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THEORETICAL CONFORMATIONAL ANALYSIS OF PHOSPHOLIPIDS

I. STUDY OF THE INTERACTIONS BETWEEN PHOSPHOLIPID MOLECULES BY USE OF SEMI-EMPIRICAL METHODS WITH THE EXPLICIT INTRODUCTION OF POLAR HEADGROUP INTERACTIONS

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We present a theoretical conformational analysis of a system composed of seven dipalmitoylphosphatidylethanolamine molecules in interaction. The combined use of classical semi-empirical methods for the polar headgroup region with mechanical statistical calculations for the aliphatic chains permits the evaluation of the free energy for a phospholipids molecule. The free energy variation in function of the mean intermolecular interchain distance gives information about the main lipid bilayer phase transition. It appears, however, necessary to take into account the hydration of the polar headgroups.

Introduction

The complexity of biological membranes has induced the use of model systems composed only of phospholipid molecules which are one of the major components of biological membranes. These model systems have been studied theoretically as well as experimentally. The theoretical investigations are primarily concerned with the thermodynamic and structural properties of the phospholipid molecules (Refs. 1, 2 and references therein).

These phospholipidic bilayer systems exhibit a phase transition between a gel state and a liquid crystal state and are characterized by a typical transition temperature [3]. The situation concerning the conformational calculations is as following: (1) The hydrophilic moiety (or polar headgroup) has been analyzed for the isolated molecule [4–10] and for molecules in interaction in a given crystal lattice [10–13]. The state of the hydrocarbon chains (completely extended or melted) is not taken into account. (2) The chains are studied in detail, but the hydrophilic moiety is more or less neglected [14–23].

In our laboratory J. Belle and B. Lemaire have developed a statistic mechanical model which allows calculation of thermodynamic and structural properties of the hydrocarbon chains [17–19]. One important parameter appears to be the distance between chains, and this can be seen on the curve of free energy versus interchain distance (Fig. 1). But these calculations do not give a satisfactory value for the transition temperature ($T_{\mathrm{t,calc}} \gg T_{\mathrm{t,exp}}$). One reason for this disagreement could be the absence of the contribution from the hydrophilic part of the phospholipid molecule.

We present an approach which combines the statistical mechanical results obtained for the chains with explicit introduction of the polar headgroup at the level of atom-atom interactions.

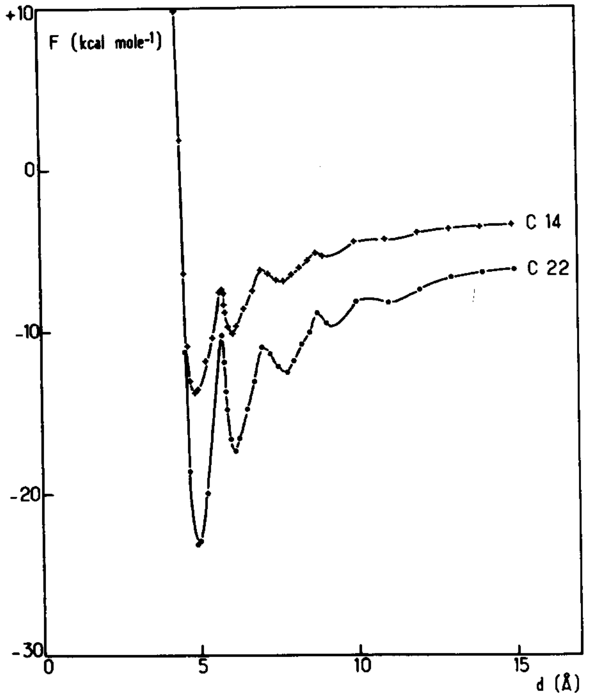

Fig. 1. Free energy curves for aliphatic chains of C_{14} and C_{22} length in function of the interchain distance d ($T = 20°C$, from J. Belle [18].

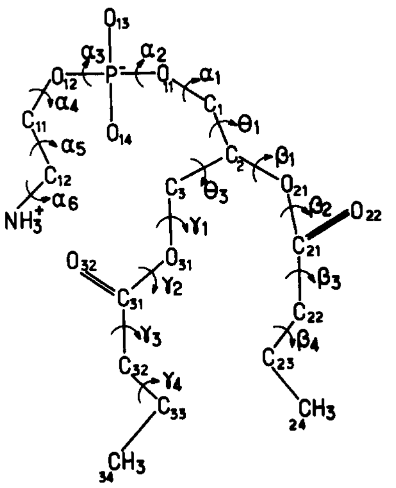

Fig. 2. Representation of various chains and torsional angles in a phospholipid molecule (β,γ-dibutyryl-L-phosphatidylethanolamine).

In this way it becomes possible to describe the state of a whole phospholipid molecule in relation to the interchain distance and temperature.

Energy calculation method

As in a previous paper [24] dedicated to the conformational analysis of the glycerol backbone of the phospholipid molecule, we have used an empirical method to obtain the total energy function, which is the sum of two terms

$$E_{\rm tot} = E_{\rm intra} + E_{\rm inter}$$

The first term, $E_{\rm intra}$, represents the intramolecular contribution for each molecule and the second term $E_{\rm inter}$ describes the intermolecular interactions between neighbouring molecules.

The intramolecular energy is considered as the sum of nonbonded, electrostatic and torsional energy terms

$$E_{\rm intra} = \sum_{i \neq j} \epsilon_{ij} \left[\left(\frac{r_{ij}^0}{r_{ij}} \right)^{12} - 2 \left(\frac{r_{ij}^0}{r_{ij}} \right)^{6} \right] + \sum_{i \neq j} \frac{q_i q_j}{\epsilon r_{ij}} + \sum_k \frac{A_k}{2} (1 \pm \cos n\theta_k)$$

where ϵ_{ij} and r_{ij}^0 are the potential well depth and position of the minimum of the energy, q is the partial atomic charge, ϵ is the dielectric constant, r_{ij} is the distance between two interacting atoms, A_k is the barrier height for rotation around the kth bond, θ_k is the dihedral angle, and n is the n-fold degeneracy of the torsional potential.

