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A β-Coupled Gauche Kink Description of the Lipid Bilayer Phase Transition†

Meyer B. Jackson

ABSTRACT: A statistical-mechanical model for a lipid bilayer was built around the assumption that β-coupled gauche kinks are the only nonstraight hydrocarbon chain configurations allowed. Packing of these chains was considered and expressions for the energy and entropy are developed based on those considerations. A solution was obtained which was not exact but was still accurate enough to be useful. In addition, an intermolecular potential was formulated which includes the attractive and repulsive forces between the hydrocarbon chains, the polar group repulsions, and hydrophobic forces. Combining the solution to the kinking model and the intermolecular potential, we obtain the total free energy of the lipid bilayer, which exhibits a first-order phase transition at a characteristic temperature. The theoretical heat of transition indicates that kinks alone can provide enough disorder for the transition to occur. Other physical quantities can be calculated, including order parameters. The order parameters calculated here give a picture of chains which are rigid near the polar group and more fluid near the methyl group.

As a model for biological membranes, lipid bilayers have been studied extensively. Though biological membranes are often more than half protein, their lipid portions have structure similar to that of pure lipids in lipid bilayers (Wilkins et al., 1971). The gel to liquid-crystal transitions seen in isolated membranes are also seen in isolated lipids (Melchior et al., 1970), and purified lipids show a transition of approximately the same magnitude, only much sharper (Hinz and Sturtevant, 1972a). In order to learn what can influence this transition and to gain a conceptual understanding of its nature, there has been some theoretical study. Assumptions concerning the transition can be tested by deriving theoretical relations which depend on those assumptions and comparing them with experimental results.

A variety of physical techniques have characterized the transition as an order–disorder transition with major changes occurring in the hydrocarbon chains. X-ray diffraction shows that the chains are straight, parallel, and arranged in hexagonal lattice in the gel phase. Above the transition temperature, the hydrocarbon chains have lost this order (Wilkins et al., 1971). Electron spin resonance (Hubbell and McConnell, 1971) and nuclear magnetic resonance (Urbina and Waugh, 1974) also show that a substantial loss of hydrocarbon chain order occurs during the phase transition.

Some investigators have suggested that, above the transition temperature, many or most of the hydrocarbon chains have β-coupled gauche kinks in them (Seelig and Niederberger, 1974; Seelig and Seelig, 1974; Trauble and Haynes, 1971). Here a kink is two gauche C–C bonds separated by one trans bond in an otherwise straight chain (Figure 1). There has been no direct detection of these kinks in bilayers, so that one can only speculate as to their importance. In this study, a theory was developed on the assumption that all nonstraight chain configurations and all the disorder of the liquid crystal can be accounted for by kinks of this type. A model is constructed and its approximate solution is used as the hydrocarbon chain disorder contribution to the free energy of the membrane.

Other theoretical work on this subject includes studies by Nagle (1973), Marcelja (1974), Jacobs et al. (1975), and McCammon and Deutch (1975). Nagle has used lattice statistics to exactly solve a planar array of infinitely long chains. In this study, a detailed picture of the critical point may have

† From the Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06510. *Received December 15, 1975.* Supported by United States Public Health Service Grant GM-00711-18.

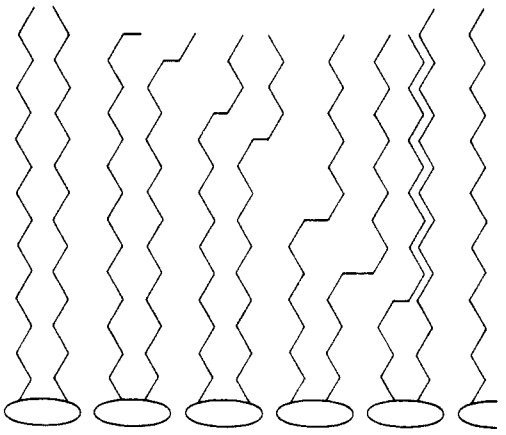

FIGURE 1: The nonstraight chains are in the β-coupled gauche kink configuration. The right-most kink is a forcing kink. Above the kink the chain is close to its neighbor. The other kinks are falling kinks which avoid close contact with neighbors by filling a vacancy created by a kinked neighbor.

been obtained by sacrificing many realistic features, since the chains are only about 20 Å long, not infinite, and a membrane has two dimensions perpendicular to the hydrocarbon chains, whereas Nagle deals with only one.

