Biochimica et Biophysica Acta, 595 (1980) 161—183

BBA 78593

A STATISTICAL MECHANICAL MODEL OF THE LIPID BILAYER ABOVE ITS PHASE TRANSITION

DAVID W.R. GRUEN

Physiological Laboratory, University of Cambridge, Downing Street, Cambridge CB2 3EG (U.K.)

(Received May 21st, 1979)

Key words: Lipid bilayer; Phase transition; Statistical model; Order parameter; Collective tilt

Summary

A statistical mechanical model of a bilayer of dipalmitoyl-3-*sn*-phosphatidylcholine molecules above their phase transition is presented. A molecular field approximation developed in previous work by Marčelja is extended by setting the molecular field at each depth in the bilayer in proportion to the average chain order at that depth. The free energy of the hydrocarbon/water interface and that due to the interaction of the polar headgroups is included in the evaluation of the statistical weights of the chain conformations.

The model gives good agreement with several independent experimental results. It resolves the dilemma posed by the experimental evidence that there is

(i) a considerable variation in order parameter along the lipid chain, but

(ii) no collective tilt in the more ordered region of the chain.

The model gives an explanation of how the lipid chains pack under these two constraints. The order parameter profile down the chain does not correspond to the profile across the bilayer.

Introduction

In the past decade, a large body of experimental knowledge has been built up concerning the thermally induced phase transitions in phospholipid bilayers. In the last five years a number of theoretical models have been developed [1—8,50—52]. Most of these are primarily interested in the transition itself. In order to simplify these models sufficiently to solve them, either exactly or

Abbreviation: DPL, dipalmitoyl-3-*sn*-phosphatidylcholine.

approximately, it is necessary to make drastic assumptions. In most cases, while this generates a 'feel' for the transition, it means that the behaviour of the model system above the transition corresponds only very approximately to the real system. In biological tissues at ambient temperatures, the lipids in cell membranes are at a temperature above the transition temperature (T_c). It is important, if model systems are to be of relevance to biology, that their behaviour above T_c should correspond as closely as possible to the real system.

Only one model (to this author's knowledge), the Marčelja model [1], is capable of giving a convincing picture of the state of the lipid chains above the thermal phase transition. This model is based on the mean field approach in which a single chain of a lipid molecule is embedded in a mean field which models the average behaviour of other chains. Using this approach, it is possible to take account of the important factors which determine the lipids' behaviour, and hence to develop a picture which agrees in many ways with experimental findings. This type of model is, however, inappropriate for the study of the phase transition itself. As Marčelja says [1]: 'In the immediate vicinity of the phase-transition temperature the results of the molecular field approximation deviate from the behaviour of real systems because the fluctuations in the value of the molecular field are neglected'.

In the present work the Marčelja model is developed to give a more detailed description of the state of dipalmitoyl-3-*sn*-phosphatidylcholine (DPL) above T_c. Considerable care is exercised in treating the hydrocarbon region. It is intended in the future to extend the model to treat the absorption of alkanes into the bilayer.

The theoretical model

In order to develop a reasonable statistical mechanical model of a bilayer it is important to take account of:

(i) the chain statistics;

(ii) the free energy associated with the hydrocarbon/water interface, and

(iii) the interaction of the headgroups.

Marčelja [1] deals with (i) in detail while taking (ii) and (iii) into account very indirectly. The Marčelja approach is to divide the energy of a hydrocarbon chain with configuration (i) into three components:

$$E^{(i)} = E^{(i)}_{int} + E^{(i)}_{disp} + PA^{(i)} \quad (1)$$

The first term takes into account the energy required to flex the chain. Each carbon-carbon bond can take up either a *trans* (t) or a *gauche* conformation (g^+, g^-). The sequences g^+g^- and g^-g^+ are forbidden for steric reasons. Marčelja assumed a bond rotation angle between *trans* and *gauche* bonds of 112.5° and an energy difference of 400 cal/mol. Here we assume perfect tetrahedral symmetry of the bond angles and an energy difference of 500 cal/mol (cf Flory [9]).

Van der Waals attraction

The second term in Eqn. 1 represents the Van der Waals attraction between chains. Only one chain can be considered at any time (the computing time

required to do otherwise is prohibitively large). Hence a molecular field is introduced which models the average behaviour of other chains. This molecular field is then set at a value which mirrors the average behaviour of the chain being considered. By analogy with the Maier-Saupe model for liquid crystals [10,53], Marčelja sets the dispersion energy as:

$$E_{\mathrm{disp}}^{(\mathrm{i})} = -\Phi \frac{n_{\mathrm{tr}}^{(\mathrm{i})}}{n} \sum_{j=1}^{n} \eta_j \tag{2}$$

and the molecular field Φ is evaluated as

$$\Phi = \frac{V}{n} \left\langle \frac{n_{\mathrm{tr}}^{(\mathrm{i})}}{n} \sum_{j=1}^{n} \eta_j \right\rangle \tag{3}$$

where

$$\eta_j = \tfrac{3}{2}\cos^2\beta_j - \tfrac{1}{2} \tag{4}$$

is the segmental order parameter of the j-th segment. β_j is the angle between the bilayer normal and the direction of the j-th chain segment which, in the case of CH_2 groups, is defined as the normal of the plane spanned by the two C—H bond vectors. In the case of the CH_3 group at the end of the chain this direction is defined as the direction of the terminal C—C bond. (These definitions for η_j were introduced by Marčelja [1] and Seelig and Niederberger [11], respectively.) The summation is performed over all segments ($j = 1,...,n$) of the chain. The angled brackets denote the thermodynamic average over all chain conformations. $n_{\mathrm{tr}}^{(\mathrm{i})}$ is the number of *trans* bonds in the chain. V is a coupling constant which models the strength of the van der Waals attraction between the chains.

The $n_{\mathrm{tr}}^{(\mathrm{i})}/n$ term was not present in the original theory which dealt with the behaviour of hydrocarbon chains in liquid crystals [12]. Without it, the dispersion energy of the system is proportional to the second power of the average order. However, dispersion energy is strongly dependent on the distance between chains while the angular dependence is weak. Salem [13] showed that for two parallel saturated hydrocarbon chains, the attractive dispersion energy between them varies as $1/D^5$ where D is the distance between them.

The present theory has no way of estimating the distance between the chains. However, as Marčelja [12] explained, this distance will increase as the order of the system falls. Because of this correlation it is possible to tie the dispersion energy to the order, and thereby implicitly take account of the large changes in energy with change in density of the system.

Clearly, the functional dependence of van der Waals energy on order will be complex. Because of its strong dependence on distance, one expects that its dependence on order will be stronger than quadratic. The introduction of the $n_{\mathrm{tr}}^{(\mathrm{i})}/n$ term achieves this result. Apart from the above plausibility argument, it can only be justified a posteriori. In the present paper, instead of introducing this term, the 3/2th power of the order is used when evaluating (i) the molecular field, and (ii) the dispersion energy. This course (which has a similar effect on the energetics of the liquid-crystal state) is taken because it is conceptually simpler and more straightforward to apply when allowing for different orders at different depths of the bilayer (see later).

Marčelja estimated V from the data of Bunn [14]. Bunn's value of 680 cal/mol of CH_2 groups is $L - RT$ where L is the molar heat of vaporization from liquid alkane to gas at the boiling point. (R is the gas constant and T is temperature.) As Bunn argues, this gives a measure of the strength of van der Waals forces in the solid, albeit an indirect one.