The intermolecular energy is the sum of nonbounded and electrostatic terms.

The parameters ϵ_{ij} and r_{ij}^0 for the Lennard-Jones function are those proposed by Scheraga et al. [25], the partial changes of the polar headgroup and glycerol have been calculated by CNDO/2 * [26,27], the barrier heights are given by Vanderkooi [5].

Hydrogen bonds may exist in the polar head, and this eventuality is taken into account by in-

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* CNDO/2, Complete Neglect of Differential Overlap, version 2.

cluding a Morse-type potential as proposed by Popov et al. [28].

The conformational energy (E_{tot}) of a phospholipid system is therefore a function of the dihedral angles and of the six external degress of freedom (three translational and three rotational) of each molecule. The atom-naming system and the definition of the torsion angles follows those proposed by Sundaralingam [29] (Fig. 2).

Results and Discussion

From the isolated phospholipid molecule to an array of seven phospholipids

Our methodological approach is quite similar to that described in a very recent work by Brasseur et al. [12]. As we will consider both the intra- and intermolecular parameters, it is necessary to adopt a step-by-step method. This implies starting with the interactions between two phospholipid molecules and then building up the array of seven molecules.

Interaction between two molecules

The intramolecular conformation of each phospholipid is chosen from those calculated in a previous paper [24] and which concern the glycerol backbone. The torsional angles of these conformation are listed in Table I. We investigated first the conformations V_1^* which is characterized by an interchain distance of 4.8 Å. The torsional angles of α_i of the α-chain are those proposed by McAlister et al. [4] for the isolated molecule: $\alpha_1 = 180°$, $\alpha_2 = 300°$, $\alpha_3 = 300°$, $\alpha_4 = 180°$, $\alpha_5 = 60°$.

The external degrees of freedom are the following: each molecule is allowed to rotate around a mean molecular axis which is parallel to the β and γ chains (Fig. 3): the distance between the two molecules can be varied (translational movement) (Fig. 3).

For each value of distance D, varying from 9.0 to 6.5 Å by steps of 0.5 Å we compute a conformational map by varying the external rotational angles, E_1 and E_2, by steps of 30° from 0° to 360°. We thus obtain six conformational maps which are condensed to one map by taking at each point (E_1, E_2) the minimum energy value from the six initial maps. The results appear in Fig. 4, the two parts of which are obtained, respectively, for $\epsilon \rightarrow \infty$ and $\epsilon = 1$. $\epsilon \rightarrow \infty$ corresponds to the absence of an electrostatic term and $\epsilon = 1$ to a vacuum environment.

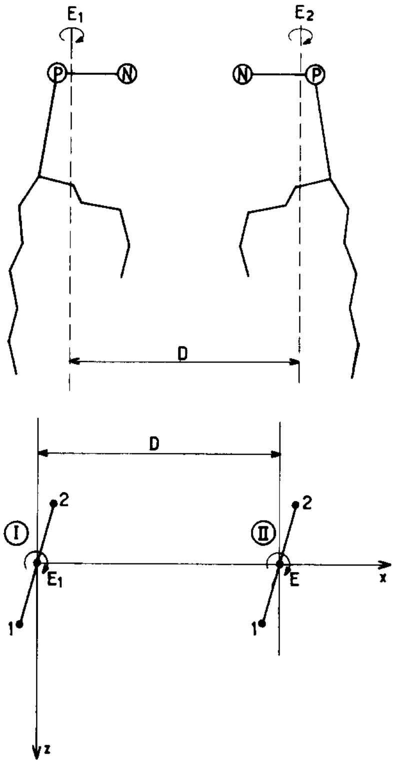

Fig. 3. Schematic representation of two phospholipid molecules in interaction. E_1 and E_2 are the angles of rotation about the molecular axis of each molecule. D represents the intermolecular distance between the two molecules.

For $\epsilon \rightarrow \infty$ the global minimum corresponds to (0, 180) and lies in a region where the interactions between the hydrophilic part of the phospholipid are predominant (denoted T). For that configuration we do not observe any interchain distance near 4.8 Å which is characteristic for the gel phase phospholipids [30,31]. Two configurations (0, 240) and (60, 180) are especially stabilized by the interaction between the hydrophobic part of the molecule ($d_{Ch-Ch} = 4.7$ Å). They lie 1 kcal · mol^{-1} above the global minimum.

The values of D are found to be, respectively, 8.5 and 7.5 Å, which compares quite well with the distance of 7.77 Å determined by X-ray diffraction [32].

