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Deuterium Order Parameters in Relation to Thermodynamic Properties of a Phospholipid Bilayer. A Statistical Mechanical Interpretation†

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ABSTRACT: The physical properties of bilayers of dipalmitoyl-3-*sn*-phosphatidylcholine are analyzed in terms of a statistical model proposed by Marčelja (S. Marčelja (1974), *Biochim. Biophys. Acta 367,* 165). The model is used to calculate the segmental order parameters of the hydrocarbon chains, the transition temperature of the crystalline → liquid crystalline phase transition, the entropy change of the transition, the bilayer thickness, and the linear thermal expansion coefficient. The theoretical predictions are in excellent agreement with experimental results obtained by deuterium magnetic resonance, differential scanning calorimetry, and X-ray diffraction. The model yields the probabilities of trans and gauche conformations and also those of more specific conformational defects like kinks or jogs.

Two recent developments in the understanding of lipid bilayers have initiated this work. Firstly, Marčelja has presented a theory for the chain ordering in lipid bilayers, which takes into account all physical forces contributing to the bilayer stability. The theory is based on a mean molecular field model, an approach which has proved extremely successful for the understanding of nematic and smectic liquid crystals (Marčelja, 1974a,b). Secondly, it has been possible to determine by means of deuterium magnetic resonance (^{2}H NMR) the chain ordering in lipid bilayers with high precision and without perturbing the bilayer structure (Charvolin et al., 1973; Seelig and Niederberger, 1974a,b; Seelig and Seelig, 1974a,b; Stockton et al., 1974). Marčelja has applied his model to the deuterium results from a soap-like bilayer (Marčelja, 1974b). The purpose of this work is to discuss the Marčelja model for bilayers of dipalmitoyl-3-*sn*-phosphatidylcholine. This bilayer shows a closer structural relationship to biological systems than soap-like bilayers and has the advantage that the thermodynamic properties and overall dimensions are well-known by differential scanning calorimetry and X-ray diffraction experiments. Our approach will be to fit the experimental deuterium magnetic resonance data by choosing appropriate parameters for the Marčelja model. Using the same set of parameters it is then possible to calculate the transition temperature for the gel → liquid-crystalline phase transition, the transition entropy of the melting process, and the bilayer thickness, and to compare these data with the corresponding experimental results. The theory will also provide information about so far unknown quantities like the probabilities of trans, gauche, kink, and jog conformations.

Theory

According to Marčelja the energy of a hydrocarbon chain with the configuration (i) in a bilayer is given by

$$E^{(i)} = E_{\text{int}}^{(i)} + E_{\text{disp}}^{(i)} + \gamma A^{(i)} \tag{1}$$

The first term, $E_{\text{int}}^{(i)}$, represents the *intramolecular* energy of the chain configuration (i) and is evaluated using the well-known rotational isomeric model (Birshtein and Ptitsyn, 1966; Flory, 1969). Each carbon–carbon bond can take up either a trans (t) or a gauche conformation (g^+, g^-), in which the latter is higher in energy by E_g = 500 cal/mol (cf. Flory, 1969). The rotations about the various bonds are not independent and sequences like g^+g^- and g^-g^+ are forbidden for steric reasons even in the free chain.

The second energy contribution, $E_{\text{disp}}^{(i)}$, arises from *intermolecular* van der Waals attractions between neighboring hydrocarbon chains. For rigid molecules in nematic

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[1] It might be argued that due to the covalent bonding of two fatty acyl chains to the same polar group the motional freedom is greatly inhibited, at least near the polar region. The deuterium NMR measurements clearly demonstrate that such an effect—if it exists—is restricted to the C-2 atoms but is no longer observable from C-3 onward. Furthermore it must be seriously questioned if the polar region is rigid at all. In any case this problem will only affect the initial orientations of the chains but will not alter the essential conclusions of this work.