The present work is an attempt to model the state of the chains in the bilayer. The physical state of the chains below T_c appears to correspond very closely to the α-crystal form of the normal paraffins [15,16]. In this form the chains pack in a hexagonal array with rotational disorder. (This fact seems to have been overlooked by the majority of workers who have modelled the gel-liquid crystal phase transition. In estimating the van der Waals energy difference between the gel and a hydrocarbon gas, they use the value 1.84 kcal/mol CH_2. This comes from Billmeyer [17], and is the energy difference between solid (with no rotational disorder) and gaseous polyethylene extrapolated to 0 K.) The area per chain for lipid chains in the gel state is quoted as 20.4 $Å^2$ [16], and for α-crystal paraffin chains 19.9 $Å^2$ [17]. This difference may be a result of polar headgroup repulsion between the lipid molecules [6].

In the Marčelja model, the α-crystal form is described by $\eta_j = 1$, for all j. (In fact, the presence of rotational disorder suggests that η may be slightly less than unity.) On melting the α-crystal three relevant changes occur:

(i) the long-range order of the system virtually disappears;

(ii) the molecules undergo a considerable number of *gauche* rotations, and

(iii) there is a significant volume increase. As already discussed, the van der Waals energy changes which occur because of the volume change are included implicitly in the theory.

As $\eta \to 0$, the system approaches liquid hydrocarbon. (Note that $\eta = 0$ does not imply that short-range order has disappeared. In liquid hydrocarbon, there is still considerable van der Waals energy. The present theory attempts to estimate the extra van der Waals energy due to the long-range order in the system.)

If we assume that the number of *gauche* bonds is determined solely by their energy, then for a long chain at 41°C *:

$$\langle E_{int} \rangle|_{\eta=0} = 180 \text{ cal/mol } CH_2 \text{ groups} \tag{5}$$

This value is probably an overestimate; most likely a slight one (see Ref. 18). We can estimate V by applying the condition:

$$(E_{int} + E_{disp})|_{\eta=1} - (E_{int} + E_{disp})|_{\eta=0} = \Delta H_{fusion} \tag{6}$$

The evaluation of ΔH_{fusion} for n-paraffins initially in the α-crystalline state is not immediately straightforward. Phillips et al. [18] quote a value of 0.74 kcal/mol of CH_2 groups, which comes from a 1953 source. As discussed by McClure [19], there is considerable disagreement about the correct value. This is mainly because insufficient purity of samples leads to confusion between the $\beta \to \alpha$ crystal transition and the melting of the α-crystal. McClure recommends

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* Analysis similar to that in Flory (Ref. 9, Chapter 3) (but assuming that sequences g^+g^- are forbidden) leads to this result. In the evaluation of Eqn. 7, the calculation was repeated at the melting temperature for each alkane.

the data of Schaerer et al. [20]. Using their data for n-alkanes C_{21}—C_{29} gives the best fit to:

$$\Delta H_{\text{fusion}} - \langle E_{\text{int}} \rangle|_{\eta=0} = 728.0\, n_{CH_3} + 355.2\, n_{CH_2} \text{ cal/mol of alkane } (r^2 = 0.9547) \quad (7)$$

The estimate of the coefficient of n_{CH_3} is subject to considerable uncertainty. A small error in $\langle E_{\text{int}} \rangle|_{\eta=0}$ produces a large change in this coefficient while altering the coefficient of n_{CH_2} only slightly. For ease of calculation, the CH_3 groups were included in the van der Waals energy sum with equal weight of the CH_2 groups. The van der Waals energy in the solid (assuming near perfect long-range order) is

$$E_{\text{disp}} = -V/2 \text{ per mol of } CH_2 \text{ groups} \quad (8)$$

(The factor 2 occurs because of double counting of the energy when summing over the whole system.) Thus from Eqns. 6—8.

$$-(0 - V/2) = 355 \quad (9)$$

and hence $V = 710$ cal/mol.

A major change from the Marčelja model in this work is the treatment of Φ. Instead of having one value at all positions in the bilayer, it is allowed to vary. Because of the conditions imposed on the first atom in the chain, and the assumption of perfect tetrahedral symmetry, the atoms in the chain lie on planes parallel to the bilayer surface spaced 1.25 Å apart. Hence, the average order at each level can be evaluated (once the statistical weight of each configuration is known). For plane i the molecular field is set as

$$\Phi_i = v(\eta^i)^{3/2} \quad (10)$$

where η^i is the average order on the plane. The van der Waals term is then

$$E_{\text{disp}} = -\sum_{j=1}^{n} \Phi_i \eta_j^i |\eta_j^i|^{1/2} \quad (11)$$

where η_j^i is the order of the j-th segment of the chain which happens to be positioned on plane i. (The form $\eta_j^i |\eta_j^i|^{1/2}$ is necessary because η_j^i can be negative.) There is no a priori reason to assume that the order across a lipid bilayer is constant; and hence a more reasonable approach is to allow the order at each level to reach its own, unique, self-consistent value. (A somewhat different approach was suggested by Schindler and Seelig [21]. We feel the present approach to be more satisfactory.)

In order for the above theoretical analysis to be valid it is important that the axis of motional averaging is identical with the bilayer normal. Studies with orientated multilayers of C-5 deuterated DPL above T_c show that the quadrupole splitting collapses if the bilayers are inclined at the magic angle with respect to the magnetic field (Seelig and Seelig [22]). Seelig concluded [23]: "The rotation of the lipid molecule as a whole and, in part, the motions about C—C bonds within the fatty acyl chains are axially symmetric about the bilayer normal". Any collective tilt of the hydrocarbon chains with a lifetime longer than approx. 10^{-6} s is excluded.

Under these conditions (i.e., the bilayer normal being the axis of motional

averaging) the η_j defined previously correspond to the order parameters measured by electron spin resonance (ESR) and DMR experiments [11]. It might still seem possible that a collective tilt exists, with a lifetime too short to be be picked up by such experiments but long enough to be detected by ESR [24,25]. Seelig [23] argues that this is not so, as it would imply that deuterium order parameters (η_j) should be smaller than the corresponding spin label order parameters. The opposite is observed. (See also Discussion.)

Steric repulsion of the chains

In order to reduce hydrocarbon-water contact, the polar headgroups are packed closely. This restricts the freedom of the lipid chains and results in them exerting a lateral pressure on their surroundings.

The third term in Marčelja's energy Eqn. 1 models this situation. It is proportional to the average cross-sectional area (i.e., the area in the plane of the bilayer) of the chain and to the value of the lateral pressure on the chain. The cross-sectional area of a chain configuration (i) is estimated as

$$A^{(i)} = \frac{A_0 L_0}{L^{(i)}} \tag{12}$$

where A_0 and L_0 are the cross-sectional area and length of the fully extended chain. $A_0 = 20.4$ Å^2 (cross-sectional area of a single chain from Tardieu et al. [16]) and $L_0 = 19.7$ Å. $L^{(i)}$ is the length of configuration (i), measured perpendicular to the membrane surface.

In Marčelja's original work, the lateral pressure P was the only free parameter. He found that a good fit was obtained for a range of experimental results when P was set at 20—25 dyn/cm. He was, however, modelling chains with a length of 9—12 C atoms. More recently the Marčelja model has been applied to DPL [21]. Both V and P were allowed to vary so as to give the best possible fit to order parameters measured along the lipid chain by DMR. The values finally chosen were $V = 590$ cal/mol and $P = 18.5$ dyn/cm.