In a more realistic treatment, Marcelja (1974) has applied the Maier–Saupe theory to lipid bilayers where a barrier to tilting one chain relative to its neighbors takes the form $\frac{3}{2}\cos^2\theta - \frac{1}{2}$. This term represents the orientation dependence of the attractive forces. The development of a mean field theory then allows calculation of thermodynamic quantities. Intermolecular repulsions are not dealt with directly, and yet that is probably the most important constraint to the conformational freedom of a chain. The advantage of the approach is that all chain configurations can be considered. In an attempt to deal more directly with interchain repulsion, Jacobs et al. (1975) and McCammon and Deutch (1975) look at fewer configurations but include terms which represent the repulsive interactions. What follows can be considered a combined approach, where an interchain repulsion energy is formulated which has a great deal in common with the one used by Jacobs et al. Here, however, the idea of McCammon and Deutch is pursued by building a model around β-coupled gauche kinks. In this paper, the assumption that kinks are the dominant contribution to disorder is essential. It is intended that the work which follows should shed some light on the validity of that assumption.

The Model

An appealing feature of the β-coupled gauche kink is that it allows chains to be packed together like spoons in a close fashion with littler interchain interaction other than the van der Waals attraction (Figure 1). A kink in one chain creates a vacancy for a neighboring chain to kink into. Without a vacancy formed by a kinked neighbor, kinking a chain would require additional energy caused by interchain repulsion. In this way, we have an analogy to an Ising model where the energy required to kink a chain has two different values depending upon whether or not one of the neighbors is kinked. A chain which is kinked into a vacancy will be said to have a falling kink and a chain which kinks by pushing into neighbors is said to have a forcing kink. In Figure 1 we see that the right-most kink is a forcing kink and the others are falling kinks.

The energy difference between a gauche and trans bond for higher straight chain alkanes in the liquid state is 500 ± 100 cal/mol (Flory, 1969). Thus, the energy of a falling kink is 1 kcal/mol. There is no expression in the literature for the repulsive interaction energy of something like a forcing kink. If one considers the polar groups to be fixed in position, then the closer a kink is to the polar group, the larger the portion of the chain is which is pushed into its neighbors. This suggests that the energy is proportional to n, where n is the number of bonds from the terminal methyl group to the first gauche bond of the kink. If the chains are more closely packed, it becomes more difficult to create a forcing kink. The energy of a forcing kink should be very high for a membrane in the gel phase, but it should decrease if the membrane expands. The simplest expression which obeys these basic requirements is $nw/(A - A_0)$ where A is the area/chain on a plane perpendicular to the chain's axis, A_0 is the value of A in the gel phase, n is as described above, and w is an arbitrary proportionality constant to be adjusted to the experimental data. This expression has the correct qualitative features. Other expressions would do as well, but this one is chosen for its simplicity.

In order for falling kinks to form, forcing kinks must exist to create vacancies for them. A forcing kink can serve as a nucleating event allowing the formation of several falling kinks. We can then consider sequences of kinks such as in Figure 1, where falling kinks occur closer and closer to the terminal methyl group. One forcing kink at n can allow for as many as $n - 1$ falling kinks. Of course, the sequence need not be linear as depicted in Figure 1, but could weave around the membrane in a snakelike fashion.

The procedure will now be to calculate the free energy of a sequence of kinks initiated by a single forcing kink. Interactions between sequences of kinks will be ignored. This will cause a slight overcounting of states due to consideration of some states with chains kinked twice in the same place. Some of the energies will be incorrect since a forcing kink could have a kinked neighbor. These are not serious effects, and should not give rise to any major errors. It is appropriate at this point to mention that the cooperative tilting of straight chains with respect to the plane of the membrane, as seen in the gel phase, is not considered. In addition, interactions between the two opposing monolayers of the bilayer are ignored.

In considering a single sequence of kinks starting with a forcing kink at position n, we are now interested in its energy and in its disorder. To obtain the disorder of a single falling kink, one must consider a vacancy created by a chain kinked at position n. Any one of six chains around the vacancy (including the already kinked chain) could kink to fill the vacancy. This contributes a factor of six to the disorder of a kink. A kinked chain has its rotational freedom reduced by a factor between three and six. Furthermore, a bond has two gauche states compared to one trans state. This yields a disorder/kink of between two and four. This number will be called P. The disorder for a forcing kink is simply two, since the only important factor here is the two gauche bonds vs. one trans. Equating the disorder of a forcing kink with P should not result in significant error, since there are many more falling kinks then forcing kinks.

