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A Theory of the Effects of Head-Group Structure and Chain Unsaturation on the Chain Melting Transition of Phospholipid Dispersions†

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ABSTRACT: We have developed statistical mechanical descriptions of the effects of head-group structure and acyl chain unsaturation on the chain melting phase transition of aqueous dispersions of bilayers containing glycerophosphocholines and glycerophosphoethanolamines. The theoretical framework is an extension of the model of Jacobs et al. [Jacobs, R. E., Hudson, B. S., & Andersen, H. C. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 3993]. There are several systematic trends in the experimental transition data for various types of phospholipids. Assumptions about the physical origins of these trends were incorporated into statistical mechanical models, which were used to calculate transition temperatures and enthalpies. The extent to which the calculated results of a model reproduce the experimental trends is taken as a measure of the validity of the assumptions on which the model is based. We found that the gross differences among the transition temperatures of phospholipids with two saturated chains, two trans-unsaturated chains, two cis-unsaturated chains, and one cis-unsaturated and one saturated chain can all be explained in terms of the effect of the double bonds on molecular shape and the subsequent effect of shape on the ability of molecules to pack together into a low-energy state at high density. The dependence of transition temperature on the location of the double bond in cis-unsaturated molecules can be understood on the same basis. The differences between the transition temperatures of glycerophosphocholines and glycerophosphoethanolamines with the same hydrocarbon chains can be explained in terms of a larger intermolecular attraction (or smaller repulsion) for the latter than for the former. These differences depend on the presence or absence of unsaturation in the hydrocarbon chains in a way that is consistent with the postulate that hydrogen bonding between glycerophosphoethanolamines is responsible for the differences.

The structure and properties of cell membranes are affected by the molecular structure of the lipids contained within it. For phospholipids, the nature of the head group, the length of the acyl chains, the degree of unsaturation of the chains, and, in the case of acidic phospholipids, the extent of deprotonation and divalent cation binding can all have an effect on the membrane's properties. One of the ways to study the relationship between lipid structure and membrane properties is to investigate the chain melting phase transition that takes place in synthetic phospholipid dispersions, which serve as simple models for the lipid domains of biological membranes (Ladbrooke & Chapman, 1969; Steim et al., 1969; Engleman, 1970; Reinert & Steim, 1970; Melchior et al., 1970; Ashe & Steim, 1971; Hubbell & McConnell, 1971; Lippert & Peticolas, 1971; Hinz & Sturtevant, 1972; Schechter et al., 1972; Nagle, 1973a,b; Marčelja, 1974; Marsh, 1974; Ranck et al., 1974; Träuble & Eibl, 1974; Overath et al., 1975; Jacobs et al., 1975; McCammon & Deutch, 1975; Linden & Fox, 1975; Morrisett et al., 1975; Cronan & Gelmann, 1975; Sklar et al., 1975, 1976, 1977; Mabrey & Sturtevant, 1976; Melchior & Steim, 1976; Thilo & Overath, 1976; Tecoma et al., 1977).

Several workers have devised statistical mechanical models to describe the chain melting phase transition in bilayers (Nagle, 1973a, 1975, 1976; Marčelja, 1974; Jacobs et al., 1975, 1977; Scott, 1975, 1977; McCammon & Deutch, 1975; Jackson, 1976). These studies confirm that the disordering of the acyl chains in the interior of the bilayer can lead to a

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phase transition, and they provide a basis for understanding the dependence of the transition temperature on chain length. The effect of variations in head group–head group interactions from one lipid to another has been studied theoretically (Nagle, 1976), as has the effect of deprotonation of acidic lipids (Jähnig, 1976; Träuble et al., 1976; Forsyth et al., 1977). In this paper we present a set of statistical mechanical models for investigating the effect of head-group structure and acyl chain unsaturation on the chain melting transition of dispersions of phospholipids.

The two most prevalent neutral (i.e., zwitterionic) phospholipid head groups are the glycerophosphoethanolamines and the glycerophosphocholines. Most cells contain saturated acyl chains as well as acyl chains with cis double bonds. There is also some interest in the physical properties of phospholipids having trans-unsaturated chains, since they are used widely in experimental manipulations of cellular lipid composition (Overath & Träuble, 1973; Esfahani et al., 1971; Tecoma et al., 1977) and since the lipids of human cells contain some trans-unsaturated chains, possibly due to the isomerization of cis bonds during cooking and processing of foods (Lehninger, 1975).

Experimental data, obtained by various workers, for the transition temperature and enthalpy of the chain melting transition for phospholipid bilayers are given in Tables I–III and Figure 3. There are a number of features of the data that would be interesting to interpret on a molecular level. (1) Within a homologous series of molecules with the same head group and similar chains, the transition temperatures and enthalpies are increasing functions of the number of carbon atoms in the acyl chains. This is most clearly seen in the series of eight diacylglycerophosphocholines (PC's)[1] with saturated chains, and it also holds for three diacylglycerophosphoethanolamines (PE's) with saturated chains. (2) A PC with a certain set of acyl chains has a transition temperature about 20 °C lower than that of the corresponding PE in most but not all cases. This holds for molecules with saturated acyl chains, and for molecules with chains having a single trans double bond. In the case of chains with a single cis double bond, however, a PC has the same transition temperature as the corresponding PE. (3) A lipid with one trans double bond near the middle of each chain has a lower transition temperature than the corresponding molecule with saturated chains. (4) A lipid with one cis double bond near the middle of each chain has an even lower transition temperature than the corresponding molecule with trans double bonds. (5) For molecules with one cis double bond in each chain (or one cis double bond in only one chain) the transition temperature and enthalpy are a sensitive function of the location of the double bond. The temperature is lowest when the double bond is in the middle of the chain, and it is increased as the double bond is moved toward either end of the chain.

The first of these features, namely, the increase of transition temperature and enthalpy with hydrocarbon chain length, is well understood as being due to the increased van der Waals attractions for longer chains, which stabilize the high-density solid phase relative to the lower density liquid phase. In this paper, we adapt the model of Jacobs et al. (1975) to describe the effect of head-group structure and of chain unsaturation upon the chain melting transition, and in so doing we provide explanations for features 2–5.

Statistical Mechanical Models for a Bilayer

The physical picture of a bilayer we use is the following. The phospholipid head groups are constrained to lie on a two-dimensional surface at the interface between an aqueous medium and a region containing the hydrocarbon chains. The head groups interact with the water and interact with each other via both short-range excluded volume repulsions and longer range dispersion and dipolar forces, which may result in either a net attraction or a net repulsion. The hydrocarbon chains also interact via short-range repulsions and long-range dispersion-type attractions. In addition, there are intramolecular energies associated with conformations of the acyl chains.

Each of the models to be discussed below takes into account some of the degrees of freedom of the molecules in the bilayer and some of the interactions. Since we are calculating the properties of a phase transition between two phases, we are primarily concerned with the differences in free energy and other thermodynamic quantities between the two phases. Any degree of freedom or contribution to the energy that has the same effect on the calculated properties of each phase will make no contribution to these differences and hence can be neglected. Therefore, by including some particular set of degrees of freedom and interactions into a model and comparing the results of the model with experiment, we can test whether these degrees of freedom and interactions are responsible for the differences between the two phases.

Many approximations are made in performing calculations for a model. Some are idealizations of the complex intermolecular and intramolecular interactions within a bilayer. Some are statistical mechanical approximations made to facilitate the calculation of the partition function. In most cases, however, we are interested in comparing the phase transitions for two or more different lipids. For example, we might want to compare the transition temperature of a PE with saturated chains with that of a PC with the same chains. Since the same types of approximations are used for both lipids, the errors will cancel to a large extent when calculating the difference between the transition temperatures.

We will first discuss the features common to all the models we will use. The degrees of freedom of a molecule are divided into two groups, denoted X and Y. X_i ($i = 1, ..., N$) denotes a set of degrees of freedom of the ith molecule, and Y_j ($j = 1, ..., 2N$) denotes a set of degrees of freedom of the jth hydrocarbon chain. (Molecule i has chains $2i - 1$ and $2i$ attached to it). X_i always includes the two-dimensional position of the head group of the molecule. It may also include an angle describing the orientation of the molecule in the plane of the bilayer (as in model CO-III). Y_j always includes a set of labels specifying the rotational isomeric state of the single bonds in each chain. Y_j may also include an angle describing the orientation of the chain (as in model CO-II) or an angle describing distortion of the chain away from the idealized rotational isomeric state (as in model CD-III). The set of all coordinates for all N molecules, $X_1, X_2, ..., X_N, Y_1, Y_2, ..., Y_{2N}$, will be abbreviated as X^N, Y^{2N}. The Hamiltonian of a bilayer containing N molecules is $H(X^N, Y^{2N})$.

In each of the models, there is some set of values of the chain variables Y_j that allows the molecules to pack together most favorably at high density. This set of values will be denoted as $Y_j^{(0)}$. In the solid phase at low temperature, the attractive forces between the hydrocarbon chains make the bilayer contract to a high density. Thus, in each model, the speci-

[1] Abbreviations used: PC, phosphatidylcholine; PE, phosphatidylethanolamine; DOPC, dioleoylphosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine; DEPC, dielaidylphosphatidylcholine; DEPE, dielaidylphosphatidylethanolamine; DSPC, distearoylphosphatidylcholine, DMPC, dimyristoylphosphatidylcholine; DMPE, dimyristoylphosphatidylethanolamine.

fication of $Y_j^{(0)}$ is equivalent to making an assumption about the structure of the high-density solid phase. $Y_j^{(0)}$ and $Y^{(0)N}$ will be called the "high-density conformation of the chains".

The Hamiltonian can be rewritten in the form:

$$H(X^N,Y^{2N}) = H(X^N,Y^{(0)2N}) + [H(X^N,Y^{2N}) - H(X^N,Y^{(0)2N})] \quad (1)$$

The first term on the right is the Hamiltonian calculated as if all the molecules retained their high-density chain conformation. The term in square brackets is the increase in energy associated with changing the chain conformations away from their high-density values, keeping the other degrees of freedom (the X^N) unchanged.

In all the models, the term in square brackets will be approximated as a sum of terms that act separately on each chain:

$$H(X^N,Y^{2N}) - H(X^N,Y^{(0)2N}) = \sum_{j=1}^{2N} h_c(Y_j;a) \quad (2)$$

where

$$a = A/N \quad (3)$$

is the area per molecule and h_c is the chain conformation Hamiltonian. Each term depends parametrically on a.

The first term in eq 1 is separated into short-range and long-range parts:

$$H(X^N,Y^{(0)2N}) = H_s(X^N,Y^{(0)2N}) + H_l(X^N,Y^{(0)2N}) \quad (4)$$

H_l contains dispersion attractions between chains and long-range attractive or repulsive interactions between head groups. H_s contains the short-range purely repulsive interactions between molecules. The effect of the long-range interactions is calculated in a mean field approximation:

$$H_l(X^N,Y^{(0)2N}) = Nh_l(a) \quad (5)$$

The quantity h_l depends on a and upon the structure of the bilayer.