Headgroups *

Marčelja did not include in his model explicit terms for the free energies due to (i) the interaction of the headgroups, and (ii) the hydrocarbon/water interface. Their presence was allowed for only in so far as they anchor the lipid chains to the bilayer surface and impose a lateral pressure on them. If the chains exert a pressure of 20—25 dyn/cm on their surroundings, then the interface (by which we mean the hydrocarbon/water interface and the head groups) is in tension by the same amount; as the experimental surface tension of solventless bilayers is apparently almost zero. (This point was made, in somewhat different ways, by Haydon and Taylor [26], by Marčelja in the Appendix of Ref. 1, and by Nagle [27].)

At the oil/water interface (where, in the absence of lipid the tension is approx. 50 dyn/cm) a pressure of approx. 50 dyn/cm must be applied to a monolayer of lipid molecules for them to be in a state similar to their state in a

* I am grateful to S.B. Hladky for convincing me that the interaction of the headgroups must be included explicitly in the calculation of free energy and surface pressure.

solventless bilayer. For the air/water interface, this pressure is also approx. 50 dyn/cm [27] (as the lipid tails in air have a residual tension), not 15—25 dyn/cm as Marčelja claims in Ref. 1 (see also Ref. 28). The data displayed in Fig. 3 of Phillips and Chapman [29] are all for membranes below the fully hydrated liquid crystal-gel phase transition temperature of 41°C and hence not directly comparable with the behaviour of the bilayer above T_c.

The data of Phillips and Chapman show that, as T increases towards T_c, the monolayer pressure at which the liquid-expanded to liquid-condensed transition occurs increases towards 50 dyn/cm. Marčelja's calculated curves show a phase transition at T_c at a pressure $P \approx 20$ dyn/cm. Marčelja's pressure P does not have a direct thermodynamic significance.

It may appear that the above discussion is at odds with the results of Albrecht et al. [30]. They studied phase transitions of monolayers of DPL molecules at the air/water interface at different temperatures and surface pressures. For the monolayer phase transition at applied surface pressure of approx. 50 dyn/cm, both the heat of transition and the change in area/molecule become zero. This is to be contrasted with the large values for these quantities for the bilayer phase transition. Albrecht et al. conclude that a bilayer should not be compared with a monolayer at 50 dyn/cm.

There are two pertinent comments.

(i) Above T_c, the area/molecule for a monolayer at an applied pressure of 50 dyn/cm agrees well with bilayer values at the same temperature. This suggests that above T_c, the state of the molecules is similar (though clearly not equivalent) in the two cases.

(ii) Albrecht et al. [30] argue that the first-order phase transition in monolayers involves finite cooperative units. To form finite domains of tilted crystalline phase in the monolayer requires that the crystalline chains extend further into the air above the monolayer than surrounding chains. (The relative shapes of crystalline and fluid chains implies this.) For the bilayer to exhibit similar behaviour would require an increase of chain-water contact around the boundary of each crystalline domain (or a perturbation of the bilayer structure to avoid this). This is, in free energy terms, considerably more unfavourable than in the monolayer case. Hence, it may not be possible to unambiguously equate the monolayer and bilayer phase transitions.

Nevertheless, for the present paper, the experimental result that the surface tension of the bilayer above T_c is almost zero, requires that the sum of surface pressures (and tensions) across the bilayer is also almost zero.

The influence of the headgroups on each other is to be taken into account explicitly. For a particular chain conformation we evaluate A_I and A_c, the areas occupied by the molecule at the hydrocarbon/water interface and by the chain, respectively. The method of evaluation of these areas is described later.

For a particular A_I, there is an area $(A_I - A_{HG})$ of hydrocarbon exposed to water. A_{HG} is the equivalent hard-disc area of the interface taken up by the headgroup, which we will assume is constant for different chain conformations (and hence for different A_I values). Different conformations (or orientations) of water and changes in energy of the hydrocarbon chains due to the local presence of water are not taken into account. Thus, in weighting different chain conformations, the free energy cost of creating this surface, not the

energy cost, must be used. For configuration (i):

$$F^{(i)}_{\text{INTERFACE}} = \gamma(A_I^{(i)} - A_{HG}) \tag{13}$$

Taking the interaction of the headgroups into account is exceedingly difficult. First, some experimental evidence.

According to Büldt et al. [31], the phosphocholine dipole is parallel to the membrane surface both above and below the phase transition. Further, just below T_c, the chains pack at an angle of 30° to the membrane surface [32]. Since at this angle, the chain packing is equivalent to perpendicular packing [15], it appears that the free energy of the system is lower with $\langle A_I \rangle = 47.1$ Å^2/lipid than with $\langle A_I \rangle = 40.8$ Å^2/lipid.

In a recent review of ^{31}P nuclear magnetic resonance (NMR) experiments, Seelig [33] provides evidence that (i) both above and just below T_c, the phosphocholine group is in rapid rotation about the bilayer normal, and (ii) even above T_c, the N-methyl protons of one headgroup are in close proximity to the phosphate group of the next.

Compensation potential measurements on spread monolayers at the air/water and oil/water interfaces suggest that there is a significant dipole in the C—O groups attached to the hydrocarbon chains [34], lying essentially perpendicular to the membrane surface. This dipole is probably in a region of smaller dielectric constant than the $(O^-\text{-P})\text{-}N^+$ dipole and hence may exert considerable influence.

Finally, as Nagle suggests [27], hydrogen bonds between headgroups may be important.

The above demonstrates that a considerable amount is known about the structure and behaviour of the headgroup. We wish to have an expression connecting the free energy of a headgroup with the area available to it. This problem is approached by Tanford [35] (who is considering the behaviour of many different lipids). He chooses $F_{HG} = \alpha_1/A_I$ when dealing with charged headgroups and $F_{HG} = \alpha_2/A_I^3$ when steric repulsion is the only force acting (where α_1 and α_2 are constants to be determined separately). Scott [36] calculates the pressure on phosphatidylcholine headgroups assuming that the overriding effect is an osmotic one. A smaller area/headgroup leads to a squeezing out of water from the polar group region and hence to a significant decrease in the number of arrangements of water molecules and headgroups in this region. (The assumption is made that the headgroups have complete freedom to occupy any position within their cell of size A_I.) This entropic effect produces a large pressure; especially for areas/lipid less than 60 Å^2. It seems reasonable to assume that as the area/headgroup decreases, the $(O^-\text{-P})\text{-}N^+$ dipoles can interact more strongly, and lower the energy of the system. This effect (which occurs because these dipoles are in the plane of the membrane rather than perpendicular to it) should be particularly strong, because as the charges approach, the shielding effect of the water molecules around them decreases. For this reason (and also because the headgroups are undoubtedly less free than assumed) one would expect that the polar headgroup contribution to the π-A curves would be considerably less steep than that predicted by Scott. In fact, to predict the observed data, Scott [36] is drawn to this conclusion (see Figs. 4 and 5 of his paper).

Nagle [27] discusses in detail how to model the headgroup interaction. He uses a general form $E_{\text{headgroup}} = -aA^{-c}$, where a and c are constants to be determined separately for each headgroup type.

In the present model, we choose

$$F_{\text{HG}} = \frac{\alpha}{A_{\text{I}}^2} \tag{14}$$

a form between the Tanford extremes, considerably less steep than the relationship which would be derived from Scott's Fig. 4, and similar in spirit to the Nagle approach. Clearly, the variation of free energy with area will be more complex than this. The results of the present calculations do not depend critically on the form chosen for F_{HG}. The important point is that the headgroups exert considerable pressure.

