Biochimica et Biophysica Acta, 735 (1983) 23–34
Elsevier

BBA 71624

THEORETICAL CONFORMATIONAL ANALYSIS OF PHOSPHOLIPIDS

II. ROLE OF HYDRATION IN THE GEL TO LIQUID CRYSTAL TRANSITION OF PHOSPHOLIPIDS

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(Received November 8th, 1982)

Key words: Phospholipid structure; Hydration; Phase transition; Conformational analysis; Statistical model; Free-energy calculation; Dipalmitoylphosphatidylethanolamine

To obtain a satisfactory agreement between computed transition temperatures and those determined experimentally, we introduce explicitly water molecules which hydrate the polar headgroup of dipalmitoylphosphatidylethanolamine molecules. The calculated free energy curves as a function of the intermolecular interchain distance and the degree of hydration of the polar groups permit the determination of the transition of the phospholipid system from the gel to the liquid crystalline phase. The detailed structure of the hydration shell is defined using the supermolecular approach.

Introduction

Phospholipid molecules, which are a major component of biological membranes, present an interesting feature. They exhibit a rich mesomorphism which is of both lyotropic and thermotropic nature. The phase diagram phospholipid water [1] exhibits a main transition temperature which is very sensitive to the degree of hydration of the phospholipids. This temperature decreases regularly when the water amount increases [1]. But the transition temperature does not fall indefinitely; it reaches a limiting value independent of the water concentration [1]. The effect of water can be interpreted as a weakening of the ionic structure of the phospholipid crystals which induces also a reduction of the dispersion forces between the hydrocarbon chains.

The phase transition of phospholipids is monitored by the temperature and the hydration. It is evident that water plays an essential role in the gel to liquid crystal transition of phospholipids.

We have seen in the preceding paper [2] that the explicit introduction of the interactions between anhydrous polar headgroups does not account for the experimentally determined transition temperature.

The difficulty of finding a reliable evaluation of the polar head contribution appears in all theoretical contributions dedicated to the phase transitions of phospholipids. In a recent review, Nagle [3] discusses the relative importance of the different features which contribute to the main lipid bilayer phase transition.

There exists a great number of works concerning the theoretical investigations of the hydrophobic part of the phospholipids (Refs. 3, 4 and references there in). The degree of complexity increases from simulations in two dimensions to Monte-Carlo type calculations which consider atom-atom interactions, as developed in our laboratory [5–7].

But the headgroup treatment has been always quite crude and has reduced to an empirical term with one or two adjustable parameters. Depending on the total energy partitioning, the headgroup component is either attractive [3], of the form $E_{\text{head}} = -a\,A^{-c}$ where a and c are parameters and A the area per molecule, or repulsive [8], of the form C/A where C is a function of temperature and the dielectric constant of the environment. Tanford [9] introduced a distinction between charged headgroups where $E_{\text{head}} = \alpha_1/A$ and non-charged ones where $E_{\text{head}} = \alpha_2/A^3$. In a recent calculation Gruen [10] has used another potential, $E_{\text{head}} = \alpha/A^2$.

Only Scott [12] has taken the water molecules explicitly into account. They are represented by a surface occupied at the polar heads' interface. But this potential is very 'hard'. The great variety of approaches concerning the headgroups led us to adopt a microscopic method to investigate the interaction between phospholipid headgroups in the anhydrous state [2]. Insomuch as the transition temperature calculated does not agree well with the experimental value, it is now necessary to complicate the system by introducing explicit water molecules.

Methods

In this work we use the same empirical potential method as in the preceding paper [2]. The energy function is of the form (see Ref. 2):

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

The intermolecular potential function involves only the first two terms.

Results and discussion

State of theoretical investigations on the isolated polar headgroup

The only studies performed to date have been based on the supermolecular approach developed by the group of A. Pullman and B. Pullman [13]. The method consists of determining the position of one or more water molecules around the solute molecule. The hydration sites are characterized by geometric parameters and a hydration energy computed by ab initio methods.