The total Hamiltonian is then

$$H(X^N,Y^{2N}) = H_s(X^N,Y^{(0)2N}) + Nh_l(a) + \sum_{j=1}^{2N} h_c(Y_j;a) \quad (6)$$

The partition function for N molecules in area A at temperature T is

$$Q(N,A,T) = (N!)^{-1} \int dX^N \int dY^{2N} \exp[-\beta H(X^N,Y^{2N})] \quad (7)$$

where

$$\beta = 1/kT \quad (8)$$

and k is Boltzmann's constant. (The limits of integration depend on the nature of the variables. Y^{2N} usually includes some discrete rather than continuous variables, and in this case $\int dY_i$ should be understood as a sum over all possible discrete values.) Using eq 6 we find

$$Q(N,A,T) = Q_s(N,A,T) \exp[-\beta N h_l(a)][q_c(a,T)]^{2N} \quad (9)$$

where

$$Q_s(N,A,T) = (N!)^{-1} \int dX^N \exp[-\beta H_s(X^N,Y^{(0)N})] \quad (10)$$

and

$$q_c(a,T) = \int dY_1 \exp[-\beta h_c(Y_1;a)] \quad (11)$$

Q_s is a partition function that includes only short-range repulsive interactions between molecules, whose chains are restricted to being in their high-density conformation $Y^{(0)N}$. The quantity q_c is a partition function for a single chain under the influence of the Hamiltonian h_c.

This scheme for calculating partition functions is a generalization of that used by Jacobs et al. (1975) in their theory of the phase transition in PC bilayers. It is a special case of the method used by Jacobs et al. (1977) in their theory of phase diagrams for bilayers composed of mixtures of PC's. The essential features that make calculations tractable are the assumptions that all long-range forces can be described in a mean field approximation as in eq 5 and that the dependence of the Hamiltonian on the chain coordinates, Y^{2N}, can be separated into individual terms for each chain, as in eq 2. These approximations, and subsequent ones to be discussed below, are crude, but the previous success of this approach leads us to believe that they will be useful for describing the effects of head-group structure and acyl chain unsaturation on the bilayer phase transition.[2]

To construct a model for a bilayer containing a particular phospholipid, we must make three choices. First, we must choose which degrees of freedom to include and which are to be regarded as X variables and which as Y variables. Secondly, we must choose the structure of the high-density phase. This determines $Y^{(0)}$ and the functional form of h_l. Thirdly, we must choose which short-range interactions to include. This affects the form of h_c and H_s.

Models for PC's and PE's Having Saturated Chains

In this section we are concerned with the effect that replacement of a choline head group by an ethanolamine head group has upon the chain melting transition. The experimental data are given in Tables I and II. A PE has roughly a 20 °C higher transition temperature than the corresponding PC when the acyl chains are saturated or have only one trans double bond, but when the chains have a cis double bond the PC and PE have the same transition temperature. Here we are primarily concerned with the case of saturated chains. Unsaturated chains will be discussed in later sections.

The ethanolamine and choline head groups are very similar in structure. Both are zwitterionic. The only difference is that an $-NH_3^+$ group on ethanolamine is replaced by an $-N(CH_3)_3^+$ group on choline. This difference can have several effects. First, the choline head group occupies a larger volume because of the methyl groups; hence, ethanolamine molecules may be able to pack together into a more dense arrangement in a bilayer. Second, the hydration of the hydrophobic quaternary ammonium group is very different from that of the primary ammonium group, which is capable of hydrogen bonding to water. Third, hydrogen bonding between head groups on different molecules is possible for PE's, but not for PC's.

Evidence from monolayer studies (Cadenhead et al., 1967; Phillips & Chapman, 1968) and from X-ray diffraction measurements suggests that PC's with saturated chains have solid-phase areas or collapse areas per molecule approximately 10–15% larger than the areas of the corresponding PE's. X-ray structural studies provide additional evidence for significant differences between PE and PC bilayers. Hitchcock et al. (1974) determined the crystal structure of 1,2-dilauroyl-DL-phosphatidylethanolamine–acetic acid. This crystal contains a lipid bilayer structure, with adjacent bilayers being separated

[2] For the models discussed in this paper, the independent variables in the partition function include the area A but not the thickness of the membrane. Thus the values of the thickness and volume and the changes in these quantities that occur in the transition cannot be calculated by this type of theory.

Table I: Experimental and Theoretical Temperatures and Enthalpies of Saturated and Trans-Unsaturated Phospholipids

	(a) transition temperatures (°C)				(b) enthalpies (kcal/mol)		
		theory				theory	
phospholipid	expt	S-I[f]	S-II[g]	S-III[h]	expt	S-I[f]	S-II[g]
PC							
12:0	~0[a,d]	3.0	2.8	2.8	4.3,[d] 1.7[c]	5.4	5.6
14:0	23,[a] 23.8[b]	24.8	24.7	24.6	6.7,[a] 6.3,[b] 6.8,[d] 5.4,[c] 5.0[j]	8.7	8.8
16:0	41,[a] 41.5,[d] 41.8[b]	(41.5)	(41.5)	(41.5)	9.6,[b] 8.7[a]	11.8	11.7
18:0	58,[a] 54.2,[b] 54.0[c]	55.2	54.9	55.0	10.7,[a] 10.8[b]	14.7	14.6
20:0		66.3	65.9	66.1		17.6	17.4
22:0	75[a]	75.6	75.2	75.4	14.9[a]	20.4	20.6
18:1 (t,9)[i]	9.5,[d] 12-14[e]	13.1	5.4		7.3[d]	10.9	11.1
PE							
12:0	29[d]	28.7	32.5	25.4	4.0[d]	6.6	6.5
14:0	47.5[d]	(47.5)	(47.5)	(47.5)	6.4[d]	9.8	9.5
16:0	60[d]	62.1	58.9	64.8	8.5[d]	12.8	12.6
18:1 (t,9)	35[d]	30.1	38.7		7.0[d]	12.2	12.6

[a] Ladbrooke & Chapman (1969). [b] Hinz & Sturtevant (1972). [c] Mabrey & Sturtevant (1976). [d] Van Dijck et al. (1976). [e] Wu & McConnell (1975). [f] For model S-I: $A_m = Na_0$, $l^{PC} = l_0$, $\gamma^b = 0.36789$, $\delta^{PC} = -6.40$, $\delta^{PE} = -3.434$. $\gamma^t = 0.42693$. [g] For model S-II:[g] $A_m = Na_0/\cos 30°$, $l^{PC} = \cos 30° l_0$, $\gamma^b = 0.31687$, $\delta^{PC} = -7.3$, $\delta^{PE} = -3.05$. $\gamma^t = 0.29372$. [h] For model S-III: $A_m = 1.1Na_0$, $l^{PC} = l_0$, $\gamma = 0.4130$, $\delta^{PC} = -6.4$, $\delta^{PE} = -5.65$. [i] Our convention in labeling acyl chain is as follows: the designation $n{:}m(t,k)$ means an n carbon chain with m double bonds of the trans type located between the kth and $k+1$st carbons in the chain. If t is replaced by c, there is a cis double bond. [j] Lentz et al. (1978).

Table II: Experimental and Theoretical Transition Temperatures and Enthalpies of Phospholipids with Cis-Unsaturated Chains

(A) Two Unsaturated Chains on Each Lipid

	temp (°C)		enthalpies (kcal/mol)	
phospholipid	expt	CD-II[d]	expt	CD-II[d]
18:1(c,9) PC	−14,[a] −21[b]	−18.2	11.2[a]	8.6
18:1(c,9) PE	−16[a]	−18.2	4.5[a]	8.6
16:1(c,9) PC	−36[a]	−23.6	9.1[a]	6.4
16:1(c,9) PE	−33.5[a]	−23.6	4.3[a]	6.4

(B) One Saturated and One Cis-Unsaturated Chain on Each Lipid

	temp (°C)			temp (°C)	
	SI/			SI/	
phospholipid	expt	CD-II	phospholipid	expt	CD-II
18:0/18:1(c,6) PC	30[b]	22.0	18:0/18:1(c,12) PC	12[b]	24.7
18:0/18:1(c,9) PC	2[c]	16.7	18:0/18:1(c,16) PC	43[b]	61.7

[a] Van Dijck et al. (1976). [b] Barton & Gunstone (1975). [c] Phillips et al. (1972). [d] Agreement with experiment for the PE's was found if the value of δ_{PE} was assumed to equal the value of δ_{PC}, which we have carried over from model S-I. For all calculations $\gamma^b = 0.36789$, $\delta^{PC} = \delta^{PE} = -6.40$, $\gamma^c = 0.3214$, and A_m is specified as described in eq 30 and 31 for parts A and B, respectively.

by acetic acid molecules. Although this is a dry crystal, it is reasonable to expect the crystal structure to be similar to that of hydrated bilayers. This is confirmed by the fact that NMR studies of the conformations of hydrated PE bilayers give results consistent with the crystal structure (Seelig & Gally, 1976). The X-ray data demonstrate the existence of intermolecular hydrogen bonding in PE bilayers, something which is impossible for PC's. Moreover, the axes of the hydrocarbon chains are approximately perpendicular to the bilayer surface in the PE crystal, whereas X-ray studies of hydrated PC bilayers (Janiak et al., 1976) indicate that the hydrocarbon chains are tilted with respect to the bilayer normal in the solid phase just below the chain melting transition. [However, see Rand et al. (1975) for a different interpretation of X-ray data.]

We have constructed statistical mechanical models to describe the effect of head-group size, head-group interactions, and chain tilting upon the chain melting phase transition. These models will now be discussed.

For phospholipids with saturated chains we will use models based on that of Jacobs et al. (1975). The X variable for a molecule is the position $\mathbf{r}$ of the head group in the plane of the bilayer. The Y variables for a chain are a set of labels α_j, $j = 1, \ldots, m$, which specify the conformations of the single bonds in the chains. A chain having n carbons has $n-1$ bonds between the carbons, but rotations about the final bond result in an equivalent conformation, so that there are $n-2 = m$ bonds for which rotations will produce different conformations. The labels α_j can have the values +1, −1, and 0, which signify gauche +, gauche −, and trans conformations, respectively [for a justification of this "rotational isomeric state" approximation, see Flory (1969)]. The energy of a gauche conformer is about 500 cal/mol above that of a trans conformer (Flory, 1969). We denote by α the vector consisting of the α_j for bonds $j = 1, \ldots, m$. $\alpha^{(0)}$ is the vector for which all the $\alpha_j = 0$; i.e., all of the single bonds are in the trans conformation. This is the high-density conformation in this model.