Looking again at a single vacancy created by kinking a chain at position n, a neighbor may or may not kink at position $n - 1$. If none of the neighbors kink at position $n - 1$, the same neighbors will have the opportunity to kink at position $n - 2$. If one neighboring chain does kink at $n - 1$, then this chain's neighbors will have a vacancy at $n - 2$. Filling or not filling a particular vacancy has no effect on the energy or disorder of the rest of the sequence of kinks, and so the probability of filling a vacancy is an independent quantity equal to $P \exp(-2E_b/kT)/[1 + P \exp(-2E_b/kT)]$, where E_b is the energy of a

gauche bond. This quantity will be denoted by the letter f and is the ratio of the number of kinks to the number of kinks plus the number of vacancies.

If we first consider a single sequence starting with a forcing kink at position n, the average number of falling kinks will be $(n-1)f$. The average energy of the sequence will then be $nw/(A-A_0) + 2f(n-1)E_b + 2E_b$. There is a contribution of $(f(n-1)+1)k \ln P$ to the entropy due to the disorder per kink.

Considering a system containing N hydrocarbon chains, we will then say that $J(n)$ is the number of sequences of kinks in that system which start with a forcing kink at position n. The fact that each sequence has $n-1$ vacancies with a varying number of kinks results in an additional contribution to the disorder. This contribution to the disorder can be determined if one considers a "level" in the membrane composed of the nth bond of every chain. One vacancy, filled or empty, is created at that level by each forcing kink at levels closer to the polar group than n. The number of filled plus empty vacancies at the nth level is then $\Sigma^m_{i=n+1}J(i)$. Thus, there are $f\Sigma^m_{n+1}J(i)$ falling kinks and $(1-f)\Sigma^m_{n+1}J(i)$ empty vacancies at the nth level. The total disorder from interchanging vacancies and falling kinks at the nth level is then $(\Sigma^m_{n+1}J(i))!/(f\Sigma^m_{n+1}J(i))!((1-f)\Sigma^m_{n+1}J(i))!$, giving a contribution to the entropy equal to $-k(f \ln f + (1-f)\ln(1-f))\Sigma^m_{n+1}J(i)$. Summing over all levels (since they are independent, it is permissible to sum) gives a contribution to the entropy of $-k(f \ln f + (1-f)\ln(1-f))\Sigma^m_{n=1}\Sigma^m_{i=n+1}J(i) = -k(f \ln f + (1-f)\ln(1-f))\Sigma^m_{n=1}(n-1)J(n)$.

The energy of the system is then

$$H = \sum_{n=1}^{m} J(n)\{nw/(A-A_0) + (n-1)f2E_b + 2E_b\} \quad (1a)$$

where m is the number of bonds in a chain at which kinks can occur. $P\Delta V$ is a small quantity for the transition, so it is not included in H. The configurational entropy is

$$\sum_{n=1}^{m} J(n)\{(n-1)(kf \ln P - fk \ln f - (1-f)k \ln(1-f)) + k \ln P\}$$

The total entropy should also include a term for the randomness in locations of the sequences among N chains equal to $k \ln (N!/\prod^m_{n=0}J(n)!)$. $J(0)$ is the number of chains without a forcing kink. This gives

$$G = \sum_{n=1}^{m} nJ(n)\{-kT \ln \epsilon\} + \sum_{n=1}^{m} J(n)\{-kT \ln f\} - kT \ln \frac{N!}{\prod_{n=0}^{m} J(n)!} \quad (1b)$$

where

$$-kT \ln \epsilon = \frac{w}{A-A_0} + 2E_b f - kTf \ln P + kT(f \ln f + (1-f)\ln(1-f)) = \frac{w}{A-A_0} - kT \ln(1 + Pe^{-2E_b/kT})$$

The model must obey the constraint $N = \Sigma^m{}_0 J(n)$. This is to say that all the chains with forcing kinks plus all the chains without forcing kinks equals N. The method of Lagrange multipliers can now be used to minimize G with respect to $J(n)$. The resulting $J(n)$'s are now the most probable values

$$J(n)_{n=1,m} = \frac{Nf\epsilon^n}{1 + f\frac{(\epsilon - \epsilon^{m+1})}{1-\epsilon}} \text{ and } J(0) = \frac{N}{1 + f\frac{(\epsilon - \epsilon^{m+1})}{1-\epsilon}} \quad (2)$$

Substituting these expressions for $J(n)$ back into eq 1b gives

$$G = -kTN \ln\left(1 + f\frac{(\epsilon - \epsilon^{m+1})}{1-\epsilon}\right) \quad (3)$$

For a given A and T, we can now calculate the contribution to free energy due to kinks.