The phosphatidylethanolamine headgroup offers two hydration centres, which are the ammonium and the phosphate groups. The principal results show that three water molecules can be attached to the ammonium group [14–16]. These water molecules lie along the direction of the N-H

TABLE I

MONOHYDRATION OF DMP$^-$

The symbols E_{ij}, E'_{ij}, E''_{ij} correspond to water bound by one hydrogen bond to oxygen i in the plane O_iPO_j. E stands for water external to the OPO angle, E′ and E_8 for water internal to this angle. In the unprimed and primed E_{ij} positions the second hydrogen of water is turned towards the PO_i axis. In the double-primed position this second hydrogen is turned away from the PO_i axis. The symbol B_{ij} corresponds to water making a bridge between oxygens i and j. From Pullman et al. [17]. θ = angle between PO_i and $O_i\ldots H$ directions. β = angle between $O_i\ldots H$ and HO_{water} directions.

Plane	Symbol	$d_{O_i\ldots H}$(Å)	θ	β	$-\Delta E$(kcal/mol)
O_1PO_3	$E_{13}E_{31}$	1.45	120	180	28.6
	B_{13}	1.75	110	143	27.1
	$E''_{13}E''_{31}$	1.50	120	180	27.4
O_3PO_2	E'_{32}	1.50	120	180	26.7
	B_{32}	1.60	116	160	25.6
	E_{32}	1.50	120	180	25.1

O_1PO_4-equivalents: $E'_{14}B_{14}E_{14}$
O_3PO_4-equivalents: $E'_{34}E_{34}$

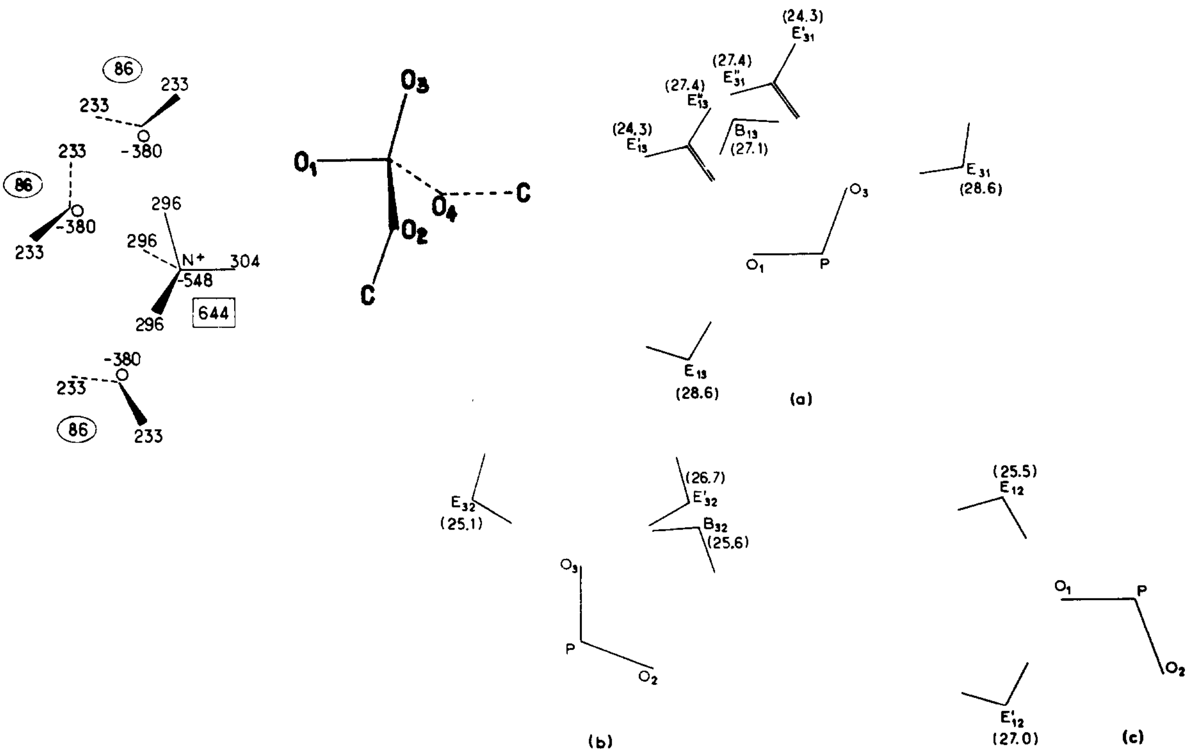

Fig. 1. Hydration scheme of the ammonium and phosphate groups (from Pullman and Armbruster [15] and Pullman et al. [17]).

bonds; the oxygen atom is found on the line which bisects the HOH angle (Fig. 1). The hydration energy is about -20 kcal · mol^{-1} and this value gives an idea of the solidity of this type of bonding.