Following Jacobs et al. (1975), we assume that the short-range interactions between molecules when they are in their high-density conformation are the same as for hard disks in two dimensions; that is, molecules repel each other infinitely strongly if the centers of their head groups come within a distance σ from each other, where σ is the diameter. It follows that Q_s is a hard disk partition function:

$$Q_s(N,A,T) = Q_{HD}(\alpha) \tag{12}$$

for which we use the results of Hoover & Ree (1968):

$$Q_{HD}(\alpha) = (\alpha^2/\rho_m)^N \exp[N(0.06 - 0.1\alpha + 0.385\alpha^2 + 0.243\alpha^3 + \ldots)] \tag{13}$$

Here $\alpha = (A - A_m)/A_m$, where A_m is the minimum area into which N disks can be compressed, and $\rho_m = N/A_m$. The two-dimensional pressure P_{HD} of the hard disk fluid is given by eq 14.

$$P_{HD}A/NkT = 2/\alpha + 1.90 + 0.67\alpha + 1.5\alpha^2 + \ldots \tag{14}$$

There are two contributions to the chain Hamiltonian. The first is an intramolecular energy of ϵ (= 500 cal/mol) for each gauche bond. The second is the work done by a chain, against the force of other chains, when it goes from its high-density all-trans conformation to another conformation. We imagine that, when a chain bends from its high-density conformation to the conformation α, its effective area increases by an amount

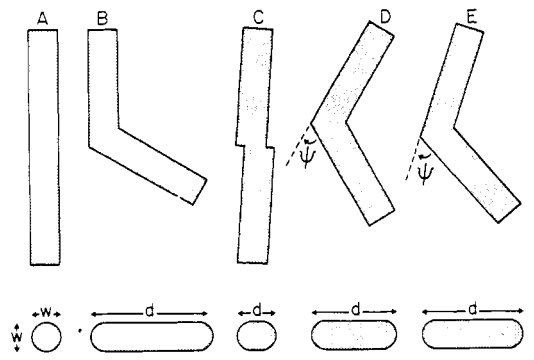

FIGURE 1: Projected areas of hydrocarbon chains. A hydrocarbon chain in the all-trans conformation is idealized as a cylinder of diameter w. Its shadow is a circle of diameter w, as in part A. In part B, a gauche rotation of one bond leads to a 60° angle between the upper and lower portions of the chain. Part C depicts a chain with one trans double bond, with all single bonds in the trans conformation, and tilted so that the area of the shadow is a minimum. Part D shows a chain with one cis double bond and tilted so that the bottom of the chain is directly below the top. The angle between the upper and lower portions of the chain is ψ. Part E depicts a chain with one cis double bond and tilted so that the projected area of the chain is a minimum. In B–E the length of the shadow is d, and $(d - w)w$ is the increase in area relative to that of the straight chain in part A.

$\Delta a_e^b(\alpha)$. (The subscript e denotes that this is an effective area increase, and the superscript b denotes that the area increase is due to bending of the chains.) Using the approximation of Jacobs et al. (1975), we have

$$h_c(\alpha) = \sum_{j=1}^{m} |\alpha_j|\epsilon + p\Delta a_e^b(\alpha) \quad (15)$$

where p is the effective pressure exerted on the chain by all the other chains in the bilayer.

The second term in eq 15 is estimated in the following way. The area of a hydrocarbon chain can be considered to be the area of the shadow cast by that chain on a plane perpendicular to the axis of the upper part of the chain. This area is smallest when the chain is in the all-trans conformation and is larger if one or more of the bonds is in a gauche conformation (see Figure 1A,B). For any conformation α, we can therefore define the actual area increase $\Delta a^b(\alpha)$ associated with chain bending. A chain does not fully occupy the area cast by its shadow; i.e., there is no reason why the shadows of different chains must not overlap. However, it is reasonable to assume that the effective area increase $\Delta a_e^b(\alpha)$ is approximately proportional to the actual area increase $\Delta a^b(\alpha)$:

$$\Delta a_e^b(\alpha) = \gamma^b \Delta a^b(\alpha) \quad (16)$$

where

$$0 < \gamma^b < 1 \quad (17)$$

The amount by which γ^b is less than unity is a measure of the extent to which the shadows of different chains may overlap. Next we assume that the pressure opposing chain bending equals (or at least is approximately proportional to) the pressure associated with the partition function Q_s, which describes the two-dimensional behavior of a bilayer whose chains are kept in their high-density conformation. Since pressure enters the partition function in the form $p\Delta a_e$, the proportionality between these two pressures can be considered to be subsumed in the area parameter γ^b. We expect that the ratio of these two pressures will be close to unity, and so eq 17 will still hold. Next we assume, for simplicity, that the actual area increase is determined solely by the position of the gauche bond closest to the carboxyl terminus of the chain. Subsequent bends could either increase or decrease the area of a chain, and as a first approximation we imagine that these effects will cancel. As shown in Figure 1B, for a bend at the ith bond in a chain:

$$\Delta a^b = (m - i + 1)wL \sin 60° \quad (18)$$

where $w = 4.85$ Å is the width of a chain, $L = 1.25$ Å is the length per methylene segment along the chain axis, and $(m - i + 1)L$ is the length of the part of the chain below the gauche bond. It is convenient to perform calculations in reduced units with areas expressed relative to a_0, which is twice the area per chain for saturated hydrocarbon chains packed at their highest density. (Thus, Na_0 would be the area of N lipid molecules whose chains form a hexagonal closest packed bilayer with their axes perpendicular to the bilayer surface.) Using the fact that at closest packing the chains are assumed to form a hexagonal array, we find that

$$\Delta a^b = 0.14(m - i + 1)a_0 \quad (19)$$

It should be noted that our use of the parameter γ^b differs from that of Jacobs et al. (1975); our γ^b equals their parameter γ divided by 0.14. We make this change to separate actual from effective quantities more explicitly, since this distinction becomes important in the models of unsaturated chains. In our usage, an area parameter is always a quantity that can be expected to range between 0 and 1; the actual area increase Δa^b is determined solely by applying geometrical considerations as in Figure 1.

These approximations yield the chain partition function shown in eq 20. In eq 20, the term 1 corresponds to the all-trans state. For each position of the first bend occurring at bond i, there are two possible gauche states with associated intramolecular energy ϵ and with a work term equal to $P_{HD}\gamma^b 0.14a_0(m - i + 1)$. Each of the subsequent $m - i$ bonds can either be trans, gauche +, or gauche −, giving rise to the factor $(1 + 2e^{-\beta\epsilon})^{m-i}$.

$$q_c = 1 + \sum_{i=1}^{m} [1 + 2\exp(-\beta\epsilon)]^{m-i} 2\exp[-\beta(\epsilon + P_{HD}a_0\gamma^b 0.14[m - i + 1])] \quad (20)$$

The long-range interactions between molecules consist of two parts, the attractive dispersion interactions between chains and the interaction between head groups. We assume that when the chains are packed to their maximum density, the dispersion energy per CH_2 or CH_3 group is the same as that in hydrocarbon crystals and that for lower densities this energy is proportional to the density.[3] Thus, the dispersion interaction contribution to h_l is $-2n\Delta H_{sub}(a_0 l_0/al)$. Here l_0 is the length of the hydrocarbon chain, and l is the thickness of the hydrocarbon region of a monolayer. The ratio l/l_0 is equal to the cosine of the angle between the bilayer normal and the axis of the hydrocarbon chain. ΔH_{sub} is the heat of sublimation per methylene group in long-chain hydrocarbon crystals. The value of ΔH_{sub} is derived from experiment (Billmeyer, 1957), and correction for vibrational degrees of freedom gives a value

[3] We note in passing that several investigators have used different functional forms for the density dependence of dispersion interactions; see Nagle (1973). We have found for our models that with suitable readjustment of the parameters δ and γ, equally good agreement with the experimental transition temperatures and slightly better agreement with enthalpies and area changes are found if the attractive interactions between chains are made proportional to the $^2/_3$ power of the density rather than the first power. The qualitative features of the models are not acutely dependent on the specific functional form of the dependence of dispersion interaction on chain separation, and the use of a first-power density dependence of the mean field energy was continued for the sake of parsimony.

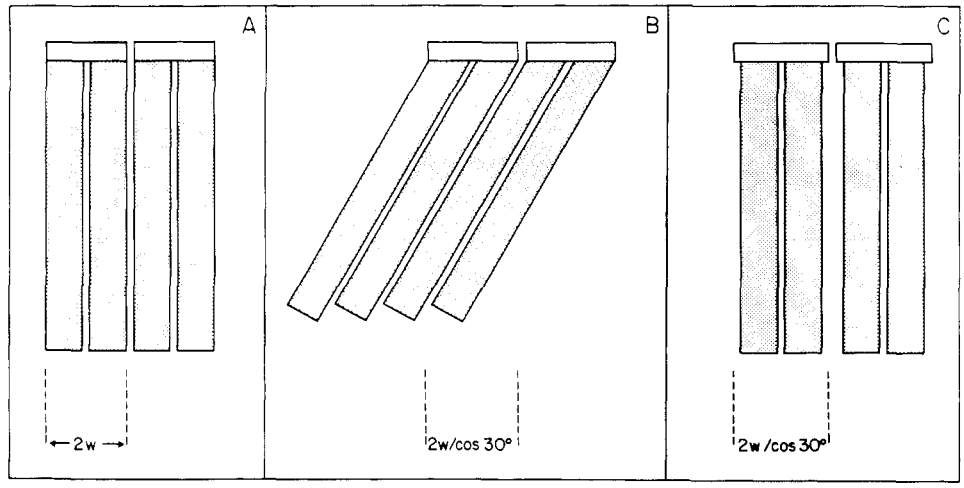

FIGURE 2: Schematics of the different models for glycerophosphocholines and glycerophosphoethanolamines in solid phases. For all models, the glycerophosphoethanolamines are regarded as packing with chains perpendicular to the bilayer plane and with $A_m = A_0$. A similar arrangement is assumed for model S-I (A) for the glycerophosphocholines. In model S-II (B), it is assumed that $A_m > A_0$ and that the chains tilt in order to preserve the same density of methylene segments and hence the same strength of dispersion attractions. This picture is similar to that described by Nagle (1976). In model S-III (C), it is assumed that $A_m > A_0$ and that such tilting does not occur.

of about 1.84 kcal/mol of methylene groups. We also assume that the head-group interaction energy is proportional to the number of head groups per unit area in the bilayer. Hence, the mean field expression for the total long-range interaction energy can be written as

$$h_l = -(a_0/a)\Delta H_{sub}(2nl_0/l + \delta) \qquad (21)$$

where δ is the strength of the head-group interaction energy per head group, when $a = a_0$, measured in units of $-\Delta H_{sub}$. When l is set equal to l_0, i.e., when no tilting of the chains away from the bilayer normal is assumed, eq 21 reduces to the formula used by Jacobs et al. (1975).