The Intermolecular Potential

An expression for the intermolecular potential must be developed which can be added to the free energy of kinking already calculated to give the total free energy. The forces to be considered are: (1) the hydrophobic force, (2) the hydrocarbon attractive and repulsive forces, and (3) the polar group repulsion.

There is compelling evidence for believing that the hydrophobic free energy is proportional to the hydrocarbon surface area which is exposed to water (Herman, 1972). The expression $q(A-A_0)$ is then suggested (Tanford, 1974), with q being 33 cal/Å^2 as determined from analyzing solubilities of alkanes in water (Herman, 1972). The hydrocarbon surface area exposed to water should be roughly equal to $A-A_0$.

The hydrocarbon chains are held together by van der Waals forces. A quantum mechanical calculation has shown that the straight alkane chains parallel and close together have an inverse fifth power attractive potential (Salem, 1962). In a study of crystalline polyethylene, a 6-12 Leonard-Jones potential was used by summing pairwise over all CH_2 groups (Brandt, 1955). The two parameters of the Leonard-Jones potential were obtained by fitting to the heat of sublimation of straight-chain alkanes at 0 K and the distances between CH_2 groups of the crystal. This potential-energy function gave an excellent prediction of experimentally determined compressibilities at higher pressures.

The 6-12 potential becomes a 5-11 potential for chains. A potential of the form $U(r) = \epsilon(\sigma/r)^5 - (\sigma/r)^{11})$ was summed pairwise over chain-chain distances in a paraffin crystal. There are three constraints which must be obeyed by this potential energy. The forces in two directions perpendicular to the chains are zero at the observed interchain distances in a crystal, and the energy at the minimum is equal to minus the heat of sublimation at 0 K. The two parameters can be determined from any two of the constraints, and they vary by less than 3% depending upon the choice of the constraints. The average σ was 4.042 Å and ϵ was 0.7226 kcal/mol CH_2.

Paraffin crystals are orthorhombic, but the lipid bilayer has chains in a hexagonal array. Paraffin crystals undergo a transition to a hexagonal lattice as the temperature is raised. In the hexagonal phase, the chains become more widely spaced with an area/chain of about 19.5 Å^2 (Muller, 1932). In this hexagonal phase, a chain can rotate about its axis, thus making its effective radius larger, but leaving the attractive force relatively unchanged. A new potential was obtained by keeping the attractive term and adjusting a single parameter in the $1/r^{11}$ repulsive potential so that the minimum now appeared at 19.5 Å^2. The result, converting from interchain distances summed pairwise to area/chain in the lattice, is $-3670/A^{2.5} + 12389000/A^{5.5}$ kcal/mol CH_2. This potential, obtained from studies of paraffin, and valid for paraffin in a hexagonal lattice, should then be reasonable for the hydrocarbon chains of the lipid bilayer.

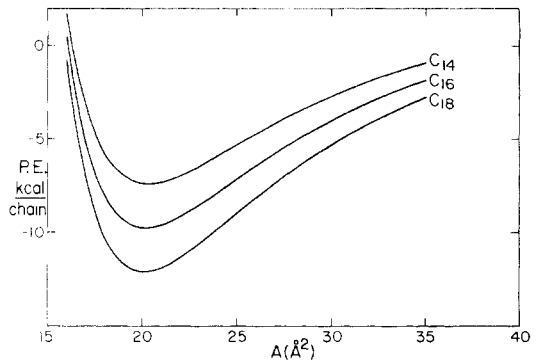

FIGURE 2: $U(A)$ is plotted vs. A for lipids with different hydrocarbon chain lengths. The minima represent the gel phases.

TABLE I: Properties of the Gel State.[a]

	A_0	$U(A_0)$
	$C = 160$	
C_{14}	20.300	−7.4450
C_{16}	20.182	−9.8023
C_{18}	20.095	−12.1656
	$C = 80$	
C_{14}	19.844	−11.4320

[a] Equation 4 was minimized for various chain lengths and for two values of C.