The hydration of the phosphate group has also been studied by various authors [17–19]. This substrate offers more hydration sites and the geometrical configurations are of varying kinds (Fig. 1). Table I shows the different types of monohydration site. Pullman et al. [20] have used these preliminary calculations to investigate the influence of hydration on the conformation of the isolated phosphatidylethanolamine molecule.

From the monohydration sites, it is possible to determine polyhydration structures which include from two to six water molecules around the phosphate group. This approach allows one to gain quite a good idea of the water structure in the vicinity of the headgroups.

But we have to take into account three important remarks which are stressed by the authors of these calculations.

(i) The equilibrium distances are generally underevaluated and the hydration energies overevaluated, especially in the case of a polar substrate. It seems reasonable to introduce a scaling factor of 0.6 to bring the energies into agreement with the available experimental values.

(ii) The calculated energies correspond, in fact, to the gas phase. It is necessary to correct these values by considering the breaking of the water dimer (in a first approximation) which necessitates about 5 kcal · mol^{-1} [21].

(iii) Last but not least, it is important to keep in mind that in all these calculations one must not attach too great a significance to the absolute energy values. But it is quite reasonable to compare different conformational and configurational states. The relative stability of these states has a physical meaning.

State of experimental studies on polar headgroup hydration

Almost all experimental results have been obtained mainly by NMR, PMR, infrared and Raman spectroscopy [22–27] and one by X-ray diffraction [28]. The picture which emerges from theses studies is that of a certain amount of water molecules tightly bound to one headgroup. This first hydration layer follows the global movements of the headgroup [23]. But the existence of strongly bound water molecules does not exclude the possibility that water molecules can alter their position between the different hydration sites within the first hydration layer. Another striking fact is the increase in the number of water molecules fixed during the phase transition of the phospholipids from the gel to the liquid crystal phase [23,25].

Hydration of the phosphatidylethanolamine headgroup in interaction

To appreciate the relative importance of the phosphate and the ammonium groups with regard to hydration, we investigated first the hydration of the phosphate and next the complete hydration of the headgroup.

(I) Hydration of the phosphate moiety

We start from the configuration calculated previously for seven phospholipid in interaction [2]. The water molecules are introduced gradually and their number varies from one to five around the phosphate group. Without going into details for the configuration of the first hydration shell, one distinguishes two major types of water molecules [17]. One type (B) is involved in two hydrogen bonds, while the second type (E) is involved in only one hydrogen bond (Fig. 2).

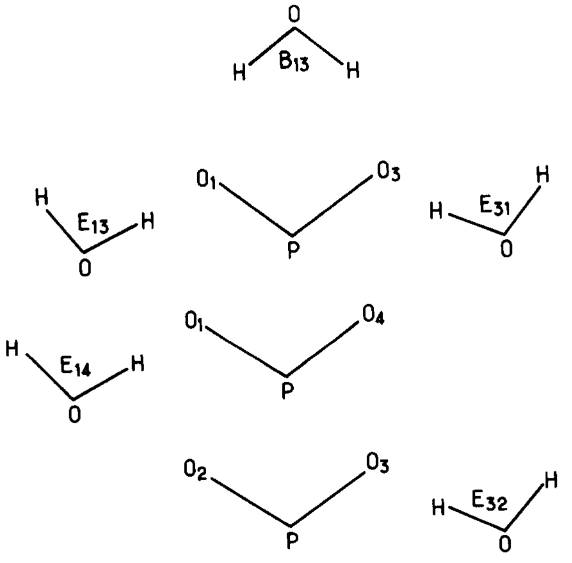

Fig. 2. B- and E-type water molecules attached to the phosphate group.