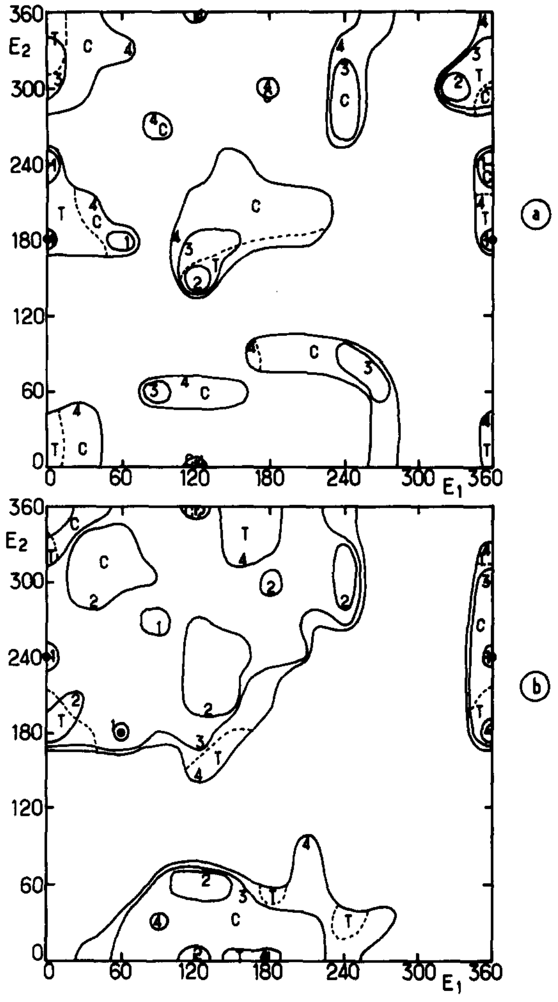

Fig. 4. Intermolecular energy maps (E_1, E_2) for two phospholipid molecules in interaction ($\epsilon \rightarrow \infty$(a) and $\epsilon = 1$(b)). Position of the energy minimum (●).

It is interesting to note that the minimum energy configuration corresponds to the two molecules oriented parallel one to the other. This reflects the situation which will be found in an array with up to seven molecules in interaction.

If we consider the situation for $\epsilon = 1$ (Fig. 4b), the two more stable configurations are now (0, 240) and (60, 180), Configuration (0, 180) is destabilized by about 2 kcal · mol^{-1}. This is due to the repulsive electrostatic contribution, which tends to favour configurations with maximum interactions between aliphatic chains.

During these calculations the intramolecular conformation is not varied. The next step consists in examining of the influence of the internal degrees of freedom. From now on we will use the value $\epsilon = 20$, which has been determined experimentally [33,34] for the phospholipid headgroup environment.

Our aim was to investigate the influence of the intermolecular interactions on the intramolecular conformation of the isolated molecule. The first torsional angle of the α-chain in the polar head is the angle θ_1. In the isolated molecule three values are commonly assumed for θ_1 (60, 180 and 300°). We have computed a conformational map $(\theta_{11}, \theta_{21})$ (where the first subscript refers to the molecule number and the second to the numbering of Sundaralingam for the torsional angles). The angles θ_{i1} are varied by steps of 20° and each conformation is minimized with regard to the intermolecular distance, D.

The configuration which is adopted corresponds to $E_1 = 0°$ and $E_2 = 240°$. It appears in Fig. 5 that the permitted conformational surface is not very important. The rotation for θ_{21} is quite easy, whereas θ_{11} is restricted between 150 and 180°. The minimum energy conformation corresponds to $\theta_{11} = 160°$, $\theta_{21} = 280°$ with $D = 7.0$ Å. The possibility of different relative orientations of the headgroup emerges and will be confirmed later for an array of headgroups in interaction.

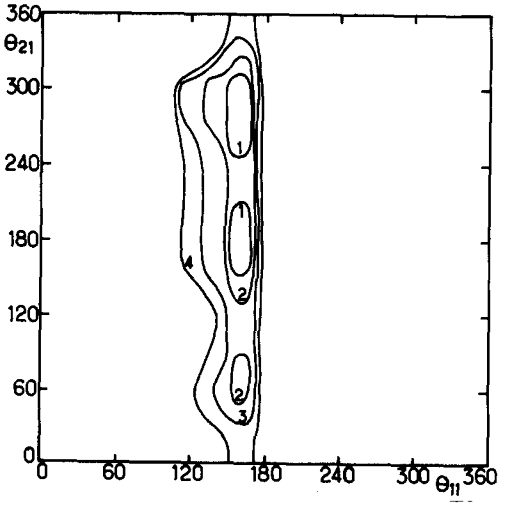

Fig. 5. Intra and intermolecular energy map $(\theta_{21}, \theta_{11})$ with $\epsilon = 20$.

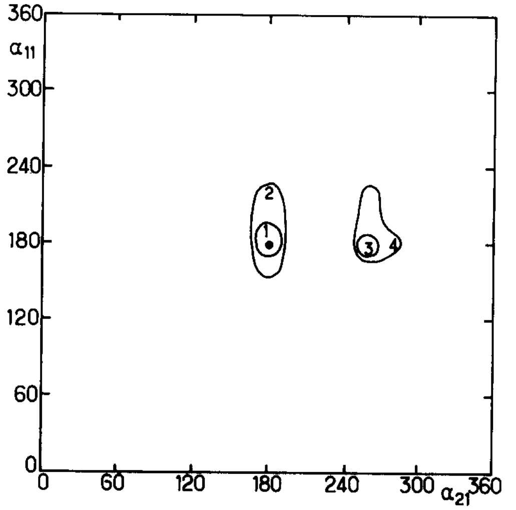

Fig. 6. Intra and intermolecular energy map $(\alpha_{21}, \alpha_{11})$ with $\epsilon = 20$.

When moving along the α-chain of the polar head, the following torsional angle is α_{i1}. In the isolated molecule the value is $\alpha_1 = 180°$. The result of the interaction between headgroups is characterized by the existence of a second energy minimum on the conformational map $(\alpha_{11}, \alpha_{21})$ (Fig. 6). There is a possibility for α_1 to move away from the *trans* form and this is observed in the crystal structures [32,35] of phospholipids.

In order to confirm these intermolecular effects we have also computed a conformational map $(\alpha_{12}, \alpha_{22})$ (Fig. 7). Two values, denoted g^+ (60°) and g^- (300°), are found in the isolated molecule. Our calculations show a certain ability of α_{12} and α_{22} around the g^- position (Fig. 7). Once more, we can verify that an environment induces modifications of the intramolecular parameters which can adopt values differing from the most stable in the isolated molecule.