The energy part of this free energy is due mainly to pairwise interaction between the heads (and hence will be double counted when a sum over all headgroups is taken). ^{31}P NMR experiments [33] suggest that the motional freedom of the headgroup just below T_{c} is not dramatically less than its freedom just above this temperature. (The ^{31}P chemical shielding anistropy varies smoothly with temperature without any discontinuity at $T = 41^{\circ}$C. At this temperature, there is a approx. 30% increase in A_{I}.) Different measurements (residual quadrupole splittings of DPL deuterated at sites in the headgroup [23]) show a discontinuity at T_{c}. The experiments are clearly difficult to interpret. The entropy contribution of the headgroup itself may be relatively insensitive to changes in area.

Water, lying between the polar heads will also contribute to the change in free energy with change in A_{I}. A change in A_{I} for one headgroup changes the number of water molecules between it and its neighbours. Hence changes in the free energy of the water molecules between the headgroups will be double counted when a sum over all the lipid molecules is taken.

For the above reasons we assume, for a system of m lipid molecules, that

$$F_{\text{HG}_{\text{total}}} = \frac{m\alpha}{2}\left\langle \frac{1}{A_{\text{I}}^2} \right\rangle \tag{15}$$

where the angled brackets denote the thermodynamic average over the chain conformations. The factor 2, although not rigorous, is a best approximation. Using this assumption we generate the pressure exerted by the headgroups:

$$\pi_{\text{HG}} = \alpha\left\langle \frac{1}{A_{\text{I}}^3} \right\rangle \tag{16}$$

A discussion of the assumptions involved in deriving this expression appears in Appendix 1.

Having determined the pressure on the headgroups π_{HG} in terms of α, we know that the pressure on the chains π_{c} must satisfy:

$$\pi_{\text{c}} = \gamma - \pi_{\text{HG}} \tag{17}$$

(from the condition that the surface tension of the film is, to a good approximation, zero). γ is the surface tension of the hydrocarbon/water interface between the headgroups. We are not dealing with two bulk phases. Nevertheless,

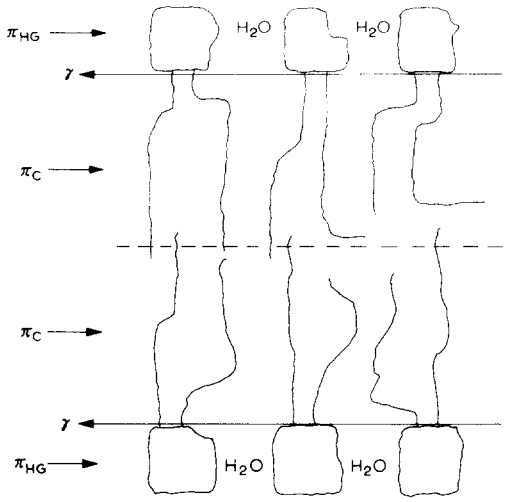

Fig. 1. Schematic surface-pressure diagram.

the best available values for γ is the bulk oil/water surface tension: $\gamma = 50$ dyn/cm.

We now tackle the problem of the evaluation of A_l and A_c for any given configuration.

A_l is the total area exposed to water by a single lipid molecule. We assume the area exposed by one chain is A_{AVE} (the average area/chain in the bilayer). The other chain's area can be evaluated explicitly. We define layer zero as the layer in which C1 (the acyl carbon attached to the first CH_2 group in the chain) sits. Layers 1 and 2 are, respectively, 1.25 Å and 2.5 Å nearer the bilayer centre. Then, for a particular conformation (i), the number of carbon atoms lying in layers 0, 1 and 2 of the bilayer ($N_{SUR}^{(i)}$) is evaluated. The chain area $A_{11}^{(i)}$ is then given as

$$A_{11}^{(i)} = C_{SUR} N_{SUR}^{(i)} \tag{18}$$

where C_{SUR} is a constant, fixed so that $\langle A_{11}^{(i)} \rangle = A_{AVE}$. The first three layers are considered because it is assumed that if a chain 'sticks out' in the second layer from the surface, this layer will form the surface as the next lipid cannot bend sufficiently to cover it. Then

$$A_l^{(i)} = A_{AVE} + A_{11}^{(i)} \tag{19}$$

(The abrupt delineation between 'surface' and 'non-surface' produces a minor artifact, the order of C-4 is artificially low (see Fig. 2). To eliminate this artifact would complicate the definition of $A_l^{(i)}$ unnecessarily.)

In evaluating the area exposed by a chain, A_c, two points need to be taken into account.

(i) Consider a bilayer with constant order at all depths. A conformation which lies parallel to the membrane surface has a larger area (to be acted on by π_c) than a conformation lying perpendicular. This statement also holds for segments of a conformation.

(ii) In regions of the membrane of large order, segments which lie parallel to the membrane surface (and hence 'stick out' from the rest of the chain), expose more surface area than segments which lie perpendicular. In regions of smaller order (further from the membrane surface), the statement is also true but to a lesser extent.

To satisfy the above conditions we define the chain area $A_c^{(i)}$ for a conformation (i) as follows.

Firstly we evaluate $A^{(i)}$ (defined previously as the Marčelja cross-sectional area). This value is then partitioned among the segments of the chain. $A_1 = 20.4$ Å^2 is allotted to the first segment (attached to the glycerol backbone, i.e. C-2). $(A^{(i)} - 20.4)/m$ Å^2 is allotted to each of the m segments which lie parallel to the membrane surface. (The value of m depends on the configuration. When $m = 0$, the configuration is all-*trans* and $A_c^{(i)} = 20.4$ Å^2.) All other segments are given zero area. $A_c^{(i)}$ is then evaluated as:

$$A_c^{(i)} = C_1 \sum_{j=1}^{n} A_j \eta_{jk} / \frac{1}{l} \sum_{x=1}^{l} \eta^x \tag{20}$$

where A_j is the j-th segment's area, η_{jk} is the average order on the plane (plane k) on which the j-th segment is sitting, η^x is the order on the x-th plane, and C_1 is a constant, independent of configuration which is fixed so that $\langle A_c^{(i)} \rangle = 1/2 \langle A_l^{(i)} \rangle = A_{AVE}$.

The sum in the numerator is over the segments of the chain ($n - 1$ CH_2 groups and a CH_3 group). The sum in the denominator is over layers in the bilayer.

The contribution which segmental areas make to the chain area in Eqn. 20 depends linearly on the order of the region in which they sit. This is the simplest assumption which satisfies point (ii) above.