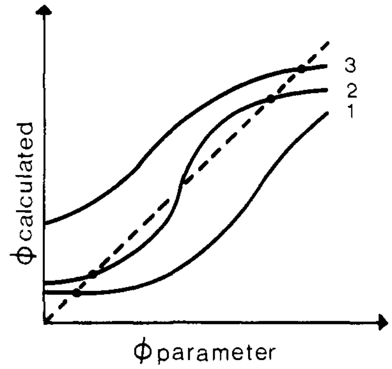

FIGURE 1: Three solutions to the self-consistent field equation (schematic). (1) High temperature ≙ liquid crystalline phase. (3) Low temperature ≙ crystalline phase. (2) Intermediate temperature ≙ phase transition.

liquid crystals this dispersion energy has been found to be (Maier and Saupe, 1958)

$$E_{\text{disp}} = -\phi(1/2)(3\cos^2\beta - 1) \qquad (2)$$

where β is the angle between the long molecular axis and the director axis of the nematic domain. ϕ is the molecular field, which may be visualized as a measure of the strength of interaction between the rod-like molecules. Marčelja has extended eq 2 to include also flexible hydrocarbon chains:

$$E_{\text{disp}}{}^{(i)} = -\phi(n_{\text{tr}}{}^{(i)}/n)\sum_{j=1}^{n}(1/2)(3\cos^2\beta_j{}^{(i)} - 1) \qquad (3)$$

Here β_j denotes the angle between the bilayer normal and the direction of the jth chain segment, which is defined as the normal on the plane spanned by the two C-H bond vectors. The summation is performed over all segments ($j = 1, \ldots, n$). $(n_{\text{tr}}{}^{(i)}/n)$ is the fraction of carbon-carbon bonds in the trans conformation.

The molecular field, ϕ, in turn depends on the average order in the lipid bilayer

$$\phi = (V_0/n)\left\langle (n_{\text{tr}}{}^{(i)}/n)\sum_{j=1}^{n}(1/2)(3\cos^2\beta_j{}^{(i)} - 1)\right\rangle \qquad (4)$$

where the angular brackets indicate the average over all chain configurations (i). V_0 is the so-called coupling constant, which enters as a free parameter in the theory. An upper limit for V_0 is 680 cal/mol, which is the melting energy per CH_2 unit of crystalline polyethylene (Schrader and Zachmann, 1970). In our calculations the best fit of the experimental data was obtained with V_0 = 590 cal/mol.

It should be pointed out that in the present model the molecular field is assumed to be constant throughout the bilayer. This does not affect the calculation of average bilayer properties like the bilayer thickness or transition entropy, but introduces some error in the calculation of the conformational probabilities at a given segment position j. A more refined model should therefore consist of a system of n coupled equations (3) with n different molecular fields ϕ_n. This problem is not yet solvable within reasonable limits of computer time.

The third energy term, $\gamma A^{(i)}$, takes into account various bilayer forces like steric repulsions, electrostatic interactions, and hydrophobic effects. $A^{(i)}$ is the effective cross-sectional area of a given chain configuration (i) and is approximated according to

$$A^{(i)} = A_0 l_0 / l^{(i)} \qquad (5)$$

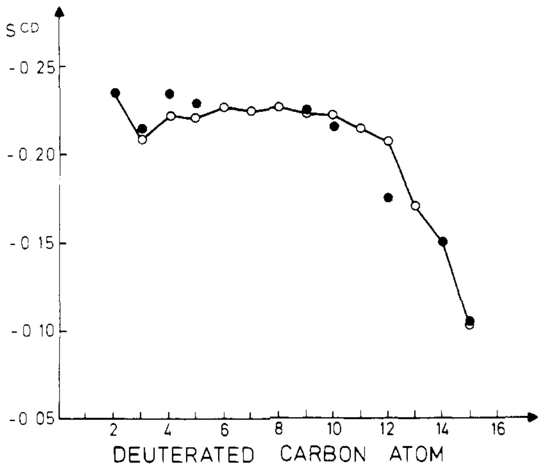

FIGURE 2: Comparison between calculated and experimental order parameters at 41°. (●) Experimental results; data taken from Seelig and Seelig (1974b).