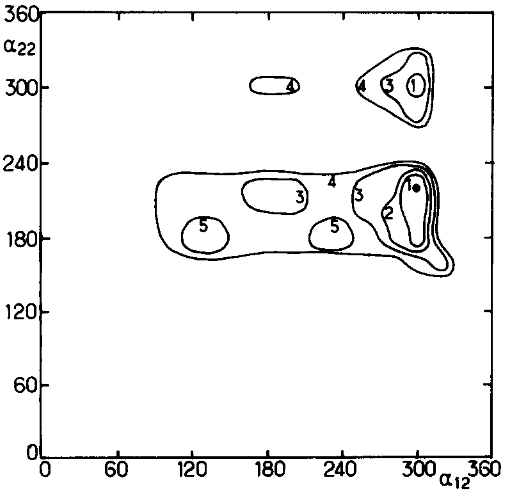

Fig. 7. Intra and intermolecular energy map $(\alpha_{22}, \alpha_{12})$ with $\epsilon = 20$.

Interaction between seven molecules

For the third molecule we can start from the packing determined for the two first molecules and study the orientation of this molecule around them. To build up our model we proceed in a way similar to that for the chain model developed in our laboratory [17–19]. We consider a central phospholipid molecule surrounded by six neighbouring molecules. The aliphatic chains are in a hexagonal array, but not so the phospholipids (they are in a quasi-hexagonal array).

Consider the projection of one molecule in the plane of the layer, the two β and γ chain axes are perpendicular to the plane (X, Z) of the layer (Fig. 8). If we try to find out all possibilities of building an hexagonal chain array around each chain we obtain Fig. 8, which is characterized by: (1) a first row of eight chains surrounding the two central ones;

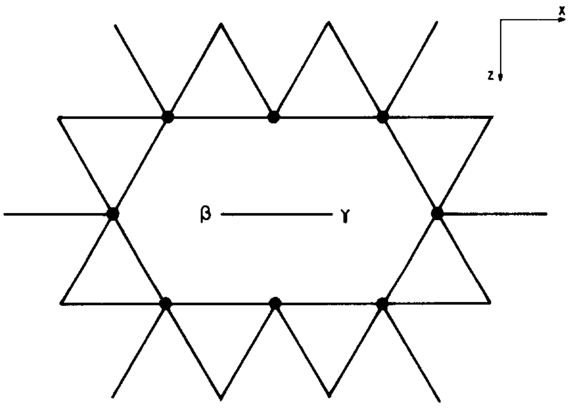

Fig. 8. Environment of a central phospholipid molecule seen from above (the aliphatic chains β and γ are perpendicular to the figure plane). ● represents the first row of the aliphatic chains of the neighbouring phospholipid molecules. The chains form an hexagonal array.

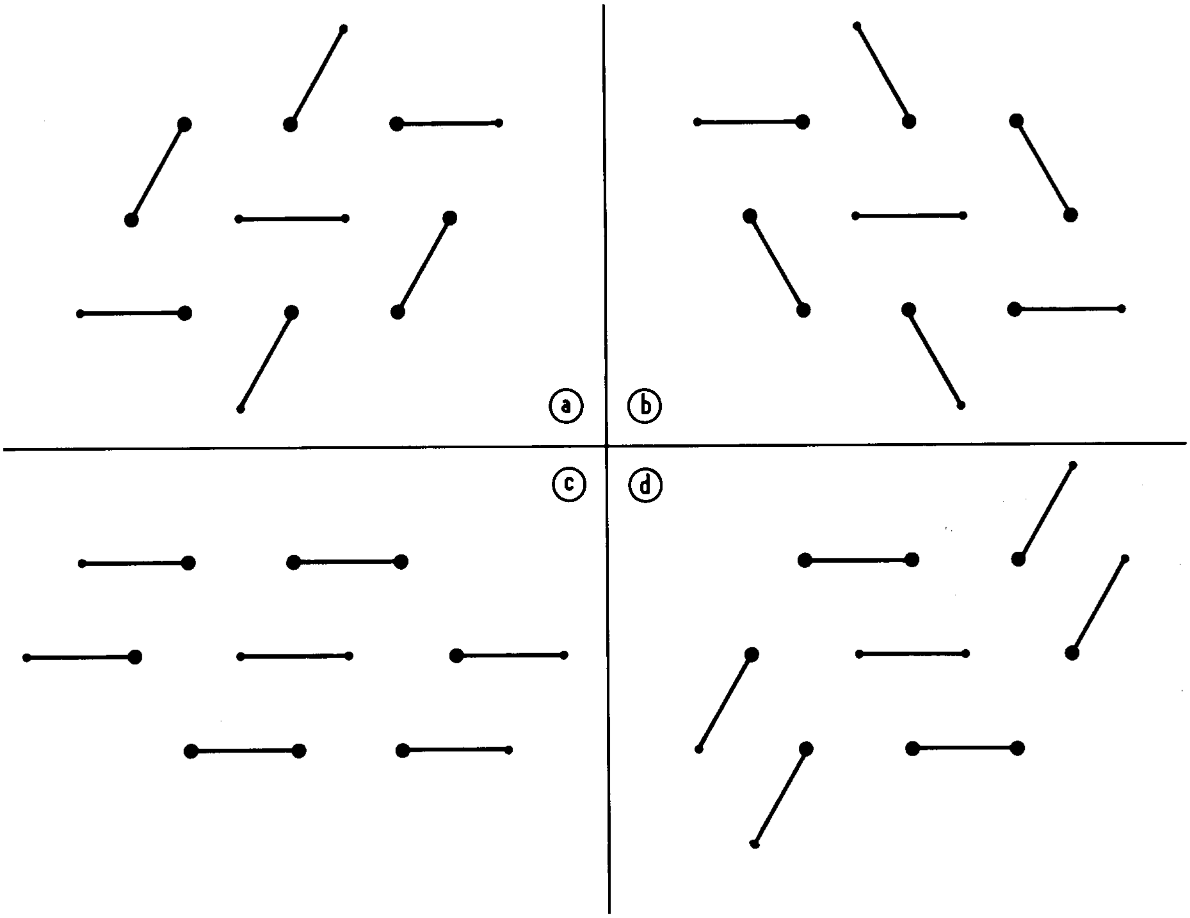

Fig. 9. Geometrically possible configurations of a system of seven molecules composed of one central molecule and six nearest-neighbour molecules. •, chains of the central molecule. ● first row of chains.