In all calculations, we consider the water molecules as bounded to each headgroup. In fact, we use the supermolecular approach to compute the intra- and intermolecular interactions in the seven-phospholipid system. We are making, of course, some simplifying assumptions, because we do not calculate for each conformation and configuration of the headgroups the new water molecule distribution which could occur. One has to limit the number of degrees of freedom in order to keep the computational time reasonable. In that way we favour the steric effects, but it is now well established that they play an important role in explaining molecular structures.

The intermolecular interactions are evaluated with a Lennard-Jones potential and a Coulomb potential for the electrostatic term with $\epsilon = 20$ (this value represents the headgroup environment fairly well). We do not consider intermolecular hydrogen bonding for the hydrated headgroup. The water molecules are strongly bounded to the polar groups and the competition will be in favour of these substrates rather than in favour of the surrounding water molecules. The hydration energy drops rapidly when going from the first to the second hydration shell (from approx. $-25\ \mathrm{kcal \cdot mol^{-1}}$ to approx. $-13\ \mathrm{kcal \cdot mol^{-1}}$) [20].

To evaluate the influence of the introduction of one water molecule on the phosphate group of the phospholipid system, we consider the perturbation induced in comparison with the non-hydrate state (zero state). The main effects concern primarily the intermolecular interactions and sometimes the intramolecular ones. The method consists, then, of minimizing the total energy by varying the internal torsional angle for each headgroup. In the next step we modify the distances between the phospholipid molecules, as for the anhydrous array, to obtain curves of energy versus the mean intermolecular interchain distance, $\langle d \rangle$.

It is interesting to compare the results obtained for the energy curves and the conformational mod-

TABLE II
PERTURBATIONS INDUCED FOR THE PHOSPHATIDYLETHANOLAMINE HEADGROUP CONFORMATION IN FUNCTION OF THE HYDRATION OF THE PHOSPHATE GROUP

Hydration	Modified torsional angles	Total
PE 7 → PE 71		0
PE 71 → PE 72	$\theta_{11}\theta_{31}\theta_{61}$	3
PE 72 → PE 73	$\theta_{11}\theta_{51}\alpha_{51}\alpha_{52}\theta_{71}\alpha_{71}$	6
PE 73 → PE 74	$\theta_{11}\alpha_{13}\alpha_{14}\theta_{31}\alpha_{63}$	5
PE 74 → PE 75	$\theta_{11}\alpha_{12}\theta_{42}\alpha_{52}\alpha_{53}\alpha_{54}\alpha_{55}\theta_{71}$ $\alpha_{71}\alpha_{72}\alpha_{74}$	11

ification of the headgroup when n_{H_2O} varies from 0 to 5.

We consider first the variations induced for the torsional angles. The reference state is the one determined for the anhydrous system [2] which we call PE7. From the Table II one can see that the total number of torsional angles which have to be modified to account for the increasing number of water molecules rises from 0 to 11 when we go from PE7 ($n_{H_2O} = 0$) to PE75 ($n_{H_2O} = 5$). If we can assume that the number of torsional angles which have to be varied reflects the degree of perturbation due to the water molecules, we can distinguish two important increases in n_{tot}: one for the transition PE2 → PE73 (3 to 6) and the second for PE74 → PE75 (5 to 11). This can be related to the fact that hydration configurations PE73 and PE75 both have a water molecule in position B_{13} (Fig. 2), which seems especially destabilizing.

A more quantitative way to evaluate the effect of hydration of the headgroups is given by the energy curves (Fig. 3). The curves all present an energy minimum which varies in position and in amount. For PE7 the minimum occurs at 5.20 Å (Fig. 3a) and there is virtually no shift for PE71 (Fig. 3b). One water molecule can be easily absorbed by the polar heads. Introducing a second water molecule (PE72) leads to a destabilization energy of about $+7$ kcal · mol^{-1} (Fig. 3c). For PE73 one observes an important effect; the minimum shifts to approx. 6.50 Å and the destabilization is about $+17.0$ kcal · mol^{-1} (Fig. 3d); the potential well is quite flat. This is due to the presence of a water molecule in bridging position B (Fig. 2) when n_{H_2O} varies from 2 to 3. On the other hand, the hydration structure of PE74 differs from that of PE73 because of the lack of a B_{13}-type water molecule. As a result, the energy curve is modified (Fig. 3e), the minimum shifts down to 5.60 Å and the energy is comparable to PE72. For PE75 we meet the same situation as for PE73 (presence of a B_{13} water molecule) and the minimum is displaced to 6.50 Å with a high energy for the well (Fig. 3f). It is interesting to notice that for that curve the repulsive part starts at a distance, $\langle d \rangle$, which is about 5.7 Å. This fact reveals the existence of important steric constraints when five hydration water molecules are present at the phosphate moiety level.