The computer calculation

An initial value is guessed for the order at each level and for α. The computer then generates all the configurations of a chain (conformations which bend backwards into the polar region are eliminated) and configuration (i) is given the 'energy':

$$E^{(i)} = E^{(i)}_{CHAIN} + F^{(i)}_{HEADGROUP} + F^{(i)}_{INTERFACE} + \Sigma \pi A \tag{21}$$

The term $\Sigma \pi A$ refers to the sum of 'πA' terms for the different layers of the bilayer, i.e. the chain region and the headgroup region. Thus for configuration (i)

$$\Sigma \pi A = \pi_c A_c^{(i)} + (\pi_{HG} - \gamma) A_l^{(i)} \tag{22}$$

Note that in general $A_c^{(i)} \neq 1/2\, A_l^{(i)}$, but $\langle A_c^{(i)} \rangle = 1/2 \langle A_l^{(i)} \rangle$. Collecting terms from Eqns. 11, 13, 14 and 22 gives:

$$\begin{aligned} E^{(i)} &= E^{(i)}_{int} - V \sum_{j=1}^{n} (\eta_{jk})^{3/2} \eta_j^k |\eta_j^k|^{1/2} + \frac{\alpha}{A_l^{(i)2}} + \gamma (A_l^{(i)} - A_{HG}) + \pi_c A_c^{(i)} + (\pi_{HG} - \gamma) A_l^{(i)} \\ &= E^{(i)}_{int} - V \sum_{j=1}^{n} (\eta_{jk})^{3/2} \eta_j^k |\eta_j^k|^{1/2} + \frac{\alpha}{A_l^{(i)2}} + \pi_{HG} A_l^{(i)} + \pi_c A_c^{(i)} - \gamma A_{HG} \end{aligned} \tag{23}$$

Terms which depend on the second 'average' chain of the lipid are all constant for different conformations of the first chain. This is also true of the term $-\gamma A_{HG}$. Hence these terms do not affect the relative weight of those conformations and are not included in evaluation of the statistical weights.

Configuration (i) is given the Boltzmann statistical weight $w^{(i)} = \exp(-E^{(i)}/kT)$ and the partition function $Z = \sum_i \exp(-E^{(i)}/kT)$ is formed. $w^{(i)}/Z$ is the probability of configuration (i). The free energy G_{SYS} for a bilayer of m lipid molecules is given as:

$$G_{SYS} = H_{SYS} - \sigma\mathcal{A}$$
$$= -m(\langle E_{disp}\rangle + 3/2\langle F_{HG}\rangle + \pi_{HG}\langle A_I\rangle + \gamma A_{HG} + 2kT \ln Z) \quad (24)$$

where H_{SYS} is the Helmholtz free energy; σ is the experimental surface tension (0 dyn/cm), and $\mathcal{A}$ is the total area of the system. (The PV term is not included in the Gibbs free energy as little 'PV work' is done.) $\langle E_{disp}\rangle$ is the thermodynamic average of E_{disp} for a chain; $\langle F_{HG}\rangle$ and $\langle A_I\rangle$ are averages for a lipid molecule. A derivation of Eqn. 24 is given in Appendix 2.

The density of packing is assumed to be the same as for liquid hydrocarbons. Hence the volume of a CH_2 group is assumed to be 27.0 Å^3 and of a CH_3 group, 54.0 Å^3 [37]. The order at each level is then evaluated as the volume weighted mean of the order of all the segments at that level. These new orders are the starting point for the next iteration. The computer generates the configurations until the order parameters fed in at the beginning of the iteration are the same (to within required accuracy, see later) as those calculated at the end. This unique (i.e., independent of starting values), self-consistent solution represents the order parameter profile across the bilayer. The average order parameters for each group in the chain are also evaluated. The value of α chosen was that value which gave the best fit of order parameters along the chain with the data of Seelig and Seelig [38]. Both the Seeligs' experiments and the present calculations were done at $T = 41°C$. The thickness of the bilayer was taken as that value which gave the most even distribution of material across the bilayer, always assuming a CH_3 group requires twice the volume of a CH_2 group. The membrane was assumed to be symmetrical in all respects about the midplane.

Results

Fig. 2 shows a comparison between calculated and measured deuterium order parameters. The order parameters S_{CD} are calculated from η_j as described in [21] (where η_j is called S_{mol}). The value of α which gave this fit was $7.09 \cdot 10^6$ cal Å^4 per mol lipid. This corresponds to $\pi_c = 27.3$ dyn/cm and $\pi_{HG} = 22.7$ dyn/cm. The calculated order parameters of the first two or three segments are sensitive to the initial chain orientation. Three initial orientations of C-2 were chosen and are categorized by Schindler and Seelig [21]. No experimental data are available so we choose relative energies for these orientations which give a good fit to the deuterium order parameters for the first two segments. Using Schindler and Seelig's notation, details are given in Table I. Imagine adding two hypothetical CH_2 groups to the beginning of the chain. If

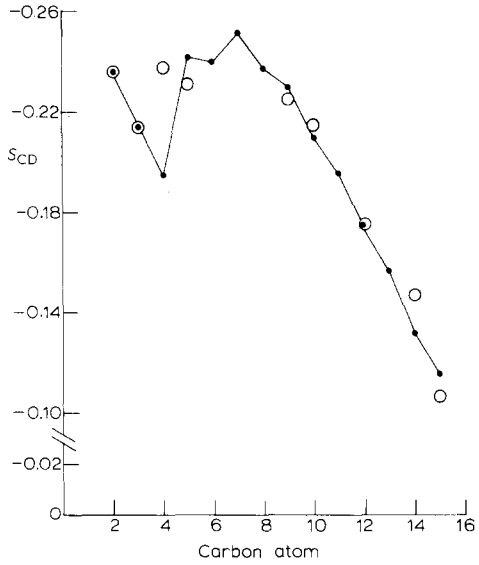

Fig. 2. Deuterium order parameters (S_{CD}) at 41°C along the lipid chain. •, calculated from the model. ○, data from Seelig and Seelig [38].

the first of these is oriented parallel to the bilayer normal the initial orientations of C-2 correspond to the sequences given in the penultimate column of Table I.

Fig. 3 shows the order parameter profile across the bilayer (i.e. at different depths rather than along the chain). The order parameter profile 'fed in' to the computer at the beginning of the final iteration is given as a dotted line. The circles represent the order parameters which emerge at the end of the iteration. As can be seen, the behaviour of the order parameter for the first few layers nearest the polar head is irregular (the order parameter at level zero is —0.125 as only the first segment of orientation III (Table I) occurs at this level). It was felt that the most reasonable approach was to average these order parameters. The dotted line represents this average value. The order parameters for the three layers nearest the centre were also averaged (dotted line). This second averaging, as can be seen, is a good approximation. It reduces the number of computer iterations necessary to find the self-consistent solution. The final result is obtained when the (averaged) order profile before and after an itera-

TABLE I

INITIAL CHAIN ORIENTATIONS

Notation	α (degrees)	β (degrees)	Conformation	Relative energies (cal/mol)
I	35.3	0	*tt/t*	0
II	35.3	60	*tt/g*	+175
III	90	60	*tg/t*	+500

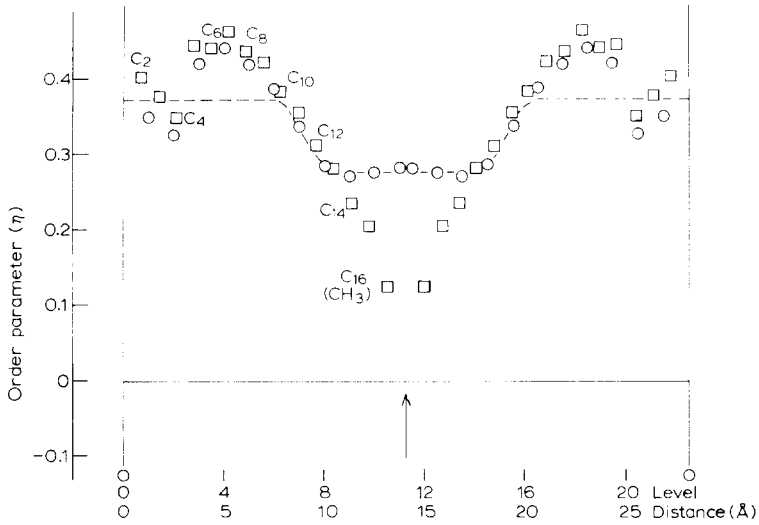

Fig. 3. Calculated order parameter profile across the bilayer. - - - - - -, order parameter profile across the bilayer. □, order parameters along the chain (drawn equally spaced from the interface to the centre). ○, order parameters which emerge after the final iteration (see text). The arrow marks the centre of the bilayer.

tion agree at all depths to within ±0.0002. The order parameters on the two sides are mirror reflections about the midplane.