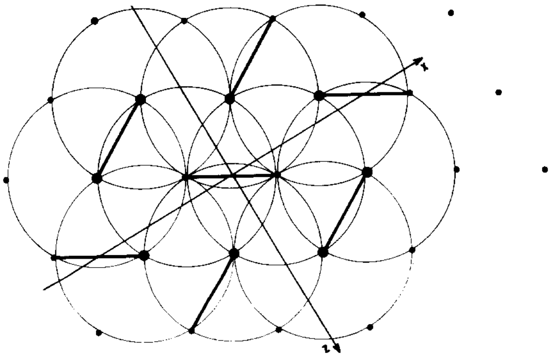

Fig. 10. Geometrical construction of an hexagonal array of aliphatic chains and of the phospholipid molecule array for an intra- and intermolecular interchain distance of 4.8 Å.

(2) different molecular configurations obtained from the eight positions of the first row of chains by generating different pairs of chains which make up a molecule.

From that general picture we can extract several environments for a central molecule surrounded by six molecules in the first row (Fig. 9). Among the different possibilities, one is of particular interest because it reproduces the structure observed by X-ray diffraction studies for phosphatidylethanolamine molecules [32]. By the mean of a simple geometrical construction we can determine the distance which separates two molecules and the angle (V) between one molecule and the X axis going through the centre of the two in the (X, Z) plane. If an interchain distance of 4.8 Å (intra- and intermolecular) is imposed one finds a dis-

TABLE I

TORSIONAL ANGLES OF THE GLYCEROL BACKBONE CONFORMATIONS USED FOR THE INTERMOLECULAR CALCULATIONS

Intramolecular interchain distance (Å)	Conformation	θ_3	β_1	β_2	β_3	β_4	γ_1	γ_2	γ_3	γ_4
4.8	V_1^*	70.0	82.4	180.0	63.8	180.0	90.8	180.0	134.0	190.6
	$K_2K_3^*$	187.4	152.9	180.0	202.2	64.4	180.0	180.0	202.5	185.6
5.4	$V_4G_2^*$	177.8	82.8	180.0	275.6	185.4	202.2	180.0	275.6	185.5
5.6	$V_4G_2^*$	178.9	78.9	180.0	278.9	182.4	193.0	180.0	282.2	184.8
5.8	$V_4G_2^*$	185.5	69.1	180.0	296.9	196.6	180.0	180.0	304.2	196.0

tance $D_X = 8.0$ Å and an angle $V = 30°$ (Fig. 10). The distance which separates two molecules in the Z direction is $D_Z = 9.6$ Å. These two values (D_X, D_Z) compare fairly well with the unit cell parameters determined by Hitchcock et al. [32] which are, respectively, $b = 7.77$ Å and $c = 9.95$ Å.

We use these results to build the array of seven phospholipid molecules. The glycerol backbone conformation is of type V_1^*, with $d_{\text{intra}} = 4.8$ Å (intramolecular interchain distance) (Table I). The conformation of the α chain corresponds to $\alpha_1 = 180°$, $\alpha_2 = \alpha_3 = 300°$, $\alpha_4 = 180°$, $\alpha_5 = 60°$ determined by McAlister et al. [4] for the isolated molecule.

Molecule 1 occupies the central position, surrounded by a row of six molecules which form a quasi-hexagonal array, whereas the chains noted 1 and 2 are in an hexagonal array (Fig. 11). We consider two positions (0 and 180°) for each molecule which gives $2^7 = 128$ possible configurations from the geometrical point of view. Energy calculations allow us to select two equivalent configurations (Fig. 11) with a lower energy: $E \approx 10^5$ kcal (to be compared with energy values of 10^7–10^{12} kcal for the other configurations). These calculations are performed with a rigid intramolecular conformation for each molecule.

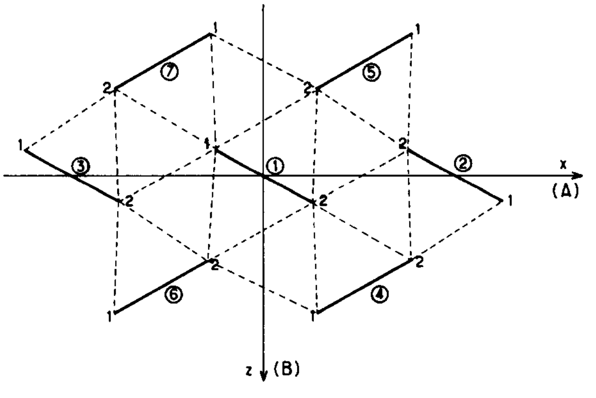

Fig. 11. View from above of seven molecules in interaction. Dashed lines represent the intermolecular interchain distances.

The energy of the array remains repulsive and to lower it we have to modify the intramolecular parameters (headgroup conformation).

It appears that a modification of the α_{11} value from 180° to 80° lowers the energy of the configuration determined earlier from 10^5 kcal to about 130 kcal. The next calculation is carried out with $\alpha_{i1} = 80°$ ($i = 2, 7$) and varying α_{11}. A new value $\alpha_{11} = 40°$ leads to an energy $E_{\text{tot}} = -39.5$ kcal · mol^{-1}. Different attempts to modify the torsional angles α_{i2} to α_{i5} do not give new results.