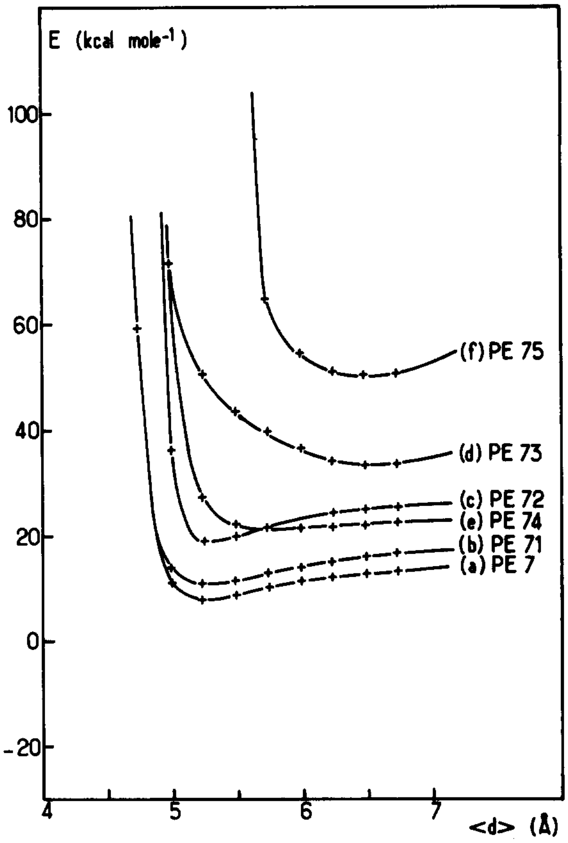

Fig. 3. Interaction energy curves of seven phosphatidylethanolamine molecules asa function of the degree of hydration (n_{H_2O} varies from 0 to 5) of the phosphate ($\epsilon = 20$).

(II) Calculation of the total free energy

In this section we present the way in which we obtain the total free energy for a phospholipid