Experimentally it has been found that the two chains of DPL are not physically equivalent [31]. The first carbon atoms of each chain are positioned at slightly different depths in the bilayer. The experimental order parameters of the first two segments of the chain are also different for the two chains. Both these effects will make a change to Fig. 3. However, as can be seen, the order parameter varies only slowly across the bilayer. Assume for example, that the first C atom of one chain is 1.9 Å further from the midplane of the bilayer than the other (as claimed in Ref. 31 for the gel state at 20°C and low water content). This would essentially lead to a superposition of two curves, out of phase by 1.9 Å. The result would be a curve similar to Fig. 3 (dotted line).

The calculation for Table II was based on evaluating the average value of |position of C_n − position of bilayer centre|. No account was taken of the difference between the two chains. This should introduce only small changes.

TABLE II

AVERAGE DISTANCE FROM BILAYER CENTRE

Data are in Å.

	Model (at 41°C)	Experimental (at 50°C) [31]
C-4	11.5	12.2 ± 1.5
C-5	10.5	10.5 ± 1.5
C-9	6.8	8.1 ± 1
C-14	3.0	3.6 ± 1
C-15	2.6	1.9 ± 1

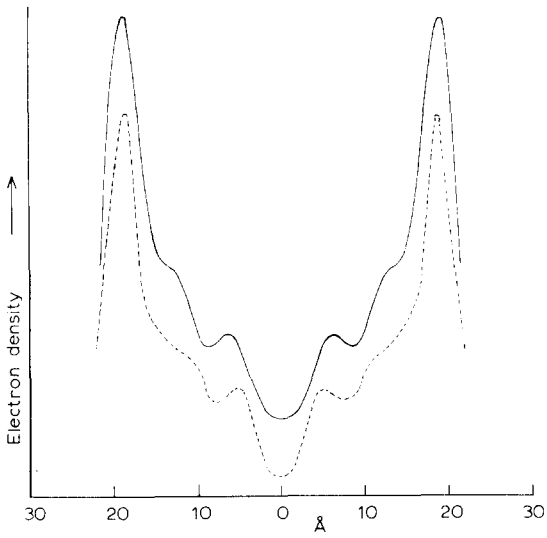

Fig. 4. Comparison of model and experimental electron density profiles. ———, model profile at 41°C. - - - - - -, experimental profile from Cain et al. [39] at 49°C and 8% hydration. The two curves are vertically offset for clarity. The origin of the horizontal scale is at the centre of the chain region.

In Fig. 4, a comparison is made between a theoretically calculated electron density profile and an experimentally determined one. To make the comparison meaningful, a headgroup was added to the model chains and water added to the headgroup region. The model used for the headgroup comes from Franks [40] and is model I in his notation. The two chains are out of step by 0.8 Å in this model. This is taken into account by 'attaching' atom C-31 (Franks' notation) at 0.4 Å nearer the bilayer centre than level zero (Fig. 3) and C-21, 0.4 Å further from the centre than level zero. The positions of all other atoms in the headgroup follow from this assumption. (If instead, the structure of the headgroup implied by Büldt et al. [31] had been used, the results would have been very similar. The importance of Fig. 4 is the comparison it allows between hydrocarbon regions, not headgroup regions.) It was further assumed that water penetrated to within 1.1 Å of level zero. The density of water was modelled by a triangle as shown in Fig. 5. This density profile is suggested by the results of a neutron diffraction experiment (on a somewhat different system) [41]. The model structure factors are evaluated from

$$F(h) = \sum_i f_i \cos(2\pi h x_i/D) \exp[-h^2 B/(4D^2)] \qquad (25)$$

where f_i is the atomic scattering factor for the i-th atom at coordinate x_i [42, 54]; B is analogous to the Debye-Waller temperature factor; h is the order number, and Σ_i denotes the sum over all atoms in a unit cell.

The value of B chosen was that value which (i) predicted the experimentally observed resolution, and (ii) gave best fit to the experimental curve. That value was $B = 310$. The magnitude of the eighth and subsequent model structure factors was smaller by a factor of greater than 3.8 than any preceding structure

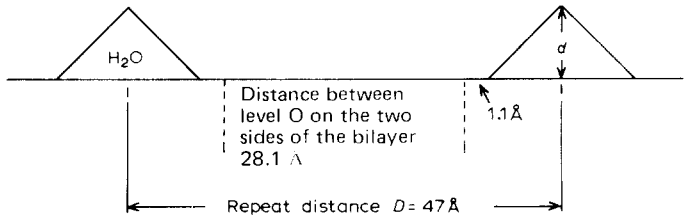

Fig. 5. Schematic water density profile. The value of D was chosen for convenience (large enough to resolve the peaks from headgroups attached to adjoining bilayers). The value of d was chosen to give a best fit to the model polar head electron density peak height of Fig. 4.

factors. (Thus, it would be expected that these orders would not be experimentally observed. They are not.) The model synthesis was achieved with seven orders and hence has resolution $D/h_{max} \approx 7$ Å as quoted in Ref. 39.

For both Table II and Fig. 4, the experiments and calculations were done at different temperatures and hydrations. An increase in temperature produces a small decrease in hydrocarbon thickness and, possibly, a small decrease in CH_3 localisation. A decrease in hydration produces closer headgroup packing [29] and, probably, and increase in order and in CH_3 localisation. The magnitudes of these effects while hard to quantify, should be small. If the experiments had been done under the same conditions as the calculations, there should be only minor changes to Table II and Fig. 4.

The thickness of the hydrocarbon region of the bilayer is assumed to be the

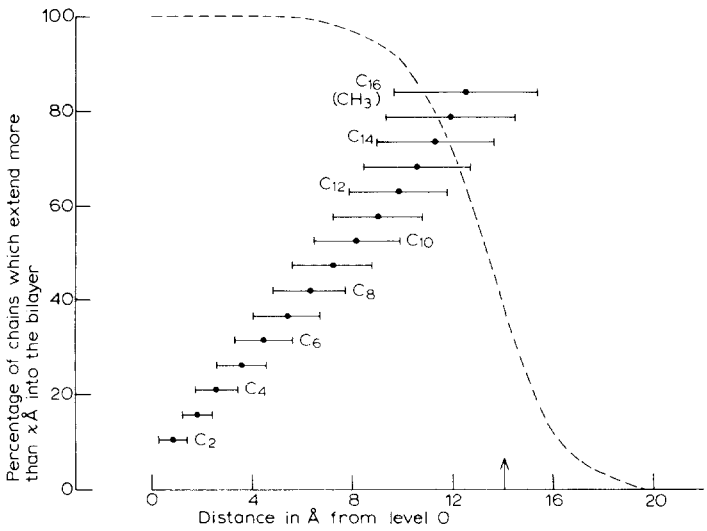

Fig. 6. Proportion of chains with headgroups on one side of the bilayer which extend further than x Å from level zero; distribution of chain segments in the bilayer, - - - - -, proportion of chains. ⊢—●—⊣, mean and S.D. of the position of C_n. The length of the bar is 2 S.D. (i.e. σ either side of the mean). The segments are shown equally spaced up the graph (the vertical scale refers only to the proportion of chains). The arrow marks the centre of the bilayer.