From these calculations one can conclude the important role played by the intramolecular conformations. There exists a coupling between the conformation of two neighbouring molecules, and the intermolecular interactions modify the conformation of the isolated molecule. Besides the influence of the polar headgroup conformation, the glycerol backbone conformation plays also an important role. Up to now we have considered molecules with the glycerol conformation V_1^* (Fig. 12b) where $\theta_3 = 60°$. We have seen that it is not very easy to minimize the intra- and intermolecular energy for an array of seven molecules of type V_1^*. If we make a comparison with the θ_3 values known for crystal structures it is interesting to note that the value $\theta_3 \approx 60°$ is observed in glycerophospha-

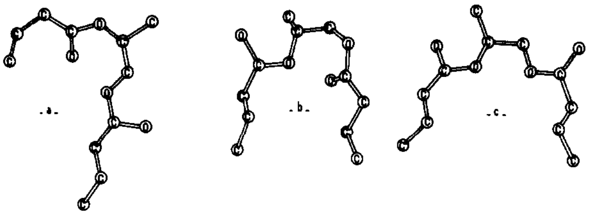

Fig. 12. Different types of glycerol backbone conformation. (a) type $K_2K_3^*$ (intermolecular interchain distance of 4.8 Å); (b) type V_1^* (intermolecular interchain distance of 4.8 Å); (c) type $V_4G_2^*$ (intermolecular interchain distance 5.4; 5.6; 5.8 Å).

tidylcholine [36] and in lysophosphatidylcholine [37] – that means in systems with no aliphatic chain or with only a single chain which do not require the same constraints to build layers as the two-chain phospholipids.

On a molecular model one can see that with $\theta_3 = 60°$ the C_2–C_1 is oriented parallel to the layer surface, whereas for $\theta_3 = 180°$ this bond points perpendicular to the surface. With the isolated molecule the two values (60° and 180°) are allowed. The inclusion of intermolecular interactions excludes one of the two values.

In fact, in the two known crystal structures of dilauroylphosphatidylethanolamine [32] and dimyristoylphosphatidylcholine [35], θ_3 has values near 180°.

Instead of glycerol conformation V_1^* we introduce now conformation $K_2^* = K_3^*$ (Table I; Fig. 12a) which is characterized by $\theta_3 = 180°$ and an intramolecular interchain distance of 4.8 Å. We start with headgroup conformation $\theta_1 = 180°$, $\alpha_1 = 180°$, $\alpha_2 = \alpha_3 = 300°$, $\alpha_4 = 180°$, $\alpha_5 = 60°$. We use the same methodology as with conformation V_1^*. In the first stage we determine the stable configurations, which are two among $2^7 = 128$ possible (Fig. 13) and in a second stage we modify the intramolecular parameters. The conformational energy appears to be very sensitive to the torsional angle α_{i4}. This angle takes different values, but remains the same in a row of molecules, and that fast allows us to build an extended array of phospholipid molecules (Fig. 14). In the central row $\alpha_{i4} = 340°$, in the next upper row $\alpha_{i4} = 300°$, in the next lower $\alpha_{i4} = 60°$ with a period of five rows. The mean energy is -42.0 kcal · mol^{-1}.

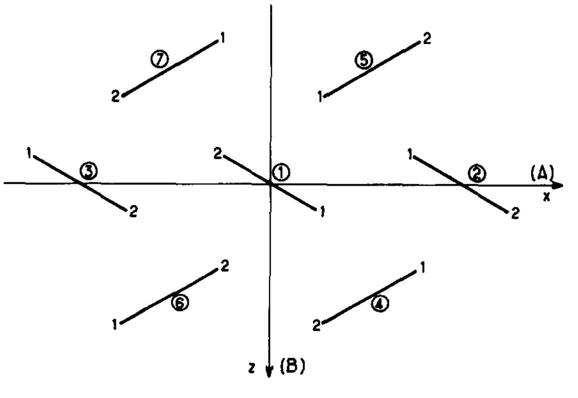

Fig. 13. Array of seven phospholipid molecules of type $K_2K_3^*$ 4.8. The molecules 1 to 7 can rotate about their molecular axis.

Variation of the intermolecular interchain distance

To connect our results concerning the hydrophilic moiety of the phospholipid molecule with those obtained by statistic mechanical calculations for the aliphatic chains (hydrophobic moiety) [18,19], it is necessary to vary the distance between chains. This is equivalent to a variation of the distance between the phospholipid molecules. We adopt the following principle: the central molecule does not move, while the six neighbouring molecules are moved apart or brought closer by means of a homothetic translation. The intramolecular conformations are kept rigid. The results are displayed in form of energy curves versus a mean distance $\langle d \rangle$ which includes the 20 intermolecular and seven intramolecular interchain distances. The total energy term consists of an intermolecular and an intramolecular contribution. At the intermolecular level we consider two possibilities for the Van der Waals term. One potential, we will call it 'soft', includes the occurrence of hydrogen bonding (EVWR), while the other, let us call it 'hard', considers all atom-atom interactions as pure Van der Waals contributions (EVWS). The difference can be seen on the energy curves which we will examine now. Fig. 15 shows the behaviour of the total energy (with EVWR or EVWS) when $\langle d \rangle$ varies. We want to stress that an energy minimized with EVWS is relevant for the existence of a molecular structure which is very satisfactory from the steric point of view. The curves (Fig. 15) all present a large energy well. The position of the minimum lies at 5.40–5.50 Å (Fig. 15). A com-