Here l_0 is the length of the hydrocarbon chain in the extended all-trans configuration with a cross-sectional area A_0 = 20.4 Å^2 (Tardieu et al., 1973). $l^{(i)}$ is the effective length of the configuration (i), that is the projected length on the bilayer normal. The factor γ in the thermodynamic energy term $\gamma A^{(i)}$ may be called lateral pressure or surface energy. It arises from hydrophobic forces which are balanced by steric and electrostatic repulsions between the hydrocarbon chains. A simple and by no means rigorous model of Parsegian predicts γ to be 18-20 dynes/cm for various soap-like bilayers (Parsegian, 1966, 1968). It is therefore reassuring that independent of these calculations the best fit of the experimental data for bilayers of dipalmitoyl-3-*sn*-phosphatidylcholine using the Marčelja model is obtained for γ = 18.5 dynes/cm. The rather precise agreement may be fortuitous, but it gives at least confidence in the order of magnitude of the surface energy.

The statistical weight $w^{(i)}$ of the configuration (i) is

$$w^{(i)} = \exp(-E^{(i)}/RT) \qquad (6)$$

The partition function Z is the summation over all configurations (i)

$$Z = \sum_i \exp(-E^{(i)}/RT) \qquad (7)$$

With these definitions the molecular field is calculated according to eq 4

$$\phi = (V_0/n)\sum_i\left[(n_{\text{tr}}{}^{(i)}/n)\exp(-E^{(i)}/RT)\times \sum_{j=1}^{n}(1/2)(3\cos^2\beta_j{}^{(i)} - 1)/Z\right] \qquad (8)$$

Since $E^{(i)}$ in itself is a function of ϕ the self-consistence condition is to find such values of ϕ which when inserted in the right side of eq 4 yield the same value of ϕ on the left side. This is illustrated schematically in Figure 1 for three different temperatures. The abscissa denotes the ϕ values which have been inserted as parameter into eq 8, while the ordinate denotes the result of the calculation. Solutions to eq 8 are only those molecular fields where $\phi_{\text{calculated}} = \phi_{\text{parameter}}$, that is, all points which fall on the straight line in Figure 2.

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[2] In these calculations the effective length of the terminal methyl group is assumed to be 2.2 Å. Previously only the effective length of a carbon-carbon bond (1.25 Å) was taken into account (Seelig and Seelig, 1974b) and this explains the small difference compared with the present result.

Table I: Initial Chain Orientations.

Notation	α (deg)	β (deg)	Conformation	Relative Energies (cal/mol)
I	35.3	0	tt/t	0
II	35.3	60	tt/g	−75
III	90	60	tg/t	+410

At high temperatures (curve 1) or low temperatures (curve 3) only one solution to eq 8 is obtained. These correspond to the liquid-crystalline and the crystalline state of the bilayer, respectively. For intermediate temperatures (curve 2) several solutions to eq 8 are obtained and the theory predicts a first-order phase transition.

Knowing the statistical weights $w^{(i)}$ and the probabilities $p^{(i)} = w^{(i)}/Z$ of the various chain configurations (i) the *average* chain conformation and other molecular averages can be calculated in the usual manner.

Results and Discussion

Deuterium Order Parameters. Figure 2 shows a comparison between calculated and measured deuterium order parameters for bilayers of dipalmitoyl-3-*sn*-phosphatidylcholine at 41°. The numerical calculations are based on the following set of parameters: bond rotation potential $E_g = 500$ (400) cal/mol; coupling constant $V_0 = 590$ (680) cal/mol; surface energy $\gamma = 18.5$ (25) dynes/cm. Perfect tetrahedral symmetry of the carbon atom bond angles was assumed in all computations. In brackets we have included the corresponding parameters for the soap-like bilayer (based on a bond angle of 112.5°; Marčelja, 1974a,b).

The calculated order parameters of the first two or three segments are rather sensitive to the initial chain orientation (1). Since no experimental data are available we have performed computer calculations to determine the chain energy as a function of the first segment orientation. In Table I we have listed those orientations which were found to correspond to minima in the average chain energy. The initial orientations are specified by two angles: α is the angle between the bilayer normal and the CO-CH_2 bond vector, while β denotes the segment direction, i.e., β is the angle between the bilayer normal and the normal on the plane spanned by the two C-H bond vectors. The three initial orientations in Table I are energetically favored because they fit exactly into the rotational isomeric scheme and allow consecutive segments to be oriented parallel to the bilayer normal. The connection with the rotational isomeric model is easily established by adding two hypothetical CH_2 groups to the beginning of the chain. If the first of these segments is oriented parallel to the bilayer normal the initial orientations correspond to the sequences given in the penultimate column of Table I.