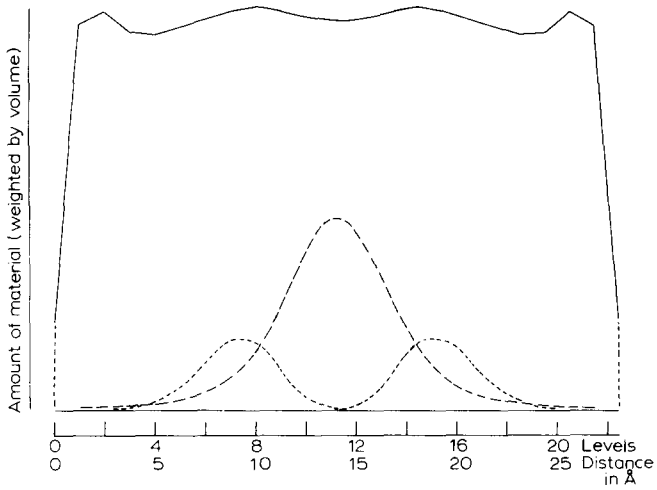

Fig. 7. Density of packing of the chains; distribution of C-11 and C-16 (CH_3) groups. ———, volume-weighted distribution of material across the bilayer. On the same scale, distribution of C-11 (- - - - -) and C-16 (— — —) groups.

average distance between the C-1—C-2 bonds on the two sides of the bilayer. The model thickness is 27.3 Å. Using the previously quoted volumes of CH_2 and CH_3 groups, this corresponds to an area/lipid of 63.3 $Å^2$ which, as discussed in Ref. 21, is in good agreement with experiments. The average number of *gauche* bonds/chain is 4.3, in agreement with earlier estimates [2,21,43]. The model predicts that 5.9% of the volume occupied by the chains whose head-groups are on one side of the bilayer is beyond the midplane. Approximately 38% of the chains extend beyond the midplane (see Fig. 6) and 0.6% of them are fully extended (all-*trans*). These three statistics give a measure of the amount of interdigitation and hence the extent to which the two sides of the bilayer can be thought of as independent.

To be a realistic theory, it is important that the chains pack in such a way as to fill up the space available to them without leaving voids or vacuum regions [44]. On the basis of segmental order parameters it might be expected that segments near the polar head could pack more closely than near the centre of the bilayer. Experimentally it is observed that there is a 4% density increase associated with the liquid-crystal to crystalline phase transition [43], where the average order of the bilayer goes from approx. 0.35 to approx. 1. The fact that the gel state has only a slightly higher density suggests that relatively small changes in order in different regions of the bilayer (see Fig. 3, dotted line) will produce much smaller changes in density. It is thus desirable, that the model predicts that the 'amount of material' (allowing for the relative volumes of CH_2 and CH_3 groups) is approximately contant at all depths. The S.D. of this quantity (plotted in Fig. 7) is 2.0% of its mean. This evenness of distribution and the agreement with experiment of the overall dimensions of the fluid chains is further evidence that the model is giving an accurate picture of the statistical distribution of the chain conformations.

Discussion

Summary of assumptions and results

The main assumptions needed in deriving the model are:

(i) the value of the parameter V;

(ii) the functional dependence of the headgroup free energy on area, and

(iii) the functional dependence of the van der Waals energy on the chain order.

Using these three assumptions and DMR data [38], the proportions of the experimental surface pressure supported by the headgroups and the chains are determined.

Of these assumptions, the first should be close to the truth, as it is based on independent experimental evidence. (There is, no doubt, a small error in the estimate of the number of *gauche* bonds in liquid hydrocarbon.) The second assumption, as already argued, is probably a best approximation. The model results are not very sensitive to this term and any form similar to Eqn. 14 would introduce little change. The third assumption is more arbitrary. The reasons for the form chosen (i.e. a power law with exponent 3/2) were given. They apply equally well for a (small) range of power laws *. Had an alternative power law within this range been used, the derived proportions of the surface pressure would have been different. Thus, the author would be sceptical of the exact numbers obtained for π_c and π_{HG}. However, given a slightly different formula linking order and energy and new derived π_c and π_{HG}, the statistical distribution of chain conformations is almost unchanged. (Using slightly different values of the parameters and different functional dependences, many computer iterations were completed. When π_c and π_{HG} were determined to give a good fit to the experimental order parameters along the chain, the other results were almost identical to those in the present paper. For example, assuming that we have overestimated the number of *gauche* bonds in liquid hydrocarbon by 5% leads to V = 730 cal/mol. Using this value of V introduces changes to the figures of the order of the thickness of the lines with which they are drawn.)

Confidence in the model should be based partly on the above, but mostly on comparison with experiments. Experimental results with which the model gives good agreement are:

(i) the order parameter profile along the lipid chain;

(ii) the dimensions of the bilayer (which is virtually assured once (i) is satisfied);

(iii) the proportion of *gauche* bonds in the chain;

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* To fit the experimental order parameters using a power law with a larger exponent would require a larger π_c. An exponent of 2.5 or larger requires that $\pi_{HG} < 0$, i.e. that the headgroups attract. There is a considerable amount of evidence, both theoretical [4,18,27] and experimental (see discussion of micelle formation together with experimental data in Tanford (Ref. 45, Chapters 6–12)) which suggests that this is not so. Nagle and Wilkinson's careful measurements [43] suggest that the volume per CH_2 group in liquid hydrocarbon and the DPL bilayer above T_c are very similar. Using their analysis, the present model predicts a volume difference of 0.2 $Å^3$ per CH_2 group. Decreasing the exponent would sharply increase this predicted difference. Further, it would require a very small value of π_c. This seems unlikely, given the substantial restrictions on the chains' movements. Within the necessarily simplistic framework of the model, the chosen power law seems reasonable.

(iv) the extent of the localisation of CH_3 groups (as measured by X-ray diffraction), and

(v) the average positions of the segments in the chain.

Finally, the model is internally consistent in that the chains pack neatly into the space available to them.

The packing of the chains

It is assumed that there is no collective tilt on any time scale. The experimental order parameter profile along the lipid chain (see Fig. 2) shows that the outer part of the chain (near the polar head) is relatively well ordered and the inner part (nearer the centre of the membrane) is increasingly disordered towards its end. The electron density profile across the bilayer (deduced from X-ray diffraction experiments, see Fig. 4) shows that the centre of the membrane is CH_3 rich. These two results led investigators [1,23,38] to regard the order profile down the lipid chain as an accurate measure of the order profile across the bilayer (i.e. at different depths).

With this identification, a problem arises as to how the chains pack. Without a collective tilt in the more ordered regions of the membrane, it seems that there must be a considerably smaller density of material in these regions than in the less well-ordered regions. This contradicts the intuitive notion that a more ordered region will be more tightly packed and hence more dense. (McConnell and McFarland [46] and Levine [47] made this point when analysing electron spin resonance data. It is also valid here.)