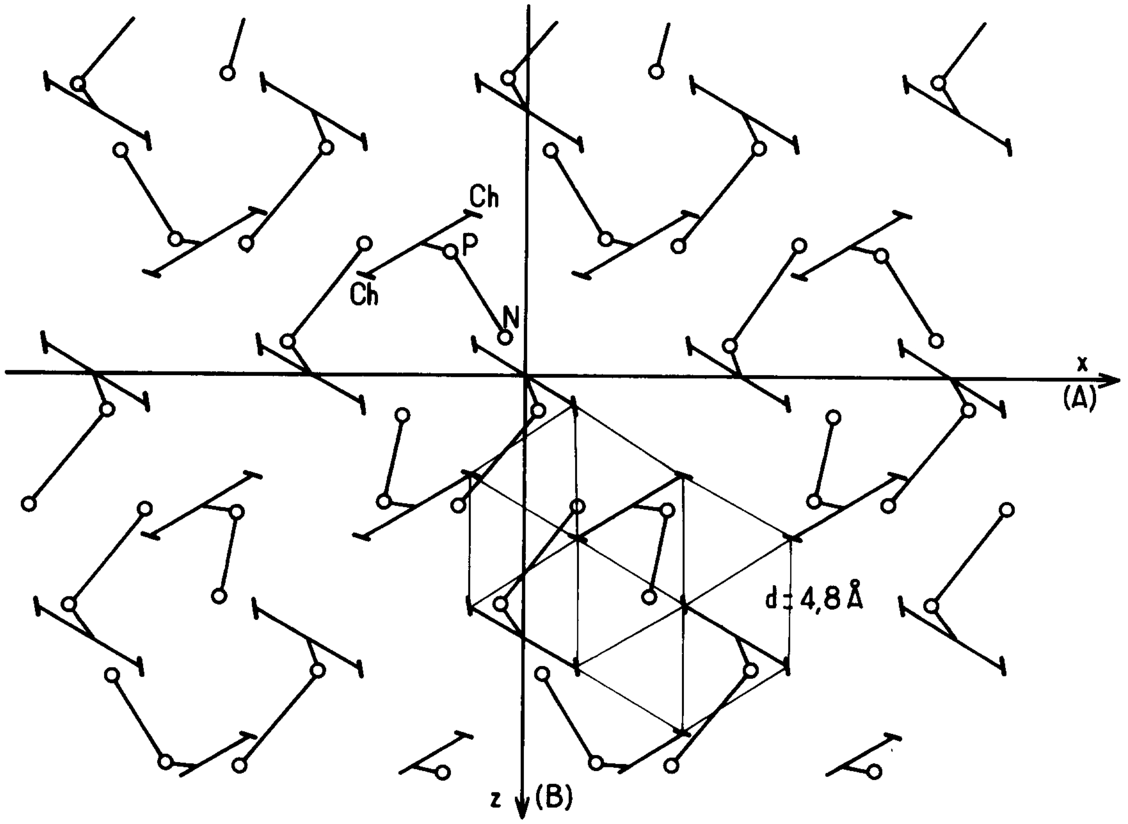

Fig. 14. View from above of an extended array of phospholipid molecules with an intra- and intermolecular interchain distance $d = 4.8$ Å. Ch: aliphatic chain planes. P and N: phosphate and ammonium groups of the polar headgroups.

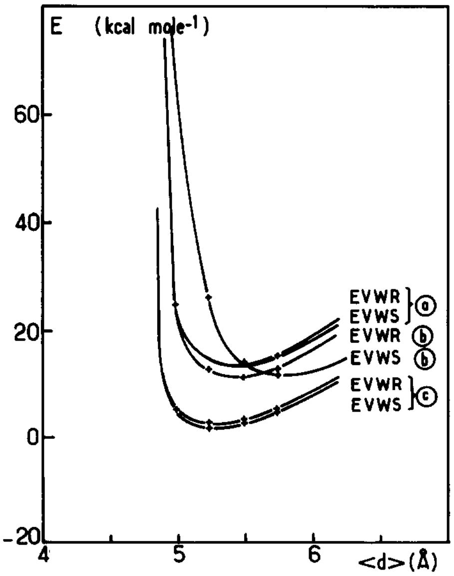

Fig. 15. Total energy curves (seven molecules of type $K_2K_3^*$ 4.8). (a) $\alpha_{13} = \alpha_{23} = \alpha_{33} = 100°$; (b) $\alpha_{13} = \alpha_{23} = \alpha_{33} = 300°$; (c) $\alpha_{14} = \alpha_{24} = \alpha_{34} = 140°$; $\alpha_{44} = \alpha_{54} = \alpha_{64} = \alpha_{74} = 220°$.

parative study with the values $\alpha_{13} = \alpha_{23} = 300°$ (as in the isolated molecule) shows a shift of the potential well to higher values (approx. 5.50–5.75 Å). Once more, we can verify the existence of a direct relationship between the variation of an intramolecular parameter and the stability of a molecular array.

A step-by-step minimization concerning the α_{i4} angles modifies the energy curves (Fig. 15) and their energy minimum is shifted to 5.25 Å. We will use this configuration in the forthcoming complete calculations which include the contribution due to the aliphatic chain. This state is considered as the anhydrous one.

Influence of the glycerol conformation

Actually, it is well known that the mean distance between aliphatic chains increases when the phospholipid layer passes from the gel phase to the liquid crystal phase [18,19,38]. This should

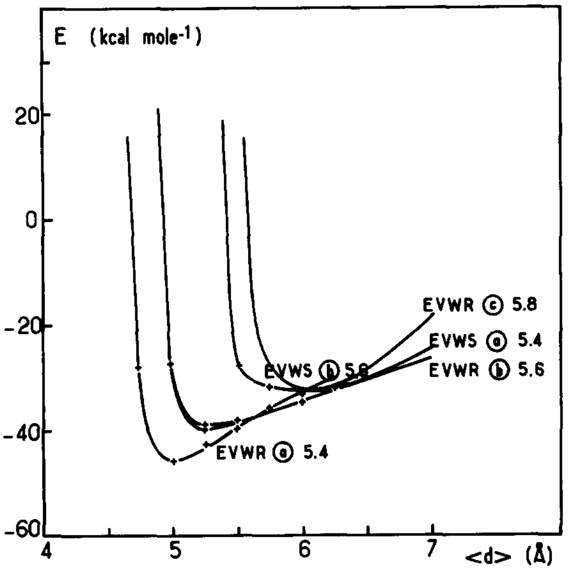

Fig. 16. Total energy curves for seven molecules in interaction. Influence of different conformations of the glycerol backbone (the intramolecular interchain distance varies from 5.4 to 5.8 Å).

necessitate a modification of the glycerol conformation which determines the intramolecular interchain distance.