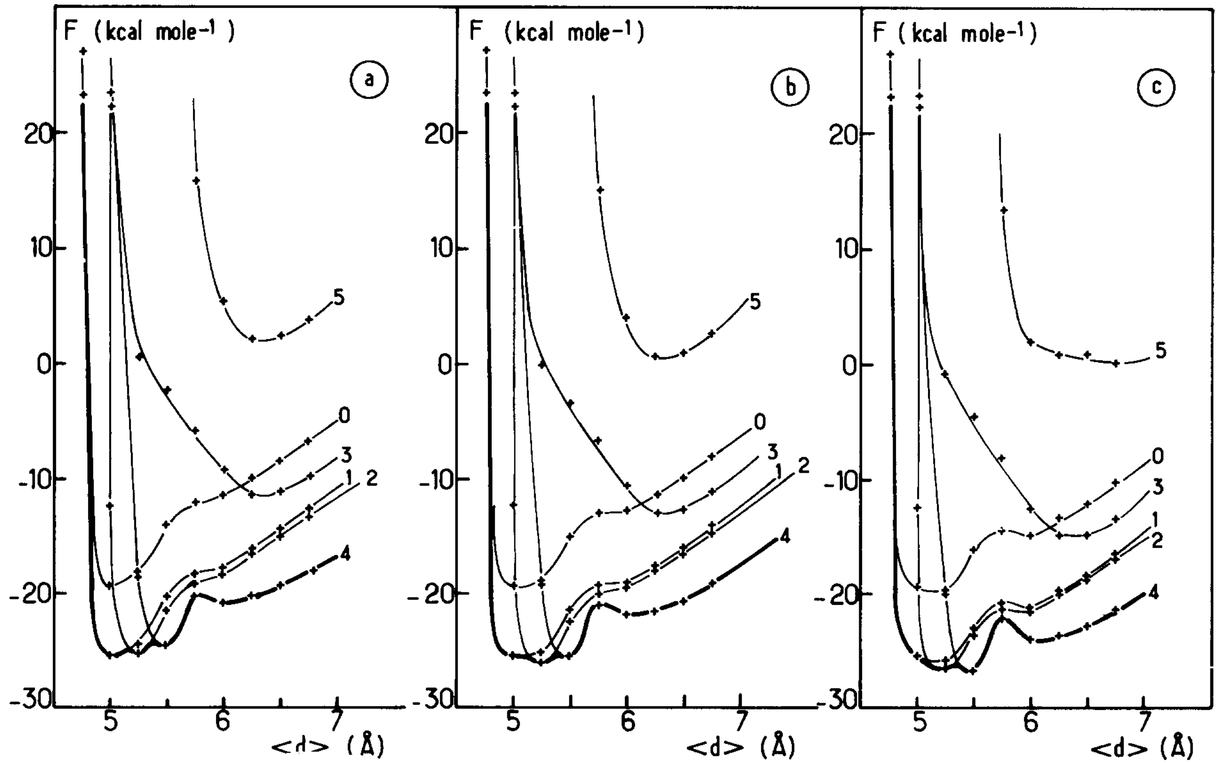

Fig. 4. Free energy curves for a dipalmitoylphosphatidylethanolamine molecule (C_{16}) in function of the intermolecular interchain distance and the degree of hydration of the phosphate (n_{H_2O} varies from 0 to 5; $\epsilon = 20$). (a) $t = 25°C$; (b) $t = 60°C$; (c) $t = 100°C$; ——— : envelope of the curves.

molecule in a layer. The contributions to the total free energy are of two types which are, respectively, energy and free energy terms.

$$F_{tot} = F_{chain} + F_{transl} + E_{head} + F_{hydr}$$

The E_{head} term corresponds to the values of the energy curves we have discussed before. We add an F_{hyd} term which includes the hydration energies computed by Pullman et al. [17] and corrected as follows (Table III). There is a scaling factor of 0.6, the energy which corresponds to the breaking of a water dimer approx. 5.0 kcal · mol^{-1}) and the entropy change when one water molecule passes from a bulk environment to the bonded state on the polar head. We obtain a relation which is:

$$\Delta F_{hydr} = \Delta E_{corr} - \Delta E_{dimer} - T\Delta S \times 10^{-3} (\text{kcal} \cdot \text{mol}^{-1})$$

where $\Delta E_{corr} = 0.6 \Delta E_{calc}$; $\Delta E_{dimer} = -5.0$ kcal · mol^{-1}; $\Delta S = -6$ e.u. *

At 25°C this equation takes the form:

$$\Delta F_{hydr} = \Delta E_{corr} + 6.8$$

For n water molecules we obtain:

$$\Delta F_{nhydr} = \Delta E_{ncorr} + \left(n_{H_2O} \times 6.8\right)$$

The values are calculated for $\Delta E_{n\ hydr}$ are significant for relative variations.

The influence of temperature arises through the

TABLE III

HYDRATION OF THE PHOSPHATE GROUP

n_{H_2O}	Configuration of water molecules	$-\Delta F_{nhydr}$ (kcal·mol^{-1})
1	B_{13}	9.5
2	$E_{13}E_{31}$	18.9
3	$B_{13}E_{13}E_{31}$	24.1
4	$E_{13}E_{31}E_{14}E_{32}$	25.4
5	$B_{13}E'_{32}E'_{14}E'_{12}E'_{34}$	26.9

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* 1e.u. = cal·mol^{-1}·K^{-1}.

contribution from F_{chain} and F_{transl}. The calculations of the free energy for a layer of alkyl chains have been developed in our laboratory [5–7] using statistical mechanical treatments (Monte Carlo – Metropolis type) with a powerful sampling method. The values of F_{chain} have been determined for three temperatures (25, 60 and 100°C) which cover an appropriate range. The translational free energy, F_{transl}, is obtained from a cellular model modified by Lemaire and Bothorel [7].