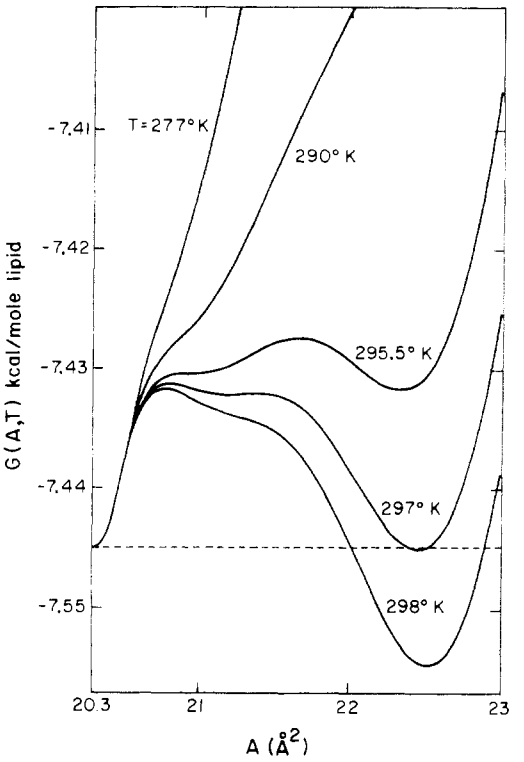

FIGURE 3: $G(A,T)$ is plotted vs. A for a variety of temperatures to illustrate the occurrence of the phase transition at the transition temperature. This is for a C_{14} lipid with $P = 3$ and $w = 0.7227$. w was adjusted to give a transition at 297 K. At the true transition temperature, $G(A)$ has a minimum which is tangent to the line $G = -7.445$.

The remaining force is the polar group repulsion. The simplest and oldest theory is the Gouy–Chapman ionic-double-layer theory (Overbeek, 1952). This theory gives C/A as the energy, where C is a function of the dielectric constant, ionic strength, and temperature. For a lipid with a zwitterionic headgroup (lecithin and phosphatidylethanolamine) C/A may still be justified because there would still be a double layer of charge formed by the two charges of the polar group. The constant C would be very hard to calculate directly, but its value must be such that the total potential of the membrane has a minimum at for the value of A seen in the gel phase, which is 20.3 Å^2 (Chapman et al., 1967). A potential of the form

$$U(A) = 13\left(\frac{-3760}{A^{2.5}} + \frac{12389000}{A^{5.5}}\right) + \frac{C}{A} + 0.033(A - A_0) \text{ kcal/mol hydrocarbon chain} \quad (4)$$

was used, where 13 is the number of hydrocarbon units in a 14 carbon fatty acid. $U(20.3\ \text{Å}^2)$ was a minimum with $C = 160$ Å^2 kcal/mol.

This potential energy function, though obtained in a roundabout empirical manner, is both simple and realistic. It has a minimum at the right place, and its shape and depth are reasonable. It is not valid for A large compared to A_0, due to penetration of water molecules in between chains. $U(A)$ is plotted in Figure 2 for C_{14}, C_{16}, and C_{18} phospholipids. The depths and areas are presented in Table I. Table I also shows the effect of lowering C to 80.

We now have a potential which includes all the important effects, and allows chain-length variation and qualitative polar group variation. The potential is completely determined without consideration of any properties of the phase transition. It can thus be applied to the phase transitions without any compromise.

The Nature of the Phase Transition

The total free energy of the membrane according to this kinking model can now be written down by combining eq 3 and 4. We now have $G(A,T)$. A as a function of T (and thus $G(T)$) can be obtained by minimizing G with respect to A for a particular value of T. In Figure 3 we see $G(A,T)$ plotted against A for several values of T. It can be seen that the minimum is $A = A_0$ for T less than a certain temperature. A new minimum appears at one temperature, indicating a phase transition which is first order.

The transition can be made to occur at a wide range of temperatures by varying w or P. P should be between 2.0 and 4.0. w is adjusted (for a value of P) so that a lipid with C_{14} fatty acids has a transition at 297 K, which is the transition temperature of dimyristoyllecithin (Hinz and Sturtevant, 1972a).

It is of interest that, in the plot of G vs. A, G does not get very high between the two minima. This suggests that under certain conditions the transition can be second order or continuous. It has been observed that certain lipid preparations from *E. coli* have distinctly second-order characteristics, as indicated by differential scanning calorimetry (Jackson and Sturtevant, 1975).

It may seem mysterious that this model can show an isothermal transition since these sequences of kinks could not include many hydrocarbon chains, and so they could not function as large cooperative units. The reason that this theory gives rise to a cooperative phase transition is that local fluctuations in the value of A are not allowed. This is equivalent to assuming that a cooperative unit includes all the molecules of a single face of a bilayer.

TABLE II: Properties of the Phase Transition.