To verify the influence of the glycerol conformation we have introduced in the molecular array, phospholipids with different intramolecular interchain distances (Fig. 12). The values of that distance vary from 5.4 to 5.8 Å and the intramolecular torsional angles are listed in Table I. The corresponding energy curves are represented in Fig. 16. Increasing the intramolecular interchain distance leads to a shift of the energy minimum (Fig. 16). For a value of 5.6 Å (intramolecular) the equilibrium distance (intermolecular) between chains becomes greater than 5.6 Å. That can be interpreted by the modification of the ratio of the headgroup region area to the chain area, which makes it more difficult to bring the molecules nearer one to another.

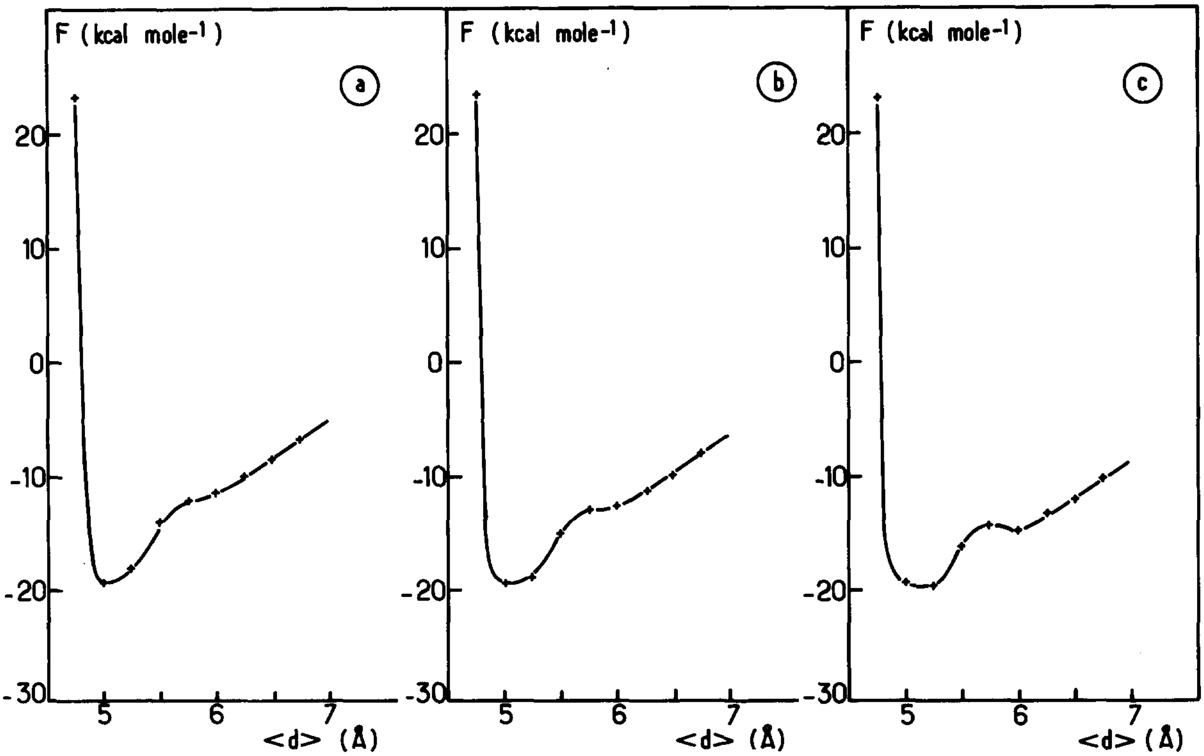

Fig. 17. Free energy curves for a dipalmitoylphosphatidylethanolamine molecule (C_{16}) as a function of the intermolecular interchain distance ($\epsilon = 20$). (a) $T = 25°C$; (b) $T = 60°C$; (c) $T = 100°C$.

Conclusion

Our calculations permit definition of the role of the intra- and intermolecular interactions, at the glycerol moiety and headgroup level, in a microarray composed of seven phosphatidylethanolamine molecules.

Unfortunately, if we consider the total free energy curves (Fig. 17) obtained by adding the energy interaction term between headgroups to the chain interaction terms (calculated by statistical mechanical calculations) we obtain a gel phase well far too deep compared with the liquid crystal well (Fig. 17). To reach the transition requires a considerable rise in temperature which would lead to much too high a transition temperature. A rough calculation gives a transition temperature of about 200°C (to compare with 60°C for the dipalmitoylphosphatidylethanolamine (C_{16}) measured by calorimetric methods). For a difference in free energy between the two wells in the order of 8 $\mathrm{kcal \cdot mole^{-1}}$ at 25°C (Fig. 17), a reasonable evaluation of the entropy gain (chain conformational + translational) gives ≈ 40 e.u. per mol, which corresponds to 40 $\Delta T \cdot \mathrm{cal \cdot mol^{-1}}$ on the energy scale. To obtain $\Delta F = 0$ we must raise the temperature with $\Delta T = 200$ K, which is obviously too much. It is well known from the phospholipid/water phase diagrams [3] that the transition temperature depends strongly on the hydration state of the polar headgroups.

Will the explicit introduction of water molecules in the polar head region improve the situation? The answer to that question is the subject of the second part of this work.

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