The experimental data in Figure 2 can only be fitted by assuming a fast equilibrium between *all three* initial orientations. Using a least-squares treatment the energies of orientations II and III relative to I as the zero reference state are found to be −75 and +410 cal/mol, respectively. The predominant contributions to explain Figure 2 are therefore due to states I and II, which are connected by a rotation of 120° around the CO-CH_2 bond vector.

Experimentally it has been found that the two fatty acyl chains in bilayers of dipalmitoyl-3-*sn*-phosphatidylcholine are not physically equivalent (Seelig and Seelig, 1974b). The most dramatic differences were found for the C-2 position of the deuterated fatty acyl chains, which gave rise to three different ^{2}H NMR signals (Seelig and Seelig, 1974a). An assignment of these resonances has become possible by synthesizing dipalmitoyl-3-*sn*-phosphatidylcholine selectively deuterated in only one of the two palmitic acyl chains. (A. Seelig, unpublished results). On the basis of these experiments, the C-2 order parameter in Figure 2 refers to the fatty acyl chain attached at position 1 of the glycerol moiety.

Attachment at position 2 produces two signals with distinctly smaller quadrupole splittings. In our model this effect can easily be accounted for by increasing the probability of orientation III. The existence of such an orientation is also supported by a recent X-ray study on single crystals of phospholipid bilayers (Hitchcock et al., 1974). The X-ray analysis shows that the initial orientation of the second chain is essentially parallel to the plane of the bilayer, that is similar to conformation III in Table I.

The theory predicts that the influence of initial orientations should level off around the third carbon atom. This is also in agreement with the experiment since only one deuterium magnetic resonance signal is observed for bilayers of dipalmitoyl-3-*sn*-phosphatidylcholine deuterated at carbon atoms 4 or 5 of the palmitic acyl chains (Seelig and Seelig, 1974b).

Thermodynamic Properties and Bilayer Dimensions. Inserting the parameters $V_0 = 590$ cal/mol and $\gamma = 18.5$ dynes/cm into eq 8 yields two solutions to the self-consistent field equation namely $\phi_{lc} = 165$ cal/mol and $\phi_c = 472$ cal/mol (at 41°). ϕ_{lc} is the molecular field of the liquid crystalline state of the hydrocarbon chains and has been used to calculate the deuterium order parameters of Figure 2. ϕ_c corresponds to the molecular field in the crystalline state. The order parameters calculated from ϕ_c are close to an almost perfect ordering of the fatty acyl chains. Repeating these calculations for different temperatures leads to the prediction of a phase transition at 39.5°. This result deviates only by 1.5° from the calorimetric value of 41° (Phillips et al., 1969) and the high precision achieved in calculating the transition temperature as well as the deuterium order parameters is probably the strongest support for the Marčelja model. Using the same parameters the melting entropy is found to be 1.15 eu per CH_2 group which compares favorably with the experimental value of 1.25 eu (Phillips et al., 1969).

The average length of a palmitic acyl chain is predicted by the Marčelja model to be 13.7 Å at 41° while the same chain in the rigid all-trans configuration measures 19.7 Å.[2] Thus a bilayer of dipalmitoyl-3-*sn*-phosphatidylcholine in the liquid-crystalline state is expected to be approximately 12 Å shorter than the same bilayer with completely extended chains. This result is in agreement with X-ray diffraction experiments which indicate a shortening of the chains by 11-12 Å (Cain et al., 1972; Chapman et al., 1967; cf. also Seelig and Seelig, 1974b).