	T_{trans} (K)	ΔH (kcal/mol	A^a
	$P = 3, w = 0.7227, C = 160$		
C_{14}	297	6.37	22.46
C_{16}	305	9.68	22.64
C_{18}	311	13.10	22.76
	$P = 3.5, w = 0.8291$		
C_{14}	297	8.55	22.88
C_{16}	304	12.12	23.02
C_{18}	309	15.78	23.11
	$P = 2.5, w = 0.6231$		
C_{14}	297	3.39	21.81
C_{16}	307	6.87	22.18
C_{18}	314	10.08	22.36
	$P = 3, w = 0.7227, C = 80$		
C_{14}	311	7.55	21.96
	Experimental Values (Hinz and Sturtevant, 1972a)		
C_{14}	297	6.26	
C_{16}	315	9.69	
C_{18}	331	10.84	

[a] Of the fluid state at T_{trans}.

Calculation of Physical Quantities

The enthalpy due to kinking can be obtained by substituting eq 2 into eq 1a, yielding:

$$\frac{H}{N} = \left(\frac{w}{A - A_0} + 2fE_b\right) f \frac{\left(\frac{\epsilon - \epsilon^{m+1}}{(1-\epsilon)^2} - \frac{m\epsilon^{m+1}}{1-\epsilon}\right)}{1 + f\frac{(\epsilon - \epsilon^{m+1})}{1-\epsilon}} + (1-f)2E_b f \frac{\frac{\epsilon - \epsilon^{m+1}}{1-\epsilon}}{1 + f\frac{(\epsilon - \epsilon^{m+1})}{1-\epsilon}}$$

This plus $U(A)$ (obtained from eq 4) times 2 (two fatty acids/lipid) gives the energy as a function of temperature. H does not change with temperature below the transition, so ΔH is the difference between $H(A',T')$ and $H(A_0,T')$ when T' is the transition temperature and A' is A at the new minimum in $G(A,T')$.

The change in transition temperature with chains of different lengths or with different polar groups is obtained by changing m or C in $G(A,T)$ and finding the temperature of transition with w already determined from the previous adjustments.

Table II shows calculated physical quantities for $P = 2.5$, 3.0, and 3.5. Experimentally determined quantities are included for the sake of comparison. It can be seen that for $P = 3.0$ the enthalpies are quite good. The temperatures of transition increase with chain length, but not by as much as they should. Table II also shows the results obtained when C is lowered to 80.

The value of A above the transition temperature is difficult to measure due to the difficulty in interpreting x-ray diffraction patterns of disordered systems. A maximum in scattering intensity is seen at 4.6 Å (Chapman et al., 1967). This would correspond to an interchain distance of 5.31 Å in a hexagonal lattice, producing a value of A equal to 24.4 Å^2. According to a theory of Warren (1933) the x-ray diffraction pattern produced by liquid paraffin has a maximum in intensity at a distance which is 8% lower than the actual interchain spacing. This would mean that the chains are 5.00 Å apart and A = 21.65 Å^2. Our calculated value for A in a C_{14} lipid, with P = 3, is 22.4 Å^2, which is too high but not alarmingly so in light of the difficulty in interpreting x-ray diffraction data of disordered systems.

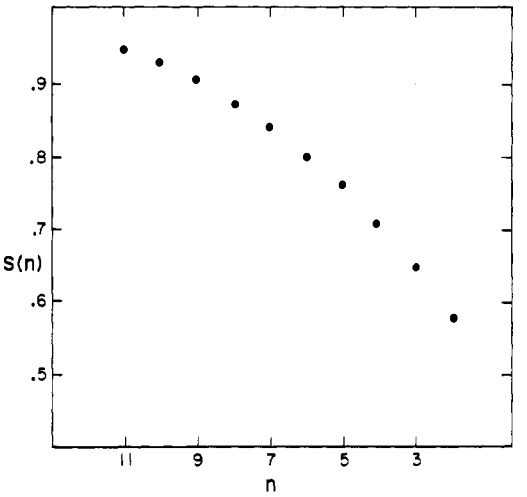

FIGURE 4: $S(n)$, the order parameter, is plotted against n, the number of the CH_2 group, counting from the terminal methyl group. n decreasing to the right is plotted for the sake of comparison with experimentally determined order parameters (Hubbell and McConnell, 1971; Seelig and Seelig, 1974) which are plotted as increasing distance from the carboxyl group, to the right.

The order parameter in a liquid crystal is $S = \langle \frac{1}{2}(3\cos^2\theta - 1)\rangle$, where θ is the angle between a molecule's axis and some fixed axis. (The fixed axis is the perpendicular to the bilayer surface, and the molecular axis at a particular CH_2 group is the perpendicular to the plane formed by the three atoms of that group.) A kink gives rise to $\theta = 60°$ for the two adjacent CH_2 groups at the kink. Using eq 3.2 one can calculate the number of kinks at each position along the chain and thus predict the order parameter. $S(n)$ for the 14 carbon chain lipid is plotted in Figure 4. It is close to one for high values of n and decreases when n becomes small. This indicates that the chains are rigid and unbent near the carboxyl group and disordered near the methyl group.