By summing up all these contributions we compute total free energy curves as a function of the mean intermolecular interchain distance, $\langle d \rangle$, and the hydration state of the polar heads. The total free energy $F_{\text{total}} = f(\langle d \rangle, n_{H_2O})$ is represented as the enveloping curve of minimum energy involving the family of six curves corresponding to $n_{H_2O} =$ 0–5 (Fig. 4).

We will now examine more in detail the results for $t = 25$°C. The free energy curves exhibit the same characteristics we have already noticed for the interactions between headgroups. When n_{H_2O} equals 3 or 5 (Fig. 4) the curves are destabilized in energy and the minimum shifts to distances ($\langle d \rangle$) greater than 6.0 Å. This is certainly due to the existence of intrinsic steric repulsions which are important and cannot be compensated by the contribution of the chains and the hydration energy. The difference in energy between curves $n_{H_2O} = 0$ and $n_{H_2O} = 1$ comes from the energy of hydration for one water molecule and that effect is maximum for $n_{H_2O} = 4$. All curves ($n_{H_2O} = 1$ to 4) exhibit two more-or-less pronounced energy wells which correspond respectively to the gel and liquid crystal state of the phospholipid system. We have already encounted this type of curve for the aliphatic chains alone [5,7]. The explicit introduction of the polar heads, in a hydrated state, preserves that feature without the need of a parametric function which varies from author to author.

The enveloping curve is characterized by the existence of a large well (4.90–5.50 Å) for the gel phase with n_{H_2O} values equal to 1, 2 and 4 and a well at approx. 6.0 Å for the liquid crystal phase with $n_{H_2O} = 4$ (Fig. 4a).

At 25°C (Fig. 4a), the gel well has a free energy which is less than that of the liquid crystal well. The phospholipid system is predicted to be in the gel phase at that temperature and that does not contradict a transition temperature of about 60°C for dipalmitoylphosphatidylethanolamine [16]. As a result of the flat energy well of the gel phase it is possible to have the coexistence of several hydrated states with one, to or four water molecules. It seems possible to introduce up to four water molecules at the phosphate moiety level even beneath the transition temperature.

Very recent experimental work by Raman and infrared spectroscopy [30,31] concerning the carbonyl stretching vibrations of the β and γ hydrocarbon chains reveals similar features. The authors show that these bands are very sensitive to hydration, temperature and presence of cholesterol. Bush et al. [31] have investigated the effect of a stepwise hydration which involves one, two and four moles of water per mole of dipalmitoylphosphatidylcholine. All measurements are realised at $T < T_{\text{transition}}$ (gel phase) and they observe a small effect when passing from 1 to 2 mol water. The perturbation is more pronounced with 4 mol water and increasing the moles of water does not modify anymore the spectra any further. This agrees with our calculations, which indicate that it is impossible to introduce a fifth water molecule on the phosphate moiety. In the crystal structure of dipalmitoylphosphatidylcholine, Pearson and Pasher [28] and Albon [32] have observed the one and two hydration states.

Increase in temperature diminishes the gap between the gel and liquid crystal wells (Fig. 4b, c), but even at $T = 100$°C we are far from the transition which would correspond to $\Delta F = 0$ (the two wells at the same free energy level).

The liquid crystal well corresponds to the tetrahydration only. Our calculations suggest that the transition is accompanied by an increase in the number of bonded water molecules, which is in agreement with the experimental results [23,25]. To a first approximation, our hydration model reflects fairly well what is known about the hydration structure of the phosphatidylcholines; but the comparison with the thermodynamical properties of these headgroups cannot be pursued because we have, in fact, ethanolamine headgroups.

(III) Hydration of the phosphate and ammonium moieties of the polar heads

It turns out that we have to take into account

TABLE IV

PERTURBATIONS INDUCED FOR THE PHOSPHATIDYLETHANOLAMINE HEADGROUP CONFORMATION IN FUNCTION OF THE HYDRATION OF THE PHOSPHATE AND AMMONIUM GROUPS

Hydration	Modified