Equations 9–21 complete the statement of the partition function for molecules with saturated hydrocarbon chains. Calculations based on these equations allow us to test the effect of chain tilting, head-group size, and head-group interactions on the phase transition.

The model employs eight parameters, ϵ, ΔH_{sub}, a_0, A_m, l_0, l, δ, and γ^b. As noted above, the first two parameters are determined from experiment to be 0.50 and 1.84 kcal/mol, respectively. X-ray diffraction measurements give a value of a_0 of about 38–40 Å; this value is subject to slight uncertainty, but since reduced area units are used in calculation of the mean field term and the hard disk expressions use only the ratio A_m/A, what is important is only a reasonable value for the ratio A_m/Na_0. The value of l_0 is determined from bond angles and bond lengths to be $1.25n$ Å, where n is the number of carbons in the chain. The values of the ratios A_m/Na_0 and l/l_0 are assumed to be 1.0 for the saturated PE's unless stated otherwise. This is equivalent to assuming that the hydrocarbon chains in ethanolamine bilayers are not tilted and that contact of the chains determines the minimum area. Different models for the saturated PC's employ different values of these ratios. In models that ascribe the differences in transition temperatures between PC's and PE's to differences in the area per molecule at closest packing, we estimate the ratio of closest packed areas as roughly the ratio of the solid-phase areas per molecule derived from X-ray diffraction patterns of Tardieu et al. (1973) and Janiak et al. (1976). This interpretation leads to values of A_m/Na_0 of about 1.1–1.15. For models that assume no tilting of the chains, l/l_0 is 1.0, and if tilting by an angle θ is assumed, then

$$l/l_0 = \cos\theta \qquad (22)$$

The parameters δ and γ^b are adjustable, subject to the constraint in eq 17. Since δ represents head-group interactions, it must be the same for all PC's regardless of chain length. The value of δ for PE's can be different from that of PC's. Since γ^b represents interactions between chains, it must be independent of the nature of the head group.

We have performed calculations for three models for saturated PE's and PC's. The models make different assumptions about the nature of the high-density conformation of the hydrocarbon chains (see Figure 2). In all models it is assumed that the chains of PE molecules are not tilted with respect to the bilayer normal and that the chains are packed together as tightly as in a hydrocarbon crystal. Thus, $A_m = Na_0$ and $l = l_0$. (In other words, the PE head group is assumed to be small enough that intermolecular packing and the minimum area are determined by the chains.) In model S-I, PC's are assumed to have the same high-density conformation as PE's. In models S-II and S-III we assume that the PC head group is large enough to affect the packing of the chains. Thus, $A_m = Na_0/\cos 30°$ (see above). In S-II the chains are assumed to tilt in such a way as to achieve the maximum packing of chains, and $l = \cos 30° l_0$. In S-III, the chains are assumed not to tilt, and $l = l_0$.

For certain values of δ and γ^b, it is found that a plot of ln Q vs. area (A) has two maxima of equal height. This implies that systems of different densities have the same temperature, pressure (zero), and Helmholtz free energy, and they can be coexisting phases at a first-order phase transition (Huang, 1967; Jacobs et al., 1975). Thus, for a given model, we fix δ and adjust γ^b until a transition is achieved at the experimental transition temperature of one member of the series of saturated PC's. The values of δ and γ^b are then fixed, the transition temperatures are calculated for the other members of the series, and the values are compared with experiment. If the fit of these transition temperatures is not adequate, a new value of δ is chosen, γ^b is adjusted to obtain fit for one chain length, and the other values are calculated and compared again with experiment. This procedure is repeated until a good fit is obtained. To incorporate the PE's into this framework, the value of γ^b is held fixed at its best value derived from the PC series. Regardless of the values of A_m and l used for the PC's, the values are set equal to Na_0 and l_0, respectively, for the PE's. Next, the value of δ, which specifies the strength of long-range head-group interactions and end effects, is ad-

justed to give good agreement with the experimental transition temperature for DMPE. The value of δ is then fixed, and the transition temperatures of DLPE and DPPE compounds are calculated and compared with experiment. Thus, a test of a set of assumptions regarding tilting and packing of PC's is that a set of parameters optimized for PC transition temperatures also give qualitative agreement with the experimental transition temperatures and enthalpies of the PE's.

All of the models possess values of the parameters γ^b and δ^{PC} that give good agreement with the transition temperatures of the PC's, and all of the models show the proper qualitative trend in calculating enthalpies which increase with chain length, although numerically the values are too high. Models S-I and S-II also give the proper dependence of transition temperature on acyl chain length for the PE's. Model S-III, using $A_m = 1.1Na_0$ for PC's, gives slightly poorer agreement, as the chain length dependence of the transition temperatures of the PE's is too steep. Larger values of A_m for PC's give a greater discrepancy. Models S-I and S-II also give equally reasonable trends in the enthalpies for the PE's; again the values are numerically too high.

These models employ physically different descriptions of the reasons for the differences in transition temperatures of the saturated PC's and PE's. Models S-I and S-II give equivalent agreement with experiment, and we regard these two models as the more plausible alternatives on physical groups. Moreover, model S-II for PC is consistent with the conclusions of Janiak et al. (1976) that the hydrocarbon chains in solid choline bilayers are tilted with respect to the normal to the bilayer. In both cases, the models can be made to fit experiment only if PE molecules are assumed to attract each other more strongly (or repel each other less strongly) than do choline molecules; i.e., the parameter δ^{PE} must be larger than δ^{PC}. (Note that the head-group interaction energy in eq 21 is proportional to $-\delta$.)

Hydrogen bonding is a plausible reason for this extra attraction in the case of PE. This possibility has been discussed by several workers and has been incorporated into a different sort of model by Nagle (1976). The extra energy of a PE bilayer at closest packing compared to a PC bilayer at closest packing is, according to eq 21, equal to $-\Delta H_{sub}(\delta^{PE} - \delta^{PC}Na_0/A_m)$, which for the parameters of model S-II equals 6.0 kcal/mol. This is of the right order of magnitude for hydrogen bonding between charged functional groups.

In summary, a model for bilayers composed of PC's and PE's with saturated chains is consistent with most of the structural data for bilayers and thermodynamic data for the chain melting transition if it has the following features: a larger head group size for PC's than PE's, tilting of the chains for the PC's but not the PE's, and less head-group repulsions (or more attractions) for PE's than PC's. Moreover, the difference in the strength of the head-group interactions is consistent with intermolecular hydrogen bonding in the case of PE's but not PC's.

Models for PC's and PE's Having Trans-Unsaturated Chains

Here we are concerned with the effect of replacing the saturated chains of a PE or a PC with chains that have one trans double bond. The experimental data for the transition temperatures and enthalpies are given in Table I. For PC with 18 carbon atoms per chain, introduction of the trans double bonds lowers the transition temperature by about 45 °C. Moreover, for PE with 18 carbons per chain and trans double bonds, the transition temperature is about 40 °C lower than what would be expected for the corresponding saturated molecule (on the basis of an extrapolation of the saturated PE data for 12-, 14-, and 16-carbon chains). Also, the transition enthalpies for unsaturated molecules are smaller than those for the analogous saturated compounds.

There are two physical differences between the saturated and unsaturated molecules that might be expected to affect the properties of the phase transition. The possible chain conformations in the vicinity of a trans double bond are different from those in the vicinity of a single bond. Also, a trans double bond distorts the shape of an otherwise saturated chain in such a way that the chains cannot pack together as well at high density. We have constructed a statistical mechanical model to describe these effects.

A space-filling model of a saturated hydrocarbon chain with all of its bonds in the trans conformation is a straight object that can be idealized as a cylinder. If the chain has a trans double bond, the part of the chain above the double bond resembles a cylinder and the part below the double bond resembles a cylinder. The axes of the two cylinders are, however, not the same. They are parallel to one another but displaced by a short distance that reflects the fact that the bond distances and bond angles near a trans double bond are not the same as those near a single bond.

Chains having trans double bonds are therefore expected to have larger projections on a plane perpendicular to the chain axis than saturated chains (see Figure 1A,C). This area increase, denoted Δa^t, is calculated from molecular models to be about $0.13a_0$ (Flory, 1969). We assume that the minimum area of a bilayer of N molecules with trans double bonds is also larger than for saturated chains. However, the difference is less than that obtained by adding together the values of Δa^t for each chain because of the cooperative packing of different molecules.

For phosphatidylethanolamines this gives rise to the following expression for A_m, the minimum area of a bilayer of N molecules with trans double bonds

$$A_m = N(a_0 + 2\gamma^t\Delta a^t) = Na_0(1 + 0.26\gamma^t) \quad (23a)$$

where γ^t is an area parameter that is expected to be between 0 and 1. A similar expression is used to describe A_m for phosphatidylcholines if we adapt model S-I to describe the trans-unsaturated species. Since we regard model S-II as having the best agreement with the structural data for saturated phosphatidylcholines, we also adapted this model to the case of trans-unsaturated phosphatidylcholines. Thus, the chains are assumed to be tilted to an angle of 30° to the bilayer normal, and the area increase due to the presence of a trans double bond equals $\Delta a^t/\cos 30°$. Thus, our expression for A_m becomes

$$A_m = Na_0(1 + 0.26\gamma^t)/\cos 30° \quad (23b)$$

As in previous models, A_m is needed for the evaluation of Q_{HD} and P_{HD}.

The chain partition function for trans-unsaturated chains is different from that of saturated chains. Rotation is absent about the double bond. The two bonds adjacent to the double bond have three rotational isomers of almost equal energy (Flory, 1969). As with other single bonds, two of the isomers result in a bend and the third keeps the chain roughly straight. We assume that effective area increases upon bending a chain are identical with the effective area increases upon bending a saturated chain at the same position. The chain partition function then becomes the sum of five terms, denoted q_1, q_2, q_3, q_4, and q_5, where q_1 refers to states with first bends occurring at least two bonds above the double bond, q_2 and q_3 refer to states with first bends occurring at the bonds imme-

diately above and below the double bond, respectively, q_4 refers to states with first bends occurring two or more bonds below the double bond, and q_5 refers to the state with no bends:

$$q_c = q_1 + q_2 + q_3 + q_4 + q_5 \quad (24)$$

where

$$q_1 = \sum_{i=1}^{k-2} 2e^{-\beta\epsilon}\xi^{m-i+1} \times 3 \times 3 \times \nu^{m-i-3}$$

$$q_2 = 2 \times 3 \times \xi^{m-k+2}\nu^{m-k-1}$$

$$q_3 = 2\xi^{m-k}\nu^{m-k-1}$$

$$q_4 = \sum_{i=k+2}^{m} 2e^{-\beta\epsilon}\xi^{m-i+1}\nu^{m-i}$$

$$q_5 = 1$$

$$\nu = (1 + 2e^{-\beta\epsilon})$$

$$\xi = \exp[-0.14\gamma^b\beta P_{HD}a_0]$$

As shown in Table I, reasonable values of the transition temperatures and enthalpies for DEPC and DEPE are obtained for model S-I (for which $\gamma^t = 0.42693$) as well as for model S-II (for which $\gamma^t = 0.29372$).

Although the numerical values of the enthalpies for both models are too high, they qualitatively agree with experiment in the sense that the enthalpies are lower than those calculated for the corresponding saturated (dioctadecanoyl) compounds but higher than those of saturated compounds with comparable transition temperatures (roughly 13 carbon chains). In summary, the interpretation we give of the lowering of the transition temperatures of these compounds is that the trans double bonds give the molecules larger areas relative to those with saturated chains. This leads to a lower density at closest packing in the solid phase, which produces a relatively greater destabilization of solid phases than fluid phases and leads to lowering of transition temperatures.

Models for PC's Having Two Cis-Unsaturated Chains

The experimental data on transition temperatures and enthalpies of PC's having two identical hydrocarbon chains each with one double bond are given in Tables I and II. The significant questions that should be answered are as follows. (1) Why does introduction of a cis double bond between the 9th and 10th carbon atoms of an 18-carbon chain PC lower the transition by the large amount of 70–75 °C? (2) Why is the transition temperature so sensitive to the location of the double bond in the acyl chain?

There are several differences between phospholipids with saturated chains and those with cis-unsaturated chains that might be responsible for the differences in their phase transition properties. The most significant is the effect of the double bond on the shape of a hydrocarbon chain. If all the bonds above and below the double bond are in their trans conformation, then the upper and lower parts of the chain, each of which is approximately cylindrical, are tilted at a 60° angle to one another. There is no conformation of the chain that is as straight as a saturated all-trans hydrocarbon chain. The double bond can affect the packing of chains at high density, thus affecting the relative stability of the solid and fluid bilayer phases. A second difference is that a cis-unsaturated chain has fewer internal rotational degrees of freedom than a saturated chain. A third difference is that the rotation of a molecule about an axis perpendicular to the bilayer surface (or of a single chain about that axis) is more severely hindered by intermolecular interactions for cis double bonded chains than for saturated chains.

If all the single bonds above and below a cis double bond are in their trans conformation, then we can imagine a plane passing through the axes of the cylinders corresponding to the upper and lower segments of the chain. An important question about the low-temperature solid phase of a bilayer is whether there are long-range correlations between these planes for the different chains in the bilayer. That is, do the planes tend to be parallel to each other or not? If they do tend to be parallel, we refer to this as an "ordered" solid phase. If they do not, we call it a "disordered" solid phase. X-ray scattering can in principle be used to decide whether the chains in a bilayer of cis-unsaturated molecules are ordered or not, but to our knowledge the experiment has not been performed. We have constructed various statistical mechanical models, some incorporating the assumption of an ordered solid phase and some assuming a disordered solid, in order to find what sorts of assumptions about bilayer structure are consistent with the observed phase-transition properties.

First let us consider disordered solid-phase models. We imagine that in a disordered model there are no long-range orientational correlations between the molecules and that each molecule is relatively free to rotate about an axis perpendicular to the surface of the membrane. The double bond affects the shape of each chain and of the molecule. This affects A_m, the minimum area into which N molecules can be compressed, which in turn affects the maximum value of the cohesive energy. A similar effect was found above to explain how trans double bonds lower the phase transition temperature. In the cis case, however, the minimum area depends upon the location of the double bond in just such a way as to explain the data of Barton & Gunstone (1975).

Suppose the kth bond in a chain is a cis double bond and that all other carbon–carbon bonds are single bonds. Then the bonds 1, 2, ..., k–2 and $k + 2$, $k + 3$, ..., m have three internal rotational states (one trans at 0° and two gauche states at ±120°). The states of the bonds $k \pm 1$ are located at angles 0 and ±60° [see Flory (1969) for a discussion of the geometry and definition of angles], with the 0° state being lowest in energy. The kth bond has, of course, only one state. If all the single bonds are in their 0° state, the upper and lower parts of the chain make an angle of about 60° with one another. For each of the four possible gauche conformations at bonds $k \pm 2$, there is a single combination of ±60°,±60° conformers at bonds $k \pm 1$ that results in an overall bend in the chain of only about 30°. No smaller angle for the overall bend is possible in the rotational isomeric approximation. Chains with 30° bends, when tilted as in parts D and E of Figure 1 have smaller projected areas than chains with 60° bends tilted in a similar fashion. In unoriented solid phases, chains with 30° bends can, therefore, attain a greater density and a greater strength of dispersion attractions. The 30° bend is destabilized by the intramolecular energy associated with the ±60°,±60° conformations of bonds $k \pm 1$ and the single gauche conformation at bond $k \pm 2$; the combined effect is an energy 3.7 kcal/mol above the corresponding all-trans chain with conformations 0°,0° at bonds $k \pm 1$. However, this may be compensated at high density by the greater dispersion interactions.

In model CD-I, we imagine that in the high-density low-temperature solid state the molecules have a 30° bend and tilt as in Figure 1D, with the methyl end of the chain directly below the bend group. In model CD-II, we imagine that the molecules have a 60° bend and that the molecules tilt in the same way. Models CD-I and CD-II use the same sets of variables as the models described above for saturated mole-

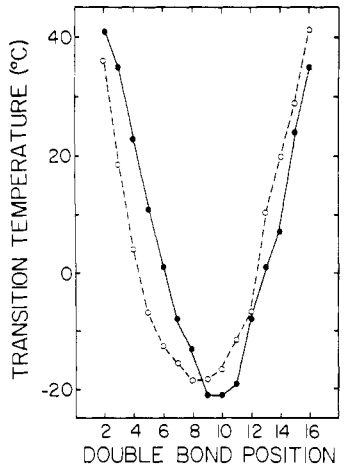

FIGURE 3: Dependence of transition temperature on double bond position for the dioctadecenoylglycerophosphocholine. Filled circles are the experimental points of Barton & Gunstone (1975). Open circles are the points calculated by using model CD-II.

cules, and several of the equations for the latter model also hold for CC-I and CD-II, such as eq 12–14 and 21. We will now discuss the chain partition functions and the calculation of A_m and l for these models.

The chain conformation partition function must take into account the effect of the double bond. The bonds adjacent to the double bond have three minima in their rotational potential as before, but the energies for these states are different from those of other single bonds. The energy λ associated with 0°,±60° conformations at bonds $k \pm 1$ is estimated by Flory (1969) to be 2.0 kcal/mol, and the energy μ associated with ±60°,±60° conformations at bonds $k \pm 1$ is estimated to be 3.2 kcal/mol. As in the case of saturated chains, we assume that the position of the first gauche bond in the chain determines the effective area increase upon chain bending, and we assume that the area increase for a bend at the ith bond is the same as the area increase for a bend at the same position in the corresponding saturated chain.

For model CD-II the chain bending/chain conformation partition function then becomes

$$q_c = \sum_{i=1}^{5} q_i \tag{25}$$

where

$$q_1 = 1$$

$$q_2 = \sum_{i=1}^{k-2} 2e^{-\beta\epsilon}\xi^{m-i+1}\nu^{m-i-3}(1 + 4e^{-\beta\mu} + 4e^{-\beta\lambda})$$

$$q_3 = \sum_{i=k+2}^{m} 2e^{-\beta\epsilon}\xi^{m-i+1}\nu^{m-i}$$

$$q_4 = 4e^{-\beta\mu}\xi^{m-k+1}\nu^{m-k-1}$$

$$q_5 = 4e^{-\beta\lambda}\xi^{m-k+1}\nu^{m-k-1}$$

In this expression, q_1 includes the state in which the bonds $k \pm 1$ are 0°,0°, respectively, and all the other single bonds are trans. The states included in q_2 have their first gauche bonds at position $i \leq k - 2$, and the states included in q_3 have their first gauche bonds at positions $i \geq k + 2$. The states included in q_4 and q_5 have conformations 0°,±60° or ±60°,0° and ±60°,±60°, respectively, at bonds $k \pm 1$. These conformations are regarded as bends occurring at the double bond position. (An alternative formulation is to regard the chain as being in its minimum area configuration when all bonds i, $i \leq k - 2$ or $i \geq k + 2$, are trans, regardless of the conformations of bonds $k \pm 1$. In this picture, q_1 becomes $[1 + 4e^{-\beta\mu} + 4e^{-\beta\lambda}]$, q_2 remains the same, q_3 is multipled by $[1 + 4e^{-\beta\mu} + 4e^{-\beta\lambda}]$, and q_4 and q_5 are omitted. Calculations with both forms of the chain partition function give virtually identical results.)

In model CD-I, in which we consider chains to have 30° bends at high densities, the chain partition function becomes

$$q_c = \sum_{i=1}^{8} q_i \tag{26}$$

where

$$q_1 = 4e^{-\beta(\epsilon+\lambda)}$$

$$q_2 = \sum_{i=1}^{k-3} 2e^{-\beta\epsilon}\xi^{m-i+1}\nu^{m-i-3}(4 + 4e^{-\beta\mu} + 4e^{-\beta\lambda})$$

$$q_3 = \sum_{i=k+2}^{m} 4e^{-\beta(\lambda+2\epsilon)}\xi^{m-i+1}\nu^{m-i}$$

$$q_4 = \sum_{i=k+2}^{m} 4e^{-\beta(\lambda+2\epsilon)}\xi^{m-i+1}\nu^{m-i}$$

$$q_5 = (1 + 2e^{-\beta\lambda} + 4e^{-\beta\mu})\xi^{m-k+1}\nu^{m-k-1}$$

$$q_6 = 2e^{-\beta\lambda}\xi^{m-k+1}(1 + e^{-\beta\epsilon})\nu^{m-k-2}$$

$$q_7 = 2e^{-\beta\epsilon}(1 + 2e^{-\beta\lambda} + 4e^{-\beta\mu})\xi^{m-k+3}\nu^{m-k-1}$$

$$q_8 = 4e^{-\beta(\epsilon+\lambda)}\xi^{m-k+3}(1 + e^{-\beta\epsilon})\nu^{m-k-2}$$

In this expression, q_1 includes the four low-area states with one gauche bond at $k + 1$ or $k - 1$ and no additional gauche bonds. The term q_2 includes all states whose first gauche bond from the top occurs at bonds $i \leq k - 3$; these are treated as bends at bond i. The terms q_3 and q_4 include states for which the bonds near and above the double bond are in one of the four minimum area conformations, but there are one or more additional gauches below the double bond, the first being at bond i. This leads to a bend in the chain at position i. Again, gauche bonds participating in low-area compensating gauche configurations are not regarded as causing bends. The terms q_5 to q_8 include all other states.

From Figure 1D, we can calculate the area of a chain projected upon the plane of the bilayer and the thickness l of the monolayer as a function of the location of the double bond. The area of the cis-unsaturated chain minus the area of a saturated chain is denoted as Δa^c and is given by

$$\Delta a^c = (d - w)w \tag{27}$$

where d is the length of the projection of the chain and w is the width of a saturated chain. The values of d and l can be calculated trigonometrically. If a and b are the lengths of the segments of the chain above and below the double bond, respectively, and ψ is the angle to which the chain is bent (i.e., 30 or 60°), then

$$d = w + (ab/l)\sin\psi \tag{28}$$

and

$$l = (a^2 + b^2 + 2ab\cos\psi)^{1/2} \tag{29}$$

As we did for trans-unsaturated chains, we assume that the effective minimum area A_m for N molecules is somewhat less than that calculated from the projection of the molecule on an axis perpendicular to the chains:

$$A_m = N(a_0 + 2\gamma^c\Delta a^c) \tag{30}$$

where Δa^c is the difference between the areas of tilted cis-unsaturated chains (bent to 60 or to 30°) and the projected areas of saturated chains, and γ^c is an area parameter, $0 < \gamma^c < 1$. Compare eq 23.

Table III: Experimental and Theoretical Transition Temperatures and Enthalpies for Dioctadecenoylglycerophosphocholines as a Function of Double Bond Position

	temp (°C)		enthalpies (kcal/mol)	
k	expt[a]	CD-II[b]	expt[a]	CD-II[b]
2	41	36.0	9.6	11.2
3	35	18.7	8.7	10.2
4	23	4.3	8.2	9.0
5	11	−6.8	7.8	8.5
6	1	−12.6	7.8	8.3
7	−8	−15.6	7.6	8.0
8	−13	−18.5	7.6	8.1
9	−21	−18.2	7.7	8.6
10	−21	−16.5	7.6	9.1
11	−19	−11.6	7.8	9.5
12	−8	−6.7	7.9	10.2
13	1	10.4	8.2	11.1
14	7	19.8	8.6	12.0
15	24	29.0	8.9	12.8
16	35	41.6	9.6	13.5

[a] Barton & Gunstone (1975). [b] $\gamma^b = 0.36789$, $\delta^{PC} = \delta^{PE} = -6.40$, $\gamma^c = 0.3214$, and A_m is specified as described in eq 30.

The parameters in these models are ϵ, ΔH_{sub}, a_0, l_0, δ, γ^b, and γ^c. Several of them, namely, ϵ, ΔH_{sub}, a_0, and l_0, are taken from structural and thermodynamic data on lipids, as discussed above. For PC's we assume that δ, the head-group interaction energy, and γ^b, the area parameter for chain bends, are the same as for model S-I. This leaves γ^c as the only new adjustable parameter. It was chosen to fit the phase-transition temperature of DOPC. (The average of several different experimental values was used.) The same value of the parameter was used to calculate the transition temperature and enthalpy for all positions of the double bond in PC's. Results of calculations using model CD-II are shown in Tables II and III and in Figure 3. The calculated transition temperatures and enthalpies show a marked dependence on the position of the double bond, both being smallest when the double bond is in the middle of the chain. The results are in qualitative agreement with the data of Barton & Gunstone (1975). (We have also performed calculations for a model in which the molecules are assumed to tilt in a slightly different way, as shown in Figure 1E. In this model the molecule tilts so as to minimize its projected area. Thus the midpoint of the lower segment of the chain is directly below the top end of the upper segment of the chain. Similar results were obtained for this model.) Calculations using model CD-I failed to produce even qualitative agreement with experiment, in the sense that calculated transition temperatures varied monotonically with double bond position, for reasons discussed elsewhere (Berde, 1978).

Now let us consider ordered solid-phase models. The rotational entropy is much lower for an ordered solid phase than for a disordered solid phase. If an ordered phase is preferred, the driving force is presumably an energetic one; i.e., the ordered phase has more favorable dispersion interactions and has a lower energy. If a chain containing a cis double bond at the kth position has all of the bonds $j < k - 2$ and $> k + 2$ in trans conformations, then the chain will have a bend of roughly 60°. These bends prevent the chains from assuming a close-packed arrangement in an ordered solid when the segments above the double bond (toward the carboxyl terminus) are oriented perpendicular to the plane of the bilayer (see Figure 4A). Several arrangements of the chains can increase the stability of the solid phase relative to this picture. If the segments of the chains above the double bond are oriented at an angle of 30° relative to the bilayer normal and

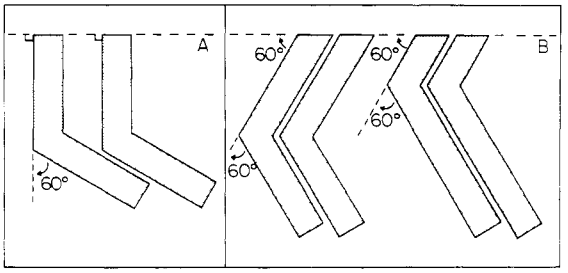

FIGURE 4: Packing arrangements of cis-unsaturated chains. As shown in A, in the absence of tilting it is impossible for the segments above and below the double bond to both be closest packed. As shown in B, tilting of chains to an angle 60° from the bilayer plane permits closest packing with an area of $2N$ chains equal to $3^{1/2}/2$ times the area of $2N$ saturated chains and a (three-dimensional) density of segments equal to that of the system of saturated chains. Note that this arrangement leads to the maximum density at closest packing for any position of the double bond in the chain.

if the planes passing through the midpoints of the carbon–carbon bonds in all of the chains are oriented parallel to each other, then it is possible for the chains to pack with as great a density as a system of saturated chains. [Any inequivalence of the chains (Seelig & Seelig, 1974, 1977) is ignored in this discussion.] It is apparent from Figure 4B that tilting of the segment above the double bond to 30° from the bilayer normal will allow the maximum packing density for any position of the double bond in the chain. Only at this degree of tilting can the segments above and below the double bond have the same cross-sectional area and simultaneously achieve closest packing. Since the driving force for formation of an ordered (rather than disordered) solid phase is the cohesive energy, it is reasonable to assume that this tilting does occur in ordered solid phases.

One way to obtain a statistical mechanical model of a bilayer composed of cis-unsaturated molecules is to construct a straightforward generalization of models S-I and S-II for saturated chains using the same set of X and Y variables. The effects of a cis double bond upon the conformational states of a hydrocarbon chain are taken into account by using eq 25 for the chain partition function. To incorporate the effects of tilting and bending of chains into the mean field term of the partition function, which should reflect the three-dimensional density of methylene segments, the factor l_0/l is set equal to $(\cos 30°)^{-1}$. Thus, when the area of the system equals A_m, the contribution to the mean field energy from attractions between chains is $-\Delta H_{sub} 2n$. That is, at closest packing the chains can assume a density and strength of dispersion attractions equal to those of saturated chains. This model is designated CO-I. This gives equations that are identical with those of model S-I except for the slight modification of the chain statistics. Also, except for minor effects due to chain statistics, the equations are almost the same for all positions of the double bond. When it is assumed that the head-group interaction parameters and area parameter are independent of the position of the double bond, the calculated transition temperatures are almost the same for all double bond positions and are approximately the same as those of the corresponding saturated molecule. This is in disagreement with the experimental data of Barton & Gunstone (1975), and thus we reject model CO-I.

If the solid phase is, in fact, ordered, there must be some important effect that is left out of CO-I. This effect must destabilize the solid, relative to the fluid, and it must be sensitive to double bond position.

One possibility is that there might be a molecular degree of freedom that is not considered in CO-I but that contributes

significantly to the difference in free energy between solid and fluid phase. The most likely candidate is a molecular rotation about an axis perpendicular to (or approximately perpendicular to) the bilayer surface. Viewed along such an axis, a phospholipid molecule or a hydrocarbon chain has a shape that is dependent upon the double bond location. Rotation about this axis is probably more severely hindered in the solid phase than in the liquid phase, and so an explicit inclusion of this degree of freedom in the partition function should tend to lower the calculated phase transition temperature to an extent that varies with double bond position. We have constructed two models, CO-II and CO-III, that take this degree of freedom into account in an approximate way. They are discussed in detail in Appendix I. Neither of them is capable of reproducing the data of Barton & Gunstone (1975), and so these models must be rejected. We can think of no other degree of freedom that could affect the phase-transition temperature in the way needed to explain the data.

Another candidate for the defect in model CO-I is the assumption that for all double bond positions it is possible for the molecules to pack together in such a way that the van der Waals cohesive energy is as large as it is for saturated hydrocarbon chains. If the double bond interferes with the packing of the chains, this would lead to a decreased van der Waals energy for the proposed ordered solid phase relative to the fluid phase and a decreased transition temperature. In particular, if this is the correct explanation, the data of Barton and Gunstone imply that the van der Waals energy must be a minimum when the cis double bond is near the center of the chain. Examination of models [for example, see Figure 3 of Barton & Gunstone (1975)] and the crystal structure of oleic acid (Abrahamsson & Ryderstedt-Nahringbauer, 1962) indicates that ordered, close-packed structures can be formed by these molecules. There seems to be no reason to expect an appreciably decreased van der Waals energy or a dependence of that energy on the double bond position.

Barton & Gunstone (1975) proposed that the interaction energy for 18 carbon chains with a double bond at position 9 is about 10% lower than when the double bond is at position 2. They based their conclusion on the calculations of Shapiro & Ohki (1974), who give values for the interaction energy of saturated hydrocarbon chains, and on the assumption that the energy of two interacting unsaturated chains can be estimated as the sum of the energies of two pairs of saturated chains with lengths appropriate to the upper and lower chain segments. The contributions due to interactions between the upper segment of one chain and the lower segment of the other are ignored. Shapiro & Ohki's calculations result in a chain–chain interaction energy for straight chains that increases with increasing chain length faster than proportional to the number of carbons for chains of less than 34 carbons. This result, in combination with the approximation made by Barton and Gunstone, leads to a minimum energy when the chain is interrupted at position 9. However, since Shapiro & Ohki use a localized bond polarizability model, the resulting nonlinear variation of energy with chain length must be due to accumulated long-range interactions. A consistent use of the model of Shapiro and Ohki should include the long-range interactions between the upper and lower chain segments. However, they were ignored by Barton & Gunstone. The simple model proposed by these authors is therefore internally inconsistent.

As a further test of these hypothesis, we have calculated the interaction energy of two cis-unsaturated 18-carbon chains using the 6–12 potential developed by Warshel & Lifson (Lifson & Warshel, 1968; Warshel & Lifson, 1970). The empirical potential function represents the intermolecular interaction as a sum of interatomic terms. This treatment results in reasonable agreement with experimental alkane crystal structures, sublimation enthalpies, and lattice vibrations (Warshel & Lifson, 1970), as well as molecular vibrational and conformational data (Lifson & Warshel, 1968). Our calculation used the experimental molecular geometry of oleic acid (Abrahamsson & Ryderstedt-Nahringbauer, 1962). The calculated interaction energy was 2.87 ± 0.02 kcal/mol for the minimum energy separation for the 18-carbon species with double bonds at position 5, 9, or 13. The value obtained for a saturated chain was 3.01 kcal/mol. We conclude that a full calculation of the interaction energy of these molecules indicates no dependence on double bond position. The lower interaction energy obtained for one unsaturated chain is less than that for saturated chains but not by an amount sufficient to result in the observed decrease in transition temperature.

By a model calculation, we estimated what reduction in interaction energy would be required to drop the transition temperature from 55 (DSPC) to −18 °C (DOPC). In this calculation we employ model S-I for DSPC and observe the reduction in transition temperature that occurs upon reducing the energy of the mean-field term, as given by eq 21. In order to reduce the transition temperature −18 °C, the interaction energy must be reduced by 30%.

We have not been able to construct any statistical mechanical model of a phospholipid bilayer with cis-unsaturated chains and an ordered solid phase that is capable of explaining their lower transition temperatures (relative to those of the corresponding saturated molecule) and the systematic variation of the transition temperatures with double bond position. Therefore, it seems to us highly unlikely that the series of cis-unsaturated diacyl PC's has an ordered solid phase with densities at closest packing that are the same for all positions of the double bond.[4]

All of the models discussed so far use the rotational isomeric state model of a hydrocarbon chain. We also constructed a model, CD-III, that describes the bending and rotation of single bonds in the chains away from the discrete states of the rotational isomeric approximation. If chains are considered to be tilted and bent to an angle of 30° as in the unoriented model, then small rotations or bends about several bonds away from their rotational minima could allow the chains to straighten, pack closer together, and regain dispersion attractions. Details of this model are given in Appendix II. It was not successful in fitting the data on transition temperature as a function of double bond position; it predicts a transition temperature that is largest when the double bond is in the middle of the chain.

In summary, we have found only one model (CD-II) for bilayers of phospholipids containing two identical cis-unsaturated hydrocarbon chains that is capable of explaining the transition-temperature data for these compounds. In this model, there are no long-range orientational correlations between the chains on different molecules. The cis double bond distorts the shape of the molecule and interferes with its ability to pack into a low-energy, high-density solid phase. This

[4] Abrahamsson & Ryerstedt-Nahringbauer (1962) determined the structure of oleic acid crystals by X-ray scattering and found a pseudoorthorhombic packing of molecules. At closest packing, the density of such a crystal is $3^{1/2}/2$ times the density of a close-packed hexagonal phase with the same spacing between chains. If DOPC bilayers also have this type of packing, this might explain the low melting temperature of DOPC bilayers compared to that of its saturated analogue DSPC. It would not shed any light on the apparently smooth dependence of melting temperature on double bond position.

interference is greatest when the double bond is in the middle of the chain, and it diminishes as the double bond is moved to either end.

Models for PC's Having One Saturated Chain and One Cis-Unsaturated Chain

The experimental data for 1-octadecanoyl-2-octadecenoyl PC's are given in Table II. There are two significant features of the data. (1) The transition temperature is dependent upon double bond position in a way that is similar to the case of two identical cis-unsaturated chains per molecule. (2) For a given position of the double bond, the transition temperature is intermediate between that of the molecule with two unsaturated chains and the molecule with two saturated chains.

To construct a model for bilayers containing these molecules, we start with model CD-II for the case of two identical unsaturated chains and imagine replacing one of the chains by a saturated chain. The molecular degrees of freedom are the same as those of model CD-II. The high-density, low-temperature state is one in which each molecule has one saturated chain that is not tilted and one unsaturated chain that is tilted, as in Figure 1D. Thus, the minimum area is

$$A_m = N(a_0 + \gamma^c \Delta a^c) \qquad (31)$$

(Compare eq 23 and 30.) Because of the saturated chain, $l/l_0 = 1$. The formula for partition function is

$$Q(N,A,T) = Q_s(N,A,T) \exp[-\beta N h_l(a)][q^c(a,T)]^N[q^s(a,T)]^N \qquad (32)$$

instead of eq 9. Here q^c is the partition function for a cis-unsaturated chain, given by eq 25, and q^s is that of a saturated chain, given by eq 20. Q_s and h_l are the same as for CD-II. This model will be referred to as S-I/CD-II, since it is a hybrid of CD-II and S-I.

All the parameters of the model are chosen to be those of S-I and CD-II without any additional adjustment. The results are given in Table II. Although the numerical values of the transition temperatures are not accurate, the minimum in the temperature as a function of double bond position is reproduced.

The physical picture on which this model is based is similar to that of CD-II. The chains form a disordered solid state, and the double bond's effect on molecular shape and molecular packing is most important for determining the transition temperature.

PE's with Cis-Unsaturated Chains

A PE with saturated chains or trans-unsaturated chains has a higher transition temperature than the corresponding PC. The most successful and reasonable models discussed above ascribe the differences to an increased attraction (or decreased repulsion) between head groups for PE's as compared with PC's. The order of magnitude of the difference was consistent with the hypothesis that it is due to intermolecular hydrogen bonding in PC's but not in PE's.

PE's with one or two cis-unsaturated chains have about the same transition temperature as the corresponding PC's (see Table II). The increased head-group attraction (or decreased repulsion) is apparently not effective in raising the transition temperatures of these PE's. One very plausible explanation is that PE molecules with cis-unsaturated chains cannot pack together tightly enough to hydrogen bond with each other effectively.

Summary

The purpose of these calculations is to find the physical basis for several regularities and trends in the experimental data on phase-transition temperatures for various neutral aqueous phospholipids. Each statistical mechanical model incorporates certain assumptions about the structure of the bilayer, about the important molecular degrees of freedom, and about the important interactions. The extent to which the results of the model reproduce the experimental trends is taken as a measure of the validity of the assumptions on which the model is based. Many approximations are made in the calculations, but we believe that they are accurate enough for the qualitative purpose for which the theories were developed.

We found that the gross differences between the transition temperatures of phospholipids with two saturated chains, two trans-unsaturated chains, two cis-unsaturated chains, and one saturated and one cis-unsaturated chain can all be explained in terms of the effect of the double bonds on molecular shape and the subsequent effect of shape on the ability of the molecules to pack together into a low-energy state at high density. The dependence of transition temperature on location of the double bond in cis-unsaturated molecules can be explained on the same basis.

The differences between the transition temperatures of PE's and PC's can be explained in terms of a larger intermolecular attraction (or smaller repulsion) for PE's than PC's. The magnitude of this difference in the interactions needed to explain the experimental data on saturated phospholipids and phospholipids with trans double bonds is consistent with the postulate that hydrogen bonding in PE bilayers is the origin of the difference. This postulate also allows us to rationalize the fact that PE's and PC's with cis double bonds have nearly the same transition temperature, even though PE's and PC's with only saturated or trans-unsaturated chains have different transition temperatures.

Appendix I: Models CO-II and CO-III

In model CO-II, the X variables of a molecule are the positions $\mathbf{r}$ of the head group, as in the model for saturated phospholipids. The Y variables for a chain are not only the set of labels α_j, $j = 1, \ldots, m$, for each of the m single bonds in the chain but also an angle ϕ describing the rotation of a chain. This is the angle between the plane passing through the upper and lower parts of the chain and the average direction of these planes for all the molecules in the bilayer. For low temperatures in the solid, each chain has a ϕ value near zero, but at higher temperatures and in the fluid phase ϕ can fluctuate. The important feature of model CO-II is that it describes, in an approximate way, the effect of this chain orientation degree of freedom on the relative free energy of the solid and fluid phases of a bilayer.

The Hamiltonian for this model is of the form given in eq 6. For H_s we use the hard disk Hamiltonian as in the model for saturated phospholipids. The mean field energy for ordered models is given in eq 21. The chain Hamiltonian h_c is assumed to be a sum of three parts:

$$h_c(Y_j;a) = \sum_{j=1}^{m} |\alpha_j|\epsilon + P\Delta a_e^b(\alpha) + P\Delta a_e^0(\phi) \qquad (33)$$

The first two terms are the same as those for saturated molecules. The third term describes the dependence of the Hamiltonian on the rotational angle ϕ. When a chain in an oriented solid phase moves through an angle ϕ (about some specified axis), it sweeps out a certain area and encounters a pressure exerted by the other chains. The actual area swept out is $\Delta a^0 = s^2\phi/2$, where the length s is the maximum width of the projection of the cis chain on a plane perpendicular to the rotation axis minus the width of a saturated chain. We assume that rotation occurs most easily about an axis that has

the property that the projection of a chain on a plane perpendicular to that axis is a minimum. Since the carboxyl terminus is linked to the head group and does not move freely, the axis that minimizes the projected area subject to this constraint must pass from the carboxyl terminus through the midpoint of the segment of the chain below the double bond. The area swept out by a chain upon rotation through a given angle will be greatest when the double bond is at positions seven or eight, and least when the double bond is at either end of the chain. The effective area increase of the chain is $\Delta a_e^0(\phi) = \gamma^0 \Delta a^0(\phi)$, where $0 < \gamma^0 < 1$ is the area parameter associated with chain rotations. Again, we assume that the pressure opposing the rotations is proportional to the hard disk pressure. This gives the last term in eq 33.

The partition function for model CO-II is given by eq 9, where now

$$q_c = q_b q_0 \tag{34}$$

The first factor is the same as the chain partition function for model CD-II, and q_0 is given by

$$q_0 = (2\pi)^{-1} \int_{-\pi}^{\pi} d\phi \exp[-\beta P_{HD} s^2 |\phi| \gamma^0/2] = (2/\pi\beta P_{HD} s^2 \gamma^0)[1 - \exp(-\beta P_{HD} s^2 \gamma^0 \pi/2)] \tag{35}$$

This first model for an oriented solid describes the chain orientation contribution to the partition function using the same type of effective pressure approximation that we have been using for chain configurations. We found that this model could not explain the data of Barton & Gunstone (1975) on transition temperatures as a function of double bond position in cis-unsaturated diacylglycerophosphocholines if it is assumed that γ^0 is independent of double bond position and of the packing density.

In model CO-II, the rotational degree of freedom was regarded as a property of each chain. The attachment of the chains to the head group might preclude this independent rotational motion of each chain. Thus, in model CO-III we imagine that the rotational motion is a property of the entire phospholipid molecule.

In model CO-III, the X variables of a molecule are the position $\mathbf{r}$ of the head group and a rotation angle ϕ for rotation of the entire molecule about an axis approximately perpendicular to the bilayer surface. The ability of the molecules to pack together at high density is enhanced if they all have a ϕ angle of ~ 0, but it is inhibited if there is some disorder in the distribution of ϕ angles. Moreover, the intermolecular interactions give each molecule a limited amount of rotational freedom. We idealize this by imagining that each molecule is an ellipse (rather than a disk) translating and rotating on a two-dimensional flat surface. We have developed an approximation for the hard ellipse partition function as a function of density and axial ratio of the ellipse. The approximation is a generalization of the cell model for hard disks and hard spheres. Details of the approximation are discussed elsewhere (Berde, 1978). In model CO-III, eq 21 gives the mean field energy and the chain partition function is given by eq 25.

Calculations were performed by using model CO-III with axial ratios for the ellipses ranging from 7 to 2. In order to produce agreement with experiment for DOPC, it was necessary to assume that the area parameter was smaller than that for saturated chains and that the longer range repulsions between head groups (as specified by the parameter δ) were greater. The axial ratio for molecules with double bonds at either end of the chain is unlikely to be less than 2, and it is unlikely that the axial ratio for the dioleyl compound is much greater than 7. For systems with this range of axial ratios with $k = 9$ in q_c, the calculated transition temperatures differed by less than 1 °C. When the value of k was changed along with the axial ratio, then the transition temperature was found to increase monotonically with double bond position, contrary to experiment.

It is reasonable to conjecture that a rotational degree of freedom, such as we have considered in models CO-II and CO-III, might have an effect on the relative free energies of the solid and fluid phases to an extent that is different for double bonds in the middle of the chains than for double bonds at either end. These approximate calculations show, however, that the effect is not large enough to explain the data of Barton & Gunstone (1975).

Appendix II: Model CD-III

This model takes into account, in an approximate way, the effect that distortions away from the idealized rotational isomeric state have upon the relative stability of solid and fluid phases. In model CD-I, the high-density, low-temperature state of the molecule has an angle of $\pi/6$ between its upper and lower segments. Model CD-III is very similar to CD-I, except that we now allow this angle, called ψ, to vary. From Figure 1D, it can be seen that the area of the shadow of a chain is related to the angle ψ. The area of the shadow is given in eq 36.

$$\pi(w/2)^2 + (d - w)w \tag{36}$$

Straightforward but lengthy trigonometric calculations show that for the type of tilting shown in Figure 1D:

$$\psi = \cos^{-1}\left\{\frac{[(d-w)^4 - \{(d-w)^2(a^2+b^2) - a^2b^2\}]^{1/2} - (d-w)^2}{ab}\right\} \tag{37}$$

where a and b are the lengths in angstroms of the segments above and below the double bond, respectively. It is clear that for any position of the double bond, $a + b = 18 \times 1.25 = 22.5$ Å for 18 carbon chains. The intramolecular energy of a chain is a minimum when $\psi = \pi/6$, and we assume for simplicity that the energy is a quadratic function of the angle. Thus, we add a Hooke's law contribution (eq 38) to the chain

$$\frac{1}{2}\kappa(\psi - \pi/6)^2 \tag{38}$$

Hamiltonian. This is taken into account in a mean field way by replacing ψ by its average value $\bar{\psi}(a/2)$, which is a function of the area per chain. This function is obtained by assuming that the area per chain is proportional to the area of the shadow of the chain. Thus, we set

$$a/a_0 = [\pi(w/2)^2 + (d - w)/w]/\pi(w/2)^2 \tag{39}$$

where we have used the fact that $d = w$ when $A = Na_0$. This determines the average value of d for any value of a, and eq 37 can then be used to obtain $\bar{\psi}$. At closest packing, $\bar{\psi} = 0$, and $\bar{\psi}$ is a monotonically increasing function of a. For areas larger than the area at which $\bar{\psi} = \pi/6$, the mean field Hooke's law energy is zero. For this model, the high-density, low-temperature phase consists of untilted chains with $\psi = 0$. Thus, $A_m = Na_0$ and $l = l_0$. The formulas for the partition function are the same as those for model CD-I, except that

$$2N\left(\frac{1}{2}\kappa\right)(\bar{\psi} - \pi/6)^2$$

is added to the mean field energy.

The parameters used in this model are δ, γ_b, A_m, l, ϵ (as defined for previous models), and κ, the Hooke's law constant. As noted earlier, A_m and l are set equal to Na_0 and l_0, respectively, and $\epsilon = 0.5$ kcal/mol. As before, $0 < \gamma_b < 1$. Previous models gave best agreement with experiment for values of δ ranging from -3 to -7, depending on the structure of the head group and the other features of the model. Since we do not envision the long-range head-group interactions to be dramatically different for this model as compared to previous cases, we explored only a limited range of values, namely from -15 to $+4$. (We would be suspicious of a calculation that showed agreement with experiment only if δ had some value outside of this range.) For a matrix of values of γ between 0 and 1 and δ between -15 and $+4$, the value of κ was chosen to give agreement with the experimental transition temperature for DOPC. The value was then fixed and transition temperatures were calculated for the other members of the series as before. For the various cases, the values of κ ranged from 4 to 100 kcal/mol·rad).

For this range of parameter values, our calculations gave a maximum value of the transition temperature when the double bond was located in the middle of the chain, precisely opposite to the experimental trend. This outcome can be interpreted physically as follows. In the solid phase, the chains are almost entirely straight, and ψ is close to zero for all positions of the double bond. Thus, the Hooke's law contribution to the free energy of the solid phase is virtually independent of double bond position. At lower densities the Hooke's law contribution to the free energy is more sensitive to double bond position, and at any given density it causes a greater increase in the free energy when the double bond is near the middle of the chain. Thus, when the double bond is near the middle of the chain the Hooke's law contribution produces a maximum destabilization of the fluid phase relative to the solid phase, and hence a maximum value of the transition temperature.

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Ribonucleic Acid Release Activity of Transcription Termination Protein ρ Is Dependent on the Hydrolysis of Nucleoside Triphosphates†

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ABSTRACT: An assay is devised to measure ρ-dependent release of RNA chains from transcription complexes. It is shown that ρ stimulates the release of RNA from isolated ternary transcription complexes consisting of nascent RNA molecules, *Escherichia coli* RNA polymerase, and T7 DNA and that this stimulation is dependent on the presence of a nucleotide substrate for ρ–NTPase. Several experiments lead to the conclusion that release is a direct consequence of a NTP hydrolysis dependent ρ action on the RNA. First, the NTP requirement is satisfied by any of the nucleotides which are substrates for ρ–NTPase but is not satisfied by nucleotides that are not hydrolyzed by ρ action. Furthermore, the fact that some of the nucleotides that do activate release are poor substrates for RNA polymerase suggests that release is not dependent upon further nucleotide addition to the nascent chain. Second, the reaction conditions for release and RNA-dependent ATPase show similar requirements for optimum activity and similar sensitivity to inhibitors. The action of ρ in release shows no selectivity with nascent T7 RNA molecules that are longer than 300 nucleotides. When the transcription complexes contain T7 RNA molecules with a length distribution from 1000 to 2500 nucleotides, all the RNA chains are released. However, when complexes are used that have T7 RNA from 50 to 500 nucleotides long, there is a significant reduction in the release of molecules shorter than 300 nucleotides. It is also shown that the released RNA molecules appear to be the same size as the corresponding RNA molecules in the complex. Since a difference as large as 12 nucleotides out of 300 could be detected in the gel system used, this result rules out any mechanism that involves release by cleavage of the nascent RNA at some point more than 12 nucleotides from the 3′ end of the RNA in the complex.

RNA polymerase from *Escherichia coli* catalyzes the polymerization of RNA from a double-helical DNA template by a processive mechanism (Krakow et al., 1976). After initiation, an RNA chain is not released from its complex with the enzyme and the DNA until its synthesis is fully terminated. Since the rate of addition of nucleotides to a nascent RNA is not uniform at all sequences in the DNA (Darlix & Fromageot, 1972; Maizels, 1973; Rosenberg et al., 1978), there will be regions where RNA polymerase will pause temporarily—even for as long as several minutes—without being released. Although the synthesis of an RNA molecule may appear to be terminated temporarily, it is not *fully* terminated until the RNA molecule is released from the transcription complex.

Release of RNA molecules, and hence full termination of RNA synthesis, can occur spontaneously at certain sites on many DNA templates in reaction mixtures containing RNA polymerase as the only enzymatic transcriptional component (Richardson, 1969; Roberts, 1976; Adhya & Gottesman, 1978). However, at other sites, termination and release depend on the presence of a protein known as ρ (Roberts, 1969, 1976; Adhya & Gottesman, 1978). From studies of the transcription of λ DNA, T7 DNA, and DNA containing the Trp attenuator gene, it has been concluded that the sites where ρ acts correspond to those regions of the DNA template where RNA polymerase pauses (Darlix & Horaist, 1975; Rosenberg et al., 1978; Fuller & Platt, 1978). This conclusion suggests the possibility that the primary function of ρ in termination could be merely to release RNA from RNA polymerase molecules that have paused during transcription (Adhya & Gottesman, 1978; Richardson, 1978; Fuller & Platt, 1978).

ρ is an RNA-dependent NTPase[1] (Lowery-Goldhammer & Richardson, 1974), and its function in termination is dependent on the presence of substrates for the ρ–NTPase (Howard & de Crombrugghe, 1976; Galluppi et al., 1976). Thus, the release of RNA in termination could be a direct consequence of the NTP hydrolysis reaction.

In this paper a membrane filter binding assay is used to measure the release of RNA from isolated transcription complexes made from the action of *E. coli* RNA polymerase on T7 DNA. The results show that ρ stimulates the release of RNA when substrates for ρ–NTPase are present. An

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[1] Abbreviations used: NTPase, nucleoside triphosphate phosphohydrolase; ATP-γ-S, adenosine 5′-*O*-(3-thiotriphosphate); App[CH_2]p, adenyl-5′-yl methylenediphosphate; App[NH]p, adenyl-5′-yl imidodiphosphate; Ap[CH_2]pp, adenosine 5′-α,β-methylenetriphosphate; ddATP, 2′,3′-dideoxyadenosine 5′-triphosphate; 3′-dATP, 3′-deoxyadenosine 5′-triphosphate, also known as cordycepin 5′-triphosphate; araATP, 9-β-D-arabinofuranosyladenine 5′-triphosphate; CpA, cytidylyl(3′–5′)adenosine; EDTA, (ethylenedinitrilo)tetracetic acid; NTP, nucleoside triphosphate.

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