Discussion

Before evaluating the success of the model in describing the phase transition of a lipid bilayer membrane, it is worthwhile to summarize the important approximations and other elements which could compromise the validity or accuracy of the calculations.

It is an order-disorder theory neglecting contributions of molecular motion to the free energy. Conformations of the hydrocarbon chain other than the β-coupled gauche or the straight chain are ignored. The interactions between different sequences of kinks are ignored. The polar groups and thus the bases of the chains are regarded as fixed, and tilting of the entire chain with respect to the normal to the surface of the bilayer is not considered. A simple functional form is assumed for the energy of a forcing kink which increases proportionally with n and is inversely proportional to $A - A_0$. Two parameters are not known precisely. w is completely arbitrary and P has 2 and 4 as its lower and upper bounds.

In comparing the heats of transition calculated for $P = 3$, agreement with experiment is good. The significance of this agreement is that β-coupled gauche kinks alone, packed in this manner, give enough disorder so that $T\Delta S$ is comparable to the measured value of ΔH. This does not prove that other configurations do not contribute, but it does demonstrate that kinks alone are enough.

An essential feature proposed in this paper is the packing scheme of kinks. A forcing kink is a nucleating configuration with high energy which creates a vacancy for another kink and thus a sequence of falling kinks. The idea of forcing and falling kinks has some impact upon the interaction of other molecules with membranes. Proteins and especially cholesterol can be seen as forming vacancies for hydrocarbon chains to fall into. The membrane would then have permanent vacancies which are basically independent of temperature. The rest of the chains would melt with a lower enthalpy. This is similar to what is observed with cholesterol (Hinz and Sturtevant, 1972b).

In Table I we see that if C of the potential energy expression is cut in half, simulating a decrease in polar group repulsion, A_0 decreases to 19.84 Å^2 and the value of $U(A_0)$ decreases to -11.43. In Table II we see that this then results in increasing the transition temperature of a C_{14} lipid to 310.6 K and the ΔH to 7.55 kcal/mol. This qualitatively explains results concerning the change in charge of the polar group (Jacobson and Papahadjopoulos, 1975).

The order parameters calculated in the liquid-crystal phase can be compared to experimental order parameters obtained from spin-resonance experiments. There is some controversy, however, with Hubbell and McConnell (1971) claiming that the chains are nearly rigid near the carboxyl group, with order diminishing gradually as the methyl group is approached. Seelig and Seelig (1974) claim that an intermediate but uniform degree of order exists along nearly two-thirds of the chain with a sudden decrease in order near the methyl group. The results of this calculation favor the first picture.

Much of what is concluded here depends on how reliably P, the disorder/kink, is estimated. A crucial step in determining P is a crude estimation of how much rotational freedom a chain loses when it kinks. If P is lower than 3, then the model does not provide enough entropy, and if P is greater than 3, the model has too much entropy. The results for $P = 2.5$ and 3.5 show that reasonable variation in P does not produce drastic variations in the predictions of the model, but they are nevertheless significant.

Quite apart from the model presented here of configurational disorder of the chains, a potential energy function was developed which is practical and can be used with any other model for chain disorder just as well. It allows quantitative study of the influence of chain length, and qualitative study of the influence of polar group variation. Predictions are made concerning the variation of A_0 in the gel phase with chain length and polar group which could be tested with very accurate x-ray measurements.

It is interesting to examine the different contributions to the ΔH of the transition. For $P = 3$, C_{14}, and $w = 0.7227$ (as in Table II), $\Delta U(A) = 1.25$ kcal/mol. Of the remaining 5.12 kcal/mol (the total ΔH is 6.37 kcal/mol) 1.28 kcal/mol can be attributed to the interchain repulsions of forcing kinks, and 3.84 kcal/mol is due to trans to gauche rotations. There are slightly less than two kinks/chain.

The recent appearance of two papers, which are similar in spirit to this one, merits some special discussion (Jacobs et al., 1975; McCammon and Deutch, 1975). The interchain repulsion developed by Jacobs et al. bears strong resemblance to the $nw/(A - A_0)$ term used here. It is interesting to note that by different arguments we both come to the conclusion that the interaction energy should be proportional to n, the number of bonds between the gauche bond closest to the methyl group and the methyl group. However, the energy is considered by Jacobs et al. to be work done against a dense gas of hard disks. The energy is here considered to be work done against the soft molecular repulsive forces of neighbors which are less mobile than the molecules of a dense gas or liquid.