The cross-sectional area per chain is calculated according to eq 5 $A = 20.4 \times 19.7/13.7 = 29.3$ Å^2, yielding a surface area per lipid molecule of 58.6 Å^2. For egg-yolk lecithin the area per polar group has been determined by X-ray diffraction to be 59-63 Å^2 (Levine and Wilkins, 1971). Finally, the linear thermal expansion coefficient of the hydrocarbon region is predicted by the theory to be -3.0×10^{-3} °K^{-1}, which is identical with the experimental result for a bilayer composed of natural lipids (Luzzati and Husson, 1962).

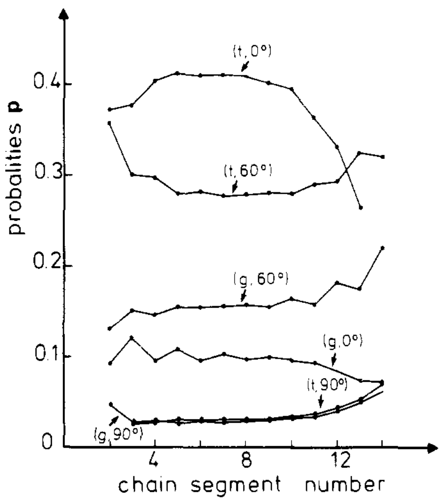

FIGURE 3: Probabilities of segment orientations as a function of the segment position in the bilayer.

Configurational Probabilities. Using the same set of parameters as defined above the probability for a carbon–carbon bond to occur in the trans conformation is found to be $p_t = 0.69$. Therefore, on the average, 9.7 bonds of a palmitic acyl chain are in the trans state while 4.3 are in the gauche state. This is in agreement with previous estimates (Nagle, 1973; Seelig and Seelig, 1974b; Marčelja, 1974b).

Assuming the first segment of the hydrocarbon chain to be parallel to the bilayer normal, it is easily seen by simple coordinate transformations that only a few segment orientations are possible on the basis of the rotational isomeric model: the segments are inclined to the bilayer normal at angles of either 0, 60, or 90°. Since the conformational state in each of these orientations may be either trans or gauche, this leads to altogether six different types of segments. We have calculated the probability for each type of segment and have plotted the results in Figure 3 as a function of the segment position in the bilayer. Figure 3 reveals two interesting features about the structure of the bilayer. Firstly, the predominant structural elements are trans segments oriented parallel to the bilayer normal ($p_{t,0°}$), while segments oriented perpendicular to this direction ($p_{t,90°}$; $p_{g,90°}$) are rather improbable. This indicates that the parallel alignment of the hydrocarbon chains is the energetically most favorable situation. Secondly, the probability of the various conformations remains almost constant throughout the bilayer up to carbon atoms 10–12. The loss of order in the central part of the bilayer is essentially due to a decrease in the number of trans-elements aligned parallel to the bilayer normal.

It is equally simple to calculate the probabilities of kinks (g^+tg^- or g^-tg^+ sequences) or jogs (2g2 jog = g^+tttg^-). These sequences are of importance since they pack very well into the bilayer and leave the chains essentially parallel to each other (Pechhold, 1968; Träuble, 1971). Previously we have concluded that the observed deuterium order parameters can be accounted for by incorporating two kinks or one 2g2 jog into the hydrocarbon chain but that a dynamic equilibrium between the two conformations seems more realistic (Seelig and Seelig, 1974b). This interpretation must be modified in view of the present calculations. The numerical computations yield approximately 0.6 kink and 0.2 (2g2) jog per palmitic acyl chain. At the same time they also predict small but nevertheless finite contributions from a large number of other conformational defects. Thus even in the region of constant order parameters it is not sufficient to postulate kinks and 2g2 jogs, but other configurations must also be included. On the other hand, the statistical model shows quite clearly that the kink and the 2g2 jog are the most important *single* contributions to the chain flexibility. These two defects together account for almost 50% of the observed disorder in the hydrocarbon chains. The calculations also indicate that the probability of a kink is smaller in the interior of the bilayer than close to the polar region.

