of the head group plays an important role in determining the thermodynamics of the bilayer transition, as evidenced in the large differences in transition temperatures between molecules with the same acyl chains and different head groups (25). The known transition temperatures for the phosphatidyl ethanolamines lie some 20° higher than those for the corresponding phosphatidyl cholines.

CONCLUSION

The important characteristics of the present calculation that distinguish it from the other calculations are the following: (i) It is a calculation for a three-dimensional, continuum (as opposed to lattice) model of a bilayer. (ii) The calculation assumes that the important interactions for determining the structure of the layer are the short range repulsive interactions between chains and between head groups, and that the longer range attractive and repulsive forces merely provide a cohesive "background" potential. (iii) The model is realistic in the sense that many of its parameters are related to measurable properties of the molecules. (This makes it easier to extend the calculation to more complicated systems, such as mixtures, and more detailed models, such as those in which the elliptical shape of the head group is taken into account.) (iv) The mathematical approximations simplify the

Table 1. Experimental and calculated phase transition properties of phosphatidyl choline

<i>n</i>	Transition temperature (°C)		ΔH (kcal/mol)		ΔA (%)
	Exp.	Calc.*	Exp.	Calc.*	
22	75†	75.1	14.9†	20.4	20.7
20		66.4		17.6	19.9
18	58,† 54.24‡	55.3	10.7,† 10.8‡	14.7	18.7
16	41,† 41.75‡	(41.75)	8.7,† 9.6‡	11.8	17.2
14	23,† 23.78‡	24.9	6.7,† 6.3‡	8.7	14.8
12	~0†	3.1		5.4	11.0
10	—	-26.5		0.8	2.0

* The calculation uses $T = 41.75^\circ$ for $n = 16$ to determine the parameter γ for a given δ and all of the experimental transition temperature data to determine the optimum value of δ in a least squares fashion, as discussed in the text. The optimized parameter values used for these calculations were $\gamma = 0.04784$ and $\delta = -6.4$.

† Ref. 6.

‡ Ref. 7.

calculations enough that it is easy and inexpensive to perform them even for long chains. (The complete calculation of Table 1 required 5 min on an IBM 360/67 computer.) (v) The method of calculation, involving computation of a free energy as a function of two variables (reduced area and temperature) and the double tangent construction, not only provides a unified description of both phases but it also guarantees that the coexisting phases have the same temperature, pressure, and chemical potential as is required for a genuine phase transition. Moreover, it guarantees that the solid-like phase is thermodynamically stable below the transition temperature and that the liquid-like phase is stable above it. The theories of Nagle, Scott, Marsh, Marčelja, and McCammon and Deutch each differ from the present theory in one or more of these five ways. The chain length dependence of the transition temperature and the magnitude of the enthalpy change were calculated correctly. Moreover, the present calculation has a physically reasonable and specific predic-

tion about the nature of the head group interactions in phosphatidyl cholines, i.e., that it is repulsive.

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