McCammon and Deutch (1975) deal with kinks and a more liquid-like disorder near the methyl group. Their assumption that molecules of a certain configuration have neighbors of the same configuration is not in agreement with the packing considerations made here. There is a serious undercounting of states owing to a failure to distinguish between chains with the same number of kinks, but with those kinks in different places along the chain. This is why the ΔH's that this theory predicts are so low.

Except for Nagle's work (Nagle, 1973), none of the theories discussed here deal directly with the influence that the configurations of one chain has on the configurational freedom of its nearest neighbors. The formulation of a statistical counting problem which deals specifically with the packing of kinked chains is thus unique.

It has recently been shown in a nuclear-spin-relaxation study that most of the fast anisotropic motion can be attributed to β-coupled gauche isomerization (Gent, M. P. N., and Prestegaard, J. H., personal communication). This study provides additional support for the proposal that the β-coupled gauche conformation is the dominant contribution to the disorder of the fluid state.

Conclusion

Within the limitations imposed by the approximations made, the model explains the experimental results fairly well. This would then support the assumptions made about β-coupled gauche kinks and the way in which they pack. Sharp disagreement between the results calculated and experiment would have made those assumptions very unlikely. The approach taken here is very different from other theoretical studies of the same problem and can be regarded as another alternative. It is important to look at complicated systems in many different ways and this model, taken as a new perspective, has provided some new insight into the nature of the lipid bilayer phase transition.

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Outer Membrane of *Salmonella typhimurium:* Accessibility of Phospholipid Head Groups to Phospholipase C and Cyanogen Bromide Activated Dextran in the External Medium†

Yoshiyuki Kamio and Hiroshi Nikaido*

ABSTRACT: Whole cells of *Salmonella typhimurium* were treated with *Bacillus cereus* phospholipase C or with CNBr-activated dextran. If phosphatidylethanolamine head groups are exposed and accessible on the outer surface of the outer membrane of these cells, it was expected that these groups would be hydrolyzed by the former agent, and become covalently coupled to the latter agent. With strains producing lipopolysaccharides of S or Rc type, results did not indicate the presence of any accessible head groups on the outer surface. In contrast, with strains that produce outer membranes containing less complete lipopolysaccharides (Rd or Re type) and reduced amounts of proteins, both methods clearly showed the presence of exposed phosphatidylethanolamine head groups. These data can be most easily explained by assuming that the outer membrane of S and Rc strains either contains all phospholipid molecules in its inner leaflet or has proteins that completely cover up the head groups at its outer surface. In either model, the reduction in the amount of outer membrane proteins in Rd or Re mutants would produce membranes with exposed phospholipid head groups. CNBr-activated dextran can be easily prepared, and reacts with high efficiency under near-physiological conditions. Its additional advantage as a nonpenetrating membrane-labeling reagent is that we can be quite confident on its impermeability because of its size, in contrast with most other reagents whose presumed impermeability is dependent only on the presence of charged groups.

The cytoplasm of gram-negative bacteria is enclosed by two membranes (Glauert and Thornley, 1969). The inner membrane corresponds to the cytoplasmic membrane, and the outer membrane, together with the underlying peptidoglycan layer, comprises the cell wall. The outer membrane is similar to many other biological membranes in that it contains phospholipids and proteins and that it gives a typical "unit membrane" profile in thin sections. The outer membrane also contains a third component, lipopolysaccharide (LPS).[1] LPS was shown to form mixed bilayers with phospholipids (Rothfield and Horne, 1967), and these results led to the hypothesis that the outer membrane is basically a mixed LPS–phospholipid bilayer with intercalated protein molecules (Schnaitman, 1971; Nikaido, 1973; Costerton et al., 1974).

Other results suggest, however, that the outer membrane is very different from phospholipid bilayer-type membranes. Specifically, both phospholipid bilayer model membranes (Mueller and Rudin, 1969; Bangham, 1972) and plasma membranes (Collander and Bärlund, 1933) allow rapid passive diffusion of hydrophobic substances, but the outer membranes of wild type *Salmonella* or *Escherichia coli* cells apparently act as penetration barriers against hydrophobic compounds (reviewed in Nikaido, 1973; see also Nikaido, 1976). Furthermore, this barrier property is influenced by the nature of LPS produced: outer membranes of "deep rough" mutants

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[1] Abbreviations used are: LPS, lipopolysaccharide; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; P_i, inorganic phosphate.