Concluding Remarks. Previous interpretations of the chain flexibility in lipid bilayers have been based on models typical for free polymer chains (Hubbell and McConnell, 1971; Seelig, 1971; Marsh, 1974). Such an approach though providing some insight into the bilayer structure remains unsatisfying since the chain–chain interactions are taken into account only indirectly. Furthermore the previous models have relied, in part, on spin-label measurements which indicate a continuous decrease of the chain ordering. This is at variance with the deuterium results of the same systems which reveal a constant order parameter in most parts of the bilayer. The discrepancy must be attributed to a perturbation of the bilayer by the spin-label group.

On the other hand, the Marčelja model explains remarkably well most of the experimental information which is at present available for liquid-crystalline bilayers of dipalmitoyl-3-*sn*-phosphatidylcholine. Nevertheless the model is far from being perfect for a number of reasons: (1) the assumption of a constant molecular field throughout the bilayer is an oversimplification; (2) the scaling factor (n_{tr}/n) has no rigorous theoretical basis; (3) the model fails to give a proper account of the crystalline state of the bilayer; (4) the small volume change of 1–3% which is associated with the crystalline → liquid crystalline phase transition has been neglected; (5) a better molecular understanding of the coupling constant V_0 and the surface energy γ would also be desirable.

In conclusion it should therefore be emphasized that the Marčelja model does not disprove other theoretical approaches (Nagle, 1973; Belle and Bothorel, 1974; Scott, 1974; Adam, 1973), but it is at present the most detailed model to be used successfully for the interpretation of experimental data.

Appendix

Deuterium Order Parameter S_{CD} and Segmental Order Parameter S_{mol}. Let us denote with α the angle between the deuterium bond vector and the bilayer normal and with β the angle between the segment direction and this axis. On the basis of the above model we need to consider only three types of segment orientations, namely $\beta = 0$, 60, and 90°. The corresponding orientations of the deuterium bond vectors are $\alpha = 90$, 35.3 and 90, and 35.3°, respectively. Thus for segments with $\beta = 60°$ the two deuterium bonds are not equivalent. Using the abbreviations $S_\zeta = (1/2)(3\cos^2\zeta - 1)$ the order parameters S_{mol} and S_{CD} are found to be

$$S_{mol} = (1/2)(3\langle\cos^2\beta\rangle - 1) = p_0S_0 + p_{60}S_{60} + p_{90}S_{90} \quad (9)$$

$$S_{CD} = (1/2)(3\langle\cos^2\alpha\rangle - 1) = p_0S_{90} + (1/2)p_{60}(S_{35.3} + S_{90}) + p_{90}S_{35.3} \quad (10)$$

Here p_ζ is the probability for a segment being oriented at the angle ζ. Taking into account

$$p_0 + p_{60} + p_{90} = 1 \tag{11}$$

the following relation between S_{mol} and S_{CD} is obtained:

$$S_{\mathrm{mol}} = -(1/8)(18S_{\mathrm{CD}} - 6p_{90} + 1) \tag{12}$$

Previously we have used the simpler relation (Seelig and Niederberger, 1974a)

$$S_{\mathrm{mol}} = -2S_{\mathrm{CD}} \tag{13}$$

which is strictly valid if the segment motion is axially symmetric and characterized by *one* order parameter. We have therefore compared eq 21 with the more accurate approach (eq 20) calculating p_{90} by means of the Marčelja model. It is found that close to the lipid-water interface the results obtained with eq 21 are about 10% too large, while in the bilayer interior both methods yield the same results. Whenever possible it is therefore advantageous to base numerical calculations on the deuterium order parameter. Following the same arguments as described previously (Seelig and Seelig, 1974b) the effective length $\langle L \rangle$ of a hydrocarbon chain with n segments can thus be calculated according to

$$\langle L \rangle = 1.25\sum_{i=1}^{n} \langle \cos \beta^{(i)} \rangle = 1.25 \sum_{i=1}^{n} (p_0{}^{(i)} + 0.5p_{60}{}^{(i)}) = 1.25\left[(n/2) - \sum_{i=1}^{n} S_{\mathrm{CD}}{}^{(i)}\right] \tag{14}$$

Here $S_{\mathrm{CD}}{}^{(i)}$ is the experimental deuterium order parameter of the ith segment.

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