The present paper provides a possible answer to this problem. In Fig. 4 the sharpness of the central trough (which is very similar in the experimental and model profiles) is a measure of how strongly the CH_3 groups are localised. The most important conclusion to be drawn from this figure is therefore, that the model gives a good description of the delocalisation of the CH_3 groups. This fact, combined with the excellent fit which the model gives to deuterium order parameters (Fig. 2) is good grounds for having confidence in the order parameter profile across the bilayer (Fig. 3, dotted line). This profile demonstrates that the difference in order between regions, while noticeable, is considerably less pronounced than previously assumed. The order at the centre is 75% of the (averaged) order near the interface! As has been shown (Fig. 7), the chains pack very neatly into the space available to them, without any tendency for lower density in the outer regions. Thus the solution to the dilemma is that a tilt is not needed for uniform packing because the order in different parts of the membrane (as opposed to different segments in the chain) is similar.

On the basis of the above, a physical picture of the behaviour of the chains can be developed. Consider the formation of a *gauche* bond in the chain. For bonds near the polar head, this will cause a large proportion of the chain to be misaligned. Thus a kink (g^+tg^- or g^-tg^+ sequence) or a jog ($2g2$ jog $= g^{\pm}ttt g^{\mp}$) is more likely than an isolated *gauche*. Towards the terminal methyl group, a *gauche* bond can form without affecting a large proportion of the chain. This is true, regardless of the depth of the bond in the bilayer. (The model calculation reveals that the probability of a kink is larger for the more ordered segments at the beginning of the chain than for segments at the end (see also [21]). Thus for segments near the polar head, it takes considerably more energy to create a

given amount of disorder than for segments near the terminal methyl group. The profile of order along the chain seems understandable. To explain the profile at different positions in the bilayer requires simply that there is sufficient delocalisation of the segments so that a proportion of the more ordered ones sit in the centre of the bilayer and a proportion of the less ordered ones sit in the outer half of the bilayer. Figs. 6 and 7 show the calculated distribution of the groups. There is considerable delocalisation.

Seelig [23] has measured the order parameters along the saturated chain of 1-palmitoyl-2-oleoyl-3-*sn*-phosphatidylcholine. He finds that they are very similar in magnitude and trend to order parameters for DPL (the measurements are done at 19° C above the respective phase transition temperatures). Further, the extent of delocalisation of the CH_3 groups (as measured by electron density profiles of similar resolution to those shown in Fig. 4, see [48] for example) in a system with both saturated and unsaturated chains (of possibly different lengths) is very similar to that shown in Fig. 4. Hence, in such systems, the author would expect an order profile across the bilayer similar to Fig. 3 (dotted line).

Addition of considerable amounts of cholesterol produces a different picture of the bilayer. The presence of 33 mol% cholesterol alters the chain-ordering profile of egg phosphatidylcholine substantially [49]. The membrane becomes considerably less fluid. The chain segments are much more localised to particular depths in the bilayer. Hence, the order parameter profile across the bilayer should be given much more accurately by the order parameter profile along the chain. Thus, in the case of considerable amounts of cholesterol, the traditional view of a substantial 'ordering gradient' across the bilayer seems justified. For lipid bilayers without cholesterol, it does not.

Conclusion

The model calculation presented in the previous sections gives a good description of the behaviour of DPL above its phase-transition temperature. The model takes account, not only of the chain statistics, but also of the hydrocarbon/water interface and the headgroups. The excellent agreement with experiment gives confidence that the calculated order parameter profile across the bilayer is realistic. The traditional view, that this profile looks like the order parameter profile down the chain, is not supported. This has important consequences for the understanding of the lipid bilayer.

Appendix 1

To accomodate a lipid molecule in the bilayer, work must be done against the pressures acting. For a lipid in configuration (i) (with chain area $A_c^{(i)}$ and interfacial area $A_I^{(i)}$) requires an amount of work

$$W = \pi_c A_c^{(i)} + (\pi_{HG} - \gamma)\, A_I^{(i)} \tag{26}$$

(the chain area of the second 'average' chain has been ignored).

For internal consistency of the model, it is necessary that

$$\pi_{HG} = -\left(\frac{\partial F_{HGT}}{\partial \mathcal{A}}\right)_{V,T,m} \tag{27}$$

where F_{HGT} is given by Eqn. 15 in the text and $\mathcal{A}$ is the total area of the system of m lipid molecules ($\mathcal{A} = m\langle A_I \rangle = 2mA_{AVE}$).

We wish to evaluate the change in F_{HGT} for a small change in $\mathcal{A}$ at constant volume and temperature. There are two extreme assumptions which can be made; the true situation probably lies between them.

Assumption I: As the total area changes from $\mathcal{A}$ to $\mathcal{A} + \Delta\mathcal{A}$, the distribution of A_I values around their changed mean remains the same. This leads straightforwardly to

$$\pi_{HG} = \alpha\left\langle \frac{1}{A_I^3} \right\rangle \tag{28}$$

Assumption II: On increasing the total area, the spread of A_I values increases noticeably. For the sake of calculation, we assume the fractional increase in S.D. is equal to the fractional increase in the mean area: $\Delta\mathcal{A}/\mathcal{A}$.

If $f(a)$ was the original probability of finding a lipid with area 'a' (where $\Sigma_{\text{all areas}}\ a\ f(a) = 2A_{AVE}$); then $f(a)/[1 + \Delta\mathcal{A}/\mathcal{A}]$) is the new probability of finding a lipid with area 'a'. Evaluating the new free energy and taking the limit $\Delta\mathcal{A} \to 0$. Leads to

$$\pi_{HG} = \frac{\alpha}{\langle A_I \rangle}\left\langle \frac{1}{A_I^2} \right\rangle \tag{29}$$

Since the spread of A_I is small, these two results (for the pressure exerted by the headgroups, π_{HG}) are similar. We used the first.

After the calculation it is possible to estimate the changes which would result if the second formula is used. To fit the order parameter data, a small (less than 5%) increase in π_c and a small change in the relative energies of the initial segment are required. Other results are not affected.

Appendix 2

As defined in standard statistical mechanical texts, the entropy for a single chain

$$S = -k \sum_i p_i \ln p_i$$

$$= -k/Z \sum_i e^{-\beta E^{(i)}}(-\beta E^{(i)} - \ln Z) \tag{30}$$

where $E^{(i)}$ is defined in Eqn. 23. (As explained in the text, the term $-\gamma A_{HG}$ is not included.) Thus

$$TS = \langle E_{int} \rangle + \langle E_{disp} \rangle + \langle F_{HG} \rangle + \pi_c\langle A_c \rangle + \pi_{HG}\langle A_I \rangle + kT \ln Z \tag{31}$$

The energy per chain is

$$U = \langle E_{int} \rangle + 1/2\langle E_{disp} \rangle \tag{32}$$

The energy and entropy contributions of the headgroups and interface cannot be separated; however, the Helmholtz free energy/ lipid is

$$H_{HEAD} + H_{INTERFACE} = 1/2\langle F_{HG} \rangle + \gamma(\langle A_I \rangle - A_{HG}) \tag{33}$$

Thus, for a system of m molecules, the Helmholtz free energy H_{SYS} is

$$H_{SYS} = m(H_{HEAD} + H_{INTERFACE}) + 2m(U - TS) \quad (34)$$

Collecting terms gives the result in the text.

Acknowledgements

I thank D.A. Haydon and S.B. Hladky for the generous amount of time they spent with me discussing the ideas in this paper. Also, N.P. Franks for much help with the electron density model. The typing would never have been completed without J.A. Goldberg. I gratefully acknowledge the support of a Postgraduate Scholarship from Shell Australia Limited.

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