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Theory of Lipid Monolayer and Bilayer Phase Transitions: Effect of Headgroup Interactions

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Summary. Headgroup and soft core interactions are added to a lipid monolayer-bilayer model and the surface pressure-area phase diagrams are calculated. The results show that quite small headgroup interactions can have biologically significant effects on the transition temperature and the phase diagram. In particular, the difference in transition temperatures of lecithins and phosphatidyl ethanolamines is easy to reproduce in the model. The phosphatidic acid systems seem to require weak transient hydrogen bonding which is also conjectured to play a role in most of the lipid systems. By a simple surface free energy argument it is shown that monolayers under a surface pressure of 50 dynes/cm should behave as bilayers, in agreement with experiment. Although the headgroup interactions are biologically very significant, in fundamental studies of the main phase transition in lipids they are secondary in importance to the hydrocarbon chain interactions (including the excluded volume interaction, the rotational isomerism, and the attractive van der Waals interaction).

The chain melting transition in lipid bilayers is of fundamental importance because it provides information from which one can estimate the amount of orientational disorder in the biologically relevant fluid phase [14]. The transition may be caused by changing thermodynamic variables other than temperature, such as pressure [28, 35], surface pressure [24], ionic strength [7, 18, 34, 37], or relative chemical potential of the various lipid components [29]. The lipid transition has effects on membrane protein clustering and mobility, which are correlated with transformation [31] and adhesion [25] of cells; on anesthesiology [2, 35] and on growth conditions for cells [24, 29].

The appropriate tool for studying phase transitions theoretically is statistical mechanics. Although lipid bilayers are rather complicated systems making complete theoretical treatment very difficult, their importance warrants current theoretical investigations [8, 13, 17, 26, 27] which help to elucidate the most important interactions between lipid molecules. It is generally agreed that rotational isomerism [8, 9, 13]

provides the disordering mechanism for the transition and that lipid intermolecular forces include van der Waals interactions as well as the excluded volume effect. In addition, Marcelja [8] has emphasized a lateral pressure, even in bilayers, which he attributes to the polar heads and the hydrophobic effect, and Scott [26] has developed models which emphasize positional disorder of the chains while retaining some orientational disorder.

The theoretical models which are proposed for study are not necessarily the closest to reality, although this is one of the goals. The other, often conflicting, goal is to find a model which one can solve with as few mathematical approximations as possible. This latter goal has been especially desirable in phase transition studies since Onsager's work [20] on the Ising model shows that the standard approximations can give qualitatively incorrect results. In the case of polymer transitions the failure of approximate methods has recently been confirmed for several exactly solvable models [15]. Accordingly, the previous theoretical work of this author on lipid bilayers has emphasized a model containing the excluded volume interaction and a slightly modified rotational isomeric interaction, which can be solved exactly, thus eliminating any mathematical uncertainty. Other theoretical work has essentially taken the rotational isomeric terms (considerably modified in [26, 27]) into account exactly, but the excluded volume effect has been treated in the mean field approximation [8] or a somewhat more accurate Bethe-type approximation [27].

In this paper additional interactions will be added to the previous model [13], to be called the basic model, in order to study the effect of headgroup interactions which distinguish between the different lipids. But first a discussion of the phenomena is in order.

Experimental Results for Monolayers

Typical isotherms for dipalmitoyl phosphatidyl choline-lecithin (DPPC) are shown in Fig. 1 on a surface pressure π versus area A plot. This Figure is a composite of the work of Phillips and Chapman [22] for the lower temperatures and of Vilallonga [38] for the higher temperatures, with reasonable agreement for 25 to 35 °C. The most recent measurements are also in reasonable agreement, but with a higher T_c [6].

The $\pi - A$ isotherms shown in Fig. 1 are usually compared to the $P - V$ isotherms of ordinary condensing fluids. Thus, one imagines a two-phase region bounded by a coexistence curve, sketched with dashed

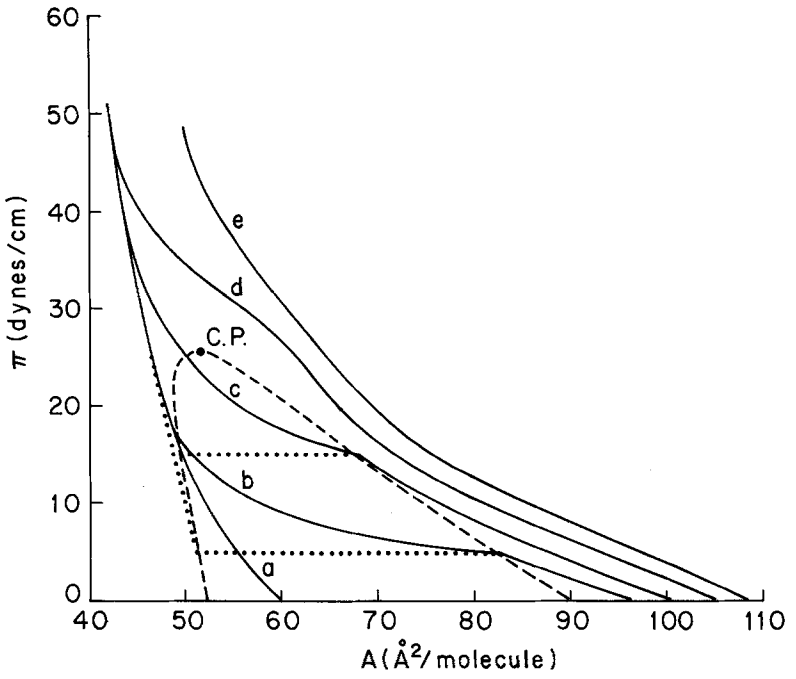

Fig. 1. The schematic experimental $\pi - A$ curves for DPPC are drawn in solid lines with temperatures (*a*) 6 °C, (*b*) 17 °C, (*c*) 26 °C, (*d*) 35 °C, and (*e*) 45 °C from references [22] and [38]. Possible ideal isotherms for 17 and 26 °C are shown with dotted lines and an ideal coexistence curve is shown with a dashed line, ending in a critical point designated C.P.

lines in Fig. 1, with a critical point on top. Since the experiments do not give flat portions to the isotherms, this interpretation is not conclusive. However, there are reasons to believe that the true isotherms for the monolayers may behave in the aforementioned standard way, shown with dotted lines in Fig. 1. First, the experiments were performed fairly rapidly so kinetic effects may disrupt the equilibrium behavior [22]. There is also a more exotic explanation possible for the nonflat isotherms in the two-phase region. Molecules near the edge of the surface have a considerably different environment than molecules in the deep interior. For example, if the walls of the trough were rough on a molecular scale and had weaker van der Waals interactions with the lipids than the lipids had with themselves, then the lipid molecules near the walls could be expected to disorder out of the all-trans ordered conformations at a higher value of π than the molecules in the interior. For many kinds of phase transitions this could be dismissed as a boundary effect which ought to be of little importance in connection with bulk thermodynamic properties. However, in these systems the excluded volume effect is very important, and this will extend the range of correlations to considerable distances, so boundary

conditions can be important. This is true of the kinds of models which have been used by this author to discuss polymer melting and the lipid transition [15]. Ways to test this possibility would be to systematically increase the size of the trough to determine whether the isotherms change slope and also, to change the materials of the walls of the trough. It may also be noted that the lack of detailed reproducibility between different workers favors this possibility. Let us therefore accept the interpretation that the monolayer isotherms have first-order phase transitions below the critical temperature T_c and no transitions above T_c. For DPPC one would estimate $35\,°C < T_c < 42\,°C$.

Comparison of Bilayers with Monolayers

It is often assumed that a bilayer is just two back-to-back monolayers with negligible interactions between the two monolayers. This author has recently added theoretical analysis [17] supporting this idea. A further test of this assumption is whether there is a correspondence of the bilayer phase transition behavior with the monolayer behavior shown in Fig. 1. Since one cannot put an external surface pressure π on bilayers, one might expect that the assumption implies that the bilayers would have thermal behavior identical to that of the monolayer at $\pi = 0$. But the monolayer at $\pi = 0$ has too low a transition temperature, $T_0 \simeq 10\,°C$. Marcelja [8] introduced the idea that bilayers correspond to monolayers under a constant, nonzero surface pressure and he chose $\pi \simeq 20$ dynes/cm. However, experiments [6, 19, 30] on DPPC now conclusively show that the transition temperature in monolayers is the same as in bilayers when an external surface pressure π_B of about 50 dynes/cm is applied to the monolayers. This, together with the assumption, means that any correspondence must be between bilayer states and monolayer states under the surface pressure $\pi_B \simeq 50$ dynes/cm (although π_B could conceivably vary with T). The fact that π_B is not zero, and even the value of π_B can be understood from the following model-independent argument, which only uses elementary thermodynamic considerations entirely independent of the assumption being tested.

There are two differences between the expansion or the creation of bilayer in bulk water and the expansion or creation of monolayer on the surface of a trough. The first difference is that an equal area of free water surface is destroyed in the monolayer case which reduces the surface free energy for the monolayer compared to the bilayer by the product of the

area times the surface tension of water which is about 70 dynes/cm near the DPPC transition. The second difference is that a hydrocarbon-air surface is created in the monolayer case and not in the bilayer case. The surface tension of *n*-octane is about 20 dynes/cm, so this difference increases the surface free energy per cm^2 for the monolayer by about 20 dynes/cm. The total effect from both differences is a surface free energy/cm^2 reduction of about 50 dynes/cm. Thus, to stabilize a monolayer at the same T and A as the bilayer (intrinsically at zero external π), assuming the same internal molecular interactions in monolayers and bilayers, *should* require an external surface pressure $\pi_B \simeq 50$ dynes/cm on the monolayer, in agreement with experiment [6, 19, 30]. Thus, the assumption that the bilayer is just two back-to-back monolayers passes this new consistency test. Furthermore, the calculations in previous papers are most relevant for bilayers which do not have these two surface effects. To translate to monolayers requires only the simple addition of $\pi_B \simeq$ 50 dynes/cm to all calculated π values.

It might be thought that a way to eliminate the second difference, i.e. the hydrocarbon-air surface tension term, is to use a hexane- or octane-water interface for the monolayer experiments rather than an air-water one. However, this has the more dramatic effect of decreasing the van der Waals attractive interactions because expansion of the monolayer is accompanied by insertion of hexane into the chain region. Thus, monolayers at an oil-water interface are not comparable to bilayers, although this is a good way to alter a fundamental energy parameter in lipid systems.

It is extremely interesting that the value of π_B is very close to (a) the critical pressure π_c for monolayers [6], (b) the equilibrium spreading pressure [19] and (c) the collapse pressure [30]. These coincidences seem significant, although the reason for them in terms of molecular models and thermodynamics is not clear to this author. The fact that $\pi_B \simeq \pi_c$ suggests that the transition in bilayers is critical like the critical point in monolayers. This is in agreement with this author's interpretation of density measurements [14]. However, perhaps the phenomenon interpreted as a critical point is only a manifestation of collapse. Thermodynamically, a monolayer subjected to π greater than π_B should transform to bilayers by simple folding into the bulk water, although such a process probably has such a high energy barrier that long-lived metastable monolayers are possible for $\pi > \pi_B$. In this paper we will take a theoretically conservative position which does not demand that the bilayer transition be rigidly tied to the monolayer critical point. Instead, if π_B is less than π_c

for the monolayers, then the transition will be first order. If π_B is greater than π_c, then the transition is technically absent, but in the case when π_B is only slightly greater *or* less than π_c, the dominant effect in bilayers will be that of a critical point. In the remainder of this paper the calculated transition temperature T_{50}, where the subscript indicates $\pi = \pi_B \simeq$ 50 dynes/cm, will be compared to the melting temperature T_m of bilayers.

Results of the Calculations on the Basic Model

The basic model developed by this author [13] has two energy parameters. First, there is the energy difference ε between trans and gauche rotations. Second, there is the van der Waals attractive energy coefficient a_{vdW}. From nonlipid work, it has been established that

$$\varepsilon = 0.5 \pm 0.1 \text{ kcal/mole } CH_2$$

and

$$a_{\text{vdW}} \simeq 1.84 \text{ kcal/mole } CH_2,$$

so there are no free parameters in the basic model [13].

In a previous paper the author has calculated the $\pi - A$ isotherms for the basic model. As has been discussed, one should add 50 dynes/cm to the calculated π for the basic model to compare to monolayers. When this is done, the result for the $\pi - A$ isotherms is shown in Fig. 2*a*. Qualitative agreement is satisfactory. There is a two-phase region for $\pi > 0$ which terminates in a critical point at $A = A_{\text{min}}$, $\pi = \pi_c$ and $T = T_c$. The details of the critical point, which is named a 3/2 order critical point [16], are different from those for usual gas-liquid critical points as is discussed in the preceding paper [17].

In the ordered phase, the isotherms become vertical lines at $A = 1$. This degeneracy of the high π, ordered phase, isotherms can easily be broken if one assumes that the lattice spacing increases with temperature. One can also make these isotherms nonvertical if one assumes that the lattice spacing decreases with increasing π. Since these reasonable modifications are easily taken into account by the reader and since they are not central to the phase transition problem, they are omitted from further discussion. Even with these modifications there remains one qualitative disagreement for the high T_c isotherms. For $T > T_c$ the isotherms have a kink at $A = A_{\text{min}}$. This is caused by the excluded volume potential which was taken to be an all-or-nothing effect. The obvious remedy is to taper the hard core, and this will be discussed in the next section.

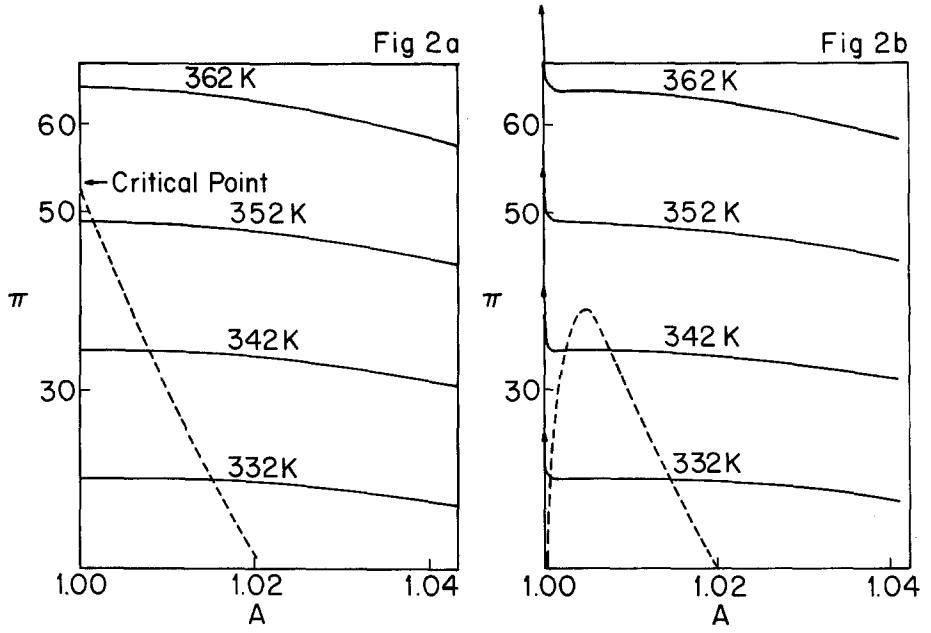

Fig. 2. (*a*) The $\pi - A$ isotherms for the basic model are shown by solid lines with the temperature in degrees Kelvin on each one. The dashed curve shows the two-phase coexistence region. The surface pressure π is given in dynes/cm and A is in relative units where $A = 1$ is the minimum area. (*b*) Same as (*a*) but with an added extended repulsive interaction, $v_r = 10^{-11}$

Quantitatively, the calculated T_c agrees well with the actual T_c considering the experimental errors in ε [13]. Other quantitative comparison between the calculated results in Fig. 2*a* and experiment is not so good. Increasing T at constant A in the disordered phase gives too large increases in π and the slope of the coexistence curve is too large. These quantitative disagreements with experiment are not surprising considering the simplifying features of the model, such as the lack of a tilting action.

Additions to the Basic Model

Previously, headgroup interactions were ignored in the basic model, partly because these interactions are smaller than the other important interactions, and also because it did not seem possible to add headgroup interactions to the infinitely long chains in the model. But with the new A variable it is indeed possible to include these interactions in the model. As an example, suppose that the headgroups are dipolar, so there is a potential which falls off as inverse cube distance between headgroups, or as $A^{-3/2}$ on average. This kind of interaction, which will be written more

generally as

$$E_{\text{headgroup}} = -a_2 A^{-c}, \tag{1}$$

may be included in the van der Waals or mean field way by adding Eq. (1) to the free energy of the basic model. Now, the chains in the model are actually infinitely long, and it is this feature which prevents a flexibility gradient [10]. However, given *this* caricature of lipid systems, the headgroup interactions have the same effect on every CH_2 group. In the model calculation of the free energy per CH_2 group the headgroup energy can therefore be divided evenly among the available rotatable CH_2 groups. Thus, the a_2 parameter equals the headgroup interaction strength of the particular lipid in question, such as DPPC or distearoyllecithin (DSPC), divided by the number of rotatable bonds. One consequence is that a_2 for the model is smaller for DSPC (32 rotatable bonds) than for DPPC (28 rotatable bonds).

There is good reason to believe that the glycerol groups may have larger excluded volumes or excluded areas than the chains, in the sense that, as lipid molecules which are oriented perpendicular to the bilayer are compressed, the first repulsion occurs between the glycerol groups at a separation r_G at which the chains are still attracting each other. One interesting consequence of this is that at low temperatures when the chains are in the all-trans conformation, it is energetically favorable for the molecules to tilt, as is shown in Fig. 3. X-ray determinations have found a 30° tilt angle in the ordered bilayer phase [32] which corresponds to a van der Waals radius for the glycerol groups about 15 % larger than the van der Waals radius for the hydrocarbon chains. The larger van der Waals radius for the glycerol group is also consistent with NMR studies which show that this is the least mobile region of the lipid molecules in the bilayer [12]. Unfortunately, the tilting of the chains, which persists above T_c in order to accommodate the flexibility gradient [10] is impossible to incorporate into this model. However, it is possible to include an extended repulsive interaction, i.e. a soft core, between glycerol groups in an area-dependent way by adding to the free energy terms like

$$\exp[a_r/(A-1)] \quad \text{or} \quad a_r(A-1)^{-6} \tag{2}$$

where the second term corresponds to an r^{-12} interaction. To complete the model we may also consider an extended repulsive interaction or soft core between the chains, such as

$$\exp[v_r/(V-1)] \quad \text{or} \quad v_r(V-1)^{-4}. \tag{3}$$

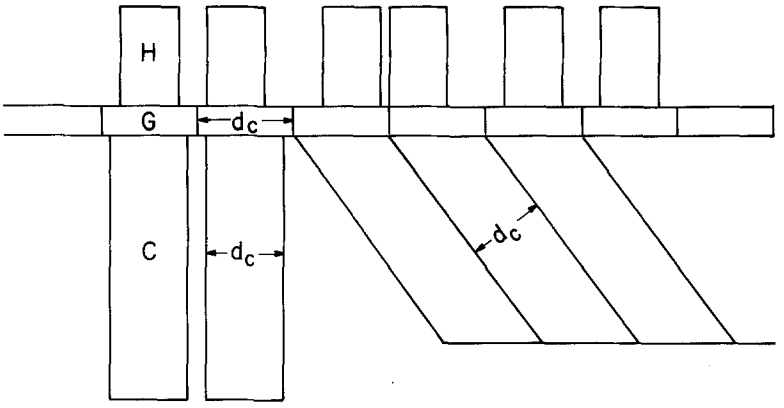

Fig. 3. A schematic representation of a monolayer. Each molecule has a head region H, a glycerol region G and a chain region C. The G regions are assumed to prohibit further lateral compression. The two chain regions on the left have not minimized their attractive van der Waals energy so it is energetically favorable for them to tilt as shown for the four chain regions on the right. Notice also the possible randomness in the headgroups which facilitates occasional hydrogen bonding discussed in the text

All of these additions, Eqs. (1), (2) and (3), to the basic model are mean field expressions. Since mean field calculations are exact only in the limit of very long-range interactions, these additions may be expected to be better for the longer range interactions [Eq. (1)], especially when c is small, and one should not have very high expectations for Eqs. (2) and (3) which are very short range. Fortunately, these mean field additions are relatively small perturbations on the basic model, and, most important, the excluded volume and rotational isomeric interactions are still treated mathematically exactly even with these added terms.

Calculations

In Fig. 2*b* is shown the $\pi - A$ diagram when an extended repulsive interaction [$v_r > 0$ in Eq. (3)] is added to the basic model. The critical point moves to A greater than one and the isotherms for $T > T_c$ have no kinks at $A = 1$. This diagram looks more conventional than the results for the basic model and probably contains much truth, although one should keep in mind the preceding discussion in the last paragraph. The case $a_r > 0$ in Eq. (2) leads to similar results. In both cases the coexistence region retains the asymmetric wedge shape exhibited by the basic model, except very near the critical point. Since the computing is much more tedious for these cases with $a_r > 0$ and/or $v_r > 0$, all the remaining calcula-

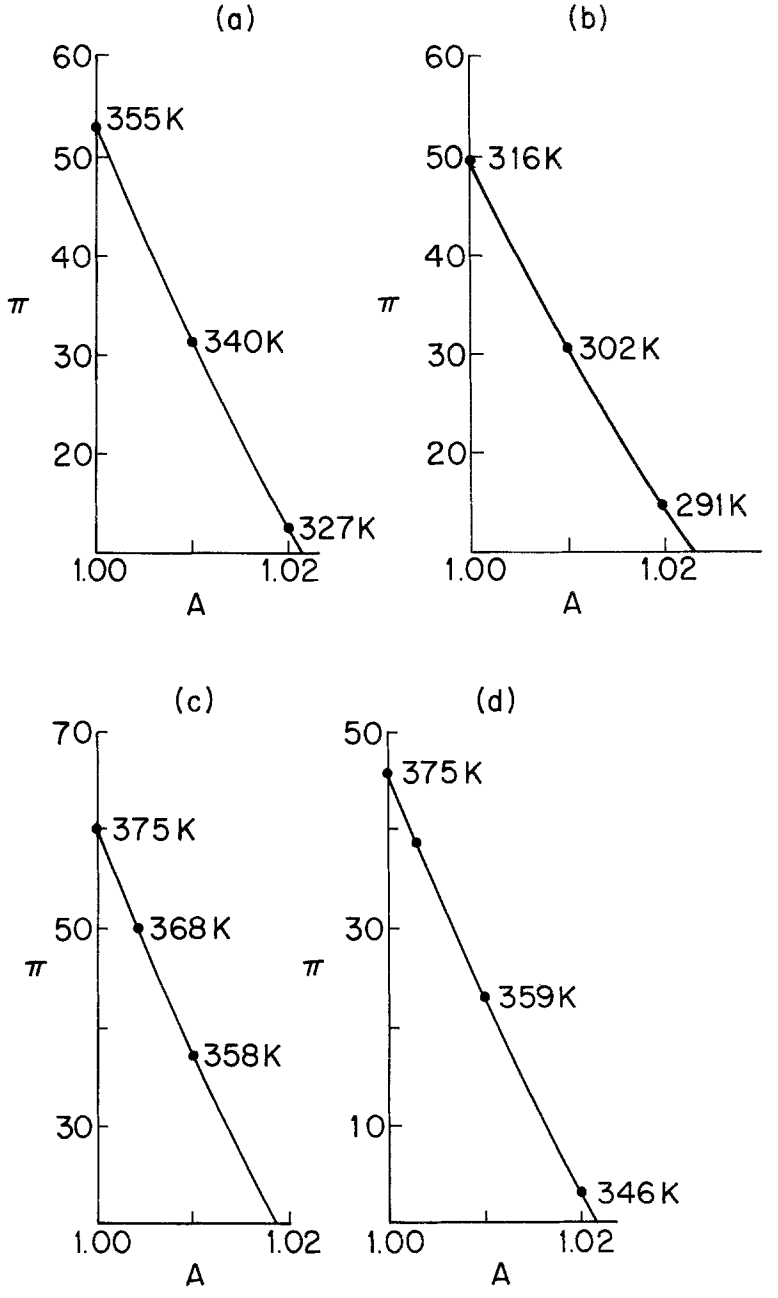

Fig. 4. The coexistence curves on $\pi - A$ diagrams for various cases where π is in dynes/cm and A is in relative units with $A = 1.00$ being the close-packed ordered state. The critical temperatures are shown as well as some other representative temperatures. The basic model has been modified as follows for each case: (*a*) basic model only, $a_{vdW} = 1.84$ kcal/mole CH_2, $\varepsilon = 0.5$ kcal/mole; (*b*) repulsive dipolar heads, $a_2 = -0.06$, $c = 1.5$ in Eq. (1); (*c*) attractive dipolar heads, $a_2 = 0.03$, $c = 1.5$ in Eq. (1); (*d*) attractive monopolar heads, $a_2 = 0.15$, $c = 0.5$ in Eq. (1)

tions will have $a_r = 0 = v_r$, but the reader can easily imagine, by analogy to the case shown, the modification produced by extended repulsive interactions.

In order to present economically the results of many calculations, diagrams such as Fig. 2*a* will be presented as shown in Fig. 4*a*. Not much information is lost because, for all the following calculations π at constant A increases more or less linearly with T and the isotherms do not change shape very much. The features of the diagram which are sensitive to changes in a_2 and c in Eq. (1) are the coexistence region and the value of T along the coexistence region, both of which are shown in the condensed figures.

It is most interesting to do calculations with values of a_2 and c which relate to the headgroup interactions in specific phospholipids. For dipolar interactions which go as r^{-3}, i.e. $A^{-3/2}$, one has $c = 3/2$ in Eq. (1). For fatty acids Phillips *et al.* [21] calculate a repulsive dipolar energy of about 1.6 kcal/mole which yields $a_2 \simeq -0.06$ kcal/CH_2 group in Eq. (1). The surface potential for DPPC is about 50 % larger than for fatty acids and the area per headgroup is doubled so the dipole moment is about three times as large. Since the distance of closest approach is larger by $\sqrt{2}$, a_2 for DPPC may be about 50 % larger than for the fatty acids. However, more water can enter between the headgroups in the DPPC case which will raise the effective dielectric constant and lower a_2, so we use an $a_2 \simeq -0.06$ kcal/CH_2 even in the DPPC case. The effect of this interaction added to the basic model is shown in Fig. 4*b*. The transition or two-phase region is pushed to lower surface pressures and lower temperatures.

DPPE, dipalmitoylethanolamine, provides an interesting contrast to DPPC. Phillips *et al.* [23] argue from X-ray evidence that the zwitterionic dipoles in lecithin (PC) are oriented perpendicular to the bilayer which gives the repulsive interaction used in the last paragraph, but they argue that the phosphotidyl ethanolamine (PE) zwitterions are oriented in the plane of the bilayer. Other evidence for this difference in orientations comes from NMR [12]. The difference in headgroup orientations must be due to stronger interactions between the amine and phosphate groups, possibly via hydrogen bonds, than between the choline and phosphate groups. The lack of a lower transition in the phosphatidylethanolamines [36] seems to be associated with this difference in headgroup interactions and orientations.

There are two ways to proceed to obtain quantitative estimates of a_2 and c for DPPE. First, if we assume that the zwitterions are point dipoles, then we can use the calculation of Phillips *et al.* [21] to obtain $a_2 \simeq$

$+0.03$ kcal/CH_2 group and $c=3/2$. The effect of this interaction added to the basic model is shown in Fig. 4*c*. However, the lengths of the dipoles are comparable to the separation between neighboring dipoles and the interaction between distant dipoles is screened, so it might be better to just include nearest neighbor monopole interactions, which gives $a_2 \simeq 0.15$ kcal/CH_2 and $c=1/2$. The effect of this interaction added to the basic model is shown in Fig. 4*d*. Both interactions give $T_c \sim 375$ °K, but the longer range $c=1/2$ depresses π_c and gives a larger two-phase region for π greater than zero.

Comparing Fig. 4*b* with Fig. 4*c* and *d* the outstanding contrast is the 50° difference in T_{50}, which is twice as much as is needed to account for the experimental difference in T_m for DPPC and DPPE bilayers. (The transition temperature T_{50} when $\pi=50$ is used to compare to the melting temperature for bilayers as was discussed earlier.) That the calculated difference is too large is not hard to rectify because the estimates of a_2 were based on electrostatic calculations for which the dielectric constant was taken to be 1; a more reasonable dielectric constant of 10 would reduce the T_{50} difference to only 6°. Another source of a T_{50} difference between DPPE and DPPC will arise shortly.

To finish this discussion of these possible electrostatic interactions, let us estimate how much enthalpy is absorbed by the headgroup upon expanding a bilayer from the all trans form to the fluid form, which typically involves an expansion of 25 % in the area A [11]. For the interactions in Fig. 4*b*, *c*, and *d*, the headgroup enthalpies ΔH_{head} are -0.48 kcal/mole, $+0.24$ kcal/mole and 0.44 kcal/mole, respectively. These energies are much smaller than the measured enthalpy of 9.2 kcal/mole for DPPC. Earlier, this author [14] performed a simple energetic calculation, ignoring ΔH_{head}, to calculate the number of gauche rotations n_g in DPPC above the bilayer transition; the present overestimates of $|\Delta H_{\text{head}}|$ only change n_g by about 1, and the more realistic values of ΔH_{head} obtained from dielectric constants greater than 1 make this change in ΔH_{head} inconsequential to the n_g calculation.

Next, let us consider dipalmitoyl phosphatidic acid (DPPA), whose bilayer transition has been shown to be strongly pH dependent, with a T_m of 67 °C for pH 6.5, for which the molecule is singly charged, $DPPA^-$ and a T_m of 58 °C for pH 9.1 for which the molecule is doubly charged $DPPA^{2-}$ [7]. This drop in T_m upon increasing the pH has been attributed to repulsive electrostatic interactions [34]. However, T_m is *higher* for both $DPPA^-$ and $DPPA^{2-}$ than for neutral DPPC. This cannot easily be accounted for by the addition of a repulsive interaction between head-

groups which only seems to lower T_{50} in the model, which seems a reasonable result on intuitive grounds.

Many years ago Alexander [1] suggested for single-chain monolayers [5] that the $\pi - A$ curves are strongly affected by hydrogen bonding between head groups. In the case of $DPPA^-$ weak transient hydrogen bonds between phosphate groups could provide a net attractive headgroup interaction which could raise T_{50}. $DPPA^{2-}$ cannot form any such direct bonds between two phosphates because the phosphates have no proton to participate in the hydrogen bond, so T_{50} would be expected to be lower than for $DPPA^-$. However, water molecules would mediate an attractive interaction between phosphates via two or more hydrogen bonds, and this could produce a net attractive interaction even in $DPPA^{2-}$.

In the case of DPPC the choline groups prevent the phosphate groups from getting close enough to form direct hydrogen bonds, although just as in $DPPA^{2-}$, water may provide an attractive interaction via two or more hydrogen bonds. In DPPE hydrogen bonds could form between the nitrogen and the phosphate oxygens and this provides another interaction which raises T_{50} for DPPE compared to DPPC. Other phospholipids such as phosphatidyl serine and phosphatidyl glycerol also may be expected to form weak transient hydrogen bonds.

It is clearly a very difficult matter to estimate the strength of hydrogen bond interactions for any of the phospholipids, and this will not be attempted. Also, the form Eq. (1) is not really sufficient to represent the highly directional and short-range hydrogen-bonded interactions. However, if we force the hydrogen-bonded interactions into the form Eq. (1), we can estimate the exponent c as follows. The distance between two headgroups is $2l_c + l_{HB}$ where l_c might be the P-O distance and l_{HB} the length of the hydrogen bond. Then the ratio of energies for two different areas is

$$\frac{U_2}{U_1} = \left(\frac{A_1}{A_2}\right)^c = \left(\frac{2l_c + l_{HB1}}{2l_c + l_{HB2}}\right)^{2c}. \tag{4}$$

Shortening the hydrogen bond from $l_{HB1} = 2.76$ Å to $l_{HB2} = 2.5$ Å gives roughly $U_2/U_1 = 2$ [3]. Thus, the exponent c can then be evaluated from Eq. (4) if l_c is given. The smallest value $c = 4.2$ is obtained if $l_c = 0$. A more reasonable value of l_c for phosphate groups is 1.5 Å and this gives $c = 7.2$. Notice that this estimate assumes that the center of the headgroup is *not* necessarily in the axial center of the molecule (*see* Fig. 3).

The result of adding a headgroup interaction of the type in Eq. (1) with $a_2 = 0.01$ kcal/mole CH_2 and $c = 4$ to the basic model is shown in Fig. 5*a*. The two-phase region above $\pi = 50$ becomes much more prominent

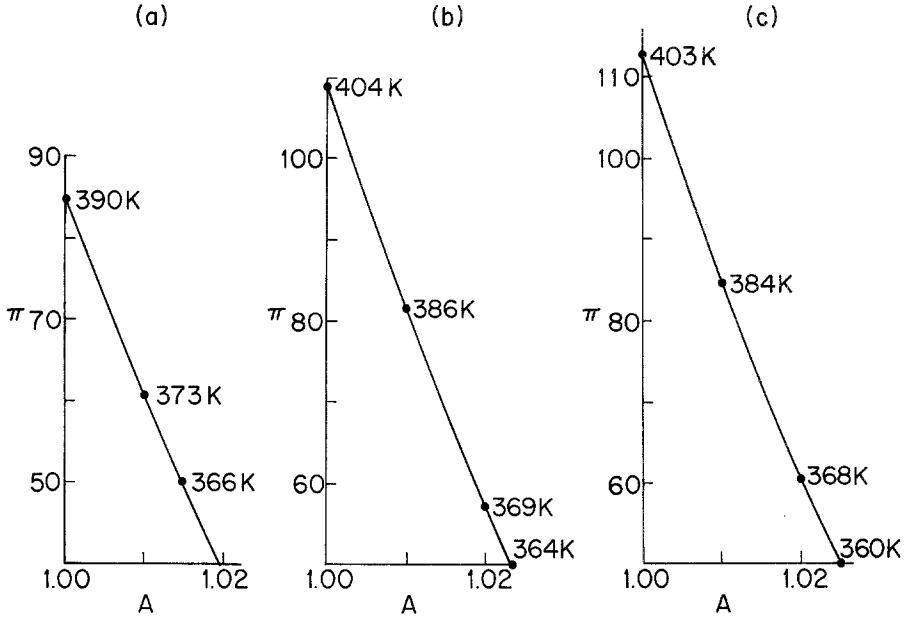

Fig. 5. Same as Fig. 4 except that the modifications to the basic model are short-range attractive head interactions with (*a*) $a_2 = 0.01$, $c = 4$ in Eq. (1); (*b*) $a_2 = 0.005$, $c = 7$ in Eq. (1); (*c*) same as (*b*) but a_{vdW} is reduced from 1.84 to 1.7 kcal/mole CH_2

and T_{50} increases about 12 degrees compared to the basic model, Fig. 4*a*. The result of adding to the basic model an interaction with $a_2 = 0.005$ kcal and $c = 7$, which is more suitable for hydrogen bonds, is shown in Fig. 5*b*, which has an even more prominent two-phase region and comparable T_{50} as in Fig. 5*a*. The values of a_2 in these two cases are remarkably small, corresponding to ΔH_{head} of only about 0.14 kcal/mole. These energies also correspond to only one hydrogen bond of length 2.76 Å for every 40 lipid molecules, which is consistent with transient and/or weak hydrogen bonds, which in turn is compatible with high lateral mobility [4]. From these calculations, it is clear that shorter-range, higher c interactions can considerably enhance the size of the two-phase region above $\pi = 0$ even with small values of a_2 and ΔH_{head}. The increase in T_{50} is comparable to DPPA versus DPPC.

Earlier, we discussed the lipid-water surface free energy involved in expanding or creating monolayers and how that differed from bilayers. There is also a surface-free-energy term common to both monolayers and bilayers, which is associated with the interaction of the headgroups with water and the disturbed hydrogen bonding at the surface. The headgroup-water interaction will clearly be different for different lipids and will be important for consideration of bilayer formation, but it will not be strongly

dependent upon area once the bilayer is formed. In addition there is an area-dependent term of the form $\pi_s A$ which accounts for the surface free energy of the water in contact with the bilayer. Measurements on black lipid membranes [33] show that π_s, although slowly varying with T, is only about one or two dynes/cm, which is negligible for our purposes. If one wishes to include this term in the calculated values of π, then π_s would be *subtracted* from π.

For our last calculation let us take the model calculated in Fig. 5*b* and change a_{vdW} from 1.84 kcal/mole to 1.7 kcal/mole. The results for this model are shown in Fig. 5*c*. The critical temperature is changed by only one degree, but the coexistence curve moves up the π axis, so that T_{50} decreases by about 4° and the area discontinuity increases by 6%. Replacing air by hexane in a monolayer system would reduce a_{vdW}, although probably far below 1.7 kcal/mole CH_2.

Discussion

The primary purpose of this paper is to further our understanding of the different properties of different lipids. But first it is necessary to discuss the methodological limitations to our efforts. The model does not produce quantitative agreement with experiment. In particular, the transition changes in area, volume, and latent heat are too small. The particular virtue of this model is that we know that the discrepancies must be in the model and not in mathematical approximations. One feature of the model which is likely to be responsible for smaller transition changes is the lack of a chain-tilting degree of freedom which is prevented because the model intrinsically has homogeneous chains of infinite length.

This model is like a cartoon or a caricature of lipid systems. It is easy to study in the sense of being mathematically solvable and, like a good cartoon, it reveals important features although it distorts them quantitatively. Clearly, the proper use of such a model is to study qualitative questions, not to try to produce numerical agreement with experiment or to evaluate phenomenological parameters by elaborate fitting to experimental results.

One important qualitative question is: can the large difference in transition temperatures of DPPC and DPPE be accounted for by known headgroup interactions? The calculations in the last section answer this question affirmatively. Electrostatic interactions alone produce effects of the correct magnitude, although this author feels that hydrogen bonding in DPPE may play at least as large a role as the electrostatic interactions. The precise mixture of the two kinds of interactions is impossible to decide

a priori and is one of those quantitative questions which this model cannot answer accurately, although with more data on DPPE, rough estimates could be given.

Another important qualitative question is: what causes the higher transition temperature in DPPA compared to DPPC? Intuitively, and according to the model calculations, this requires a more attractive headgroup interaction in DPPA than in DPPC, which is contrary to a purely electrostatic picture. Hydrogen bonding in DPPA could account for this difference, but the presence of too many hydrogen bonds is contrary to the lateral mobility picture of lipid systems [4]. The calculations resolve this dilemma by showing that large changes in transition temperatures and phase diagrams can be produced by very weak and transient hydrogen bonds which should not materially affect the lateral mobility. Also, the model has a hard time producing a two-phase region for $\pi > 50$ and it is encouraging that a little hydrogen bonding can also enlarge the two-phase region. This and general considerations suggest that hydrogen bonding between headgroups, both direct and indirect via water, may be important to varying degrees in all the phospholipids, with the least occurring in the lecithins.

The next qualitative question which can be discussed is the relative importance of the various interactions in lipid systems. Changes in headgroup interactions, which can be induced by changes in pH, Ca^{++} or other ionic levels, produce changes in transition temperatures of the order of 10° which is very important biologically. However, this is small compared to 300° on a fundamental Kelvin scale. The most important determinant of T_c in the model is the value of the rotational isomeric energy ε. Just the experimental error in ε amounts to about a 60 K change in T_c, and this should be kept in mind in examining the results of the calculations shown in Figs. 4 and 5. Another measure of the relative importance of the different interactions is the relative energy changes in going through the transition. The energy change of the rotational isomeric term is about 3.5 kcal/mole, and the van der Waals term changes by about 6 kcal/mole for DPPC [14]. In comparison, all the headgroup interactions considered change by less than 0.5 kcal/mole. A previous energetic calculation of the change in the number of gauche rotations at the transition assumed that headgroup interactions could be ignored [14]. The results of this paper support that assumption.

In conclusion, study of this model yields answers to interesting qualitative questions about lipid systems, including the effects of headgroup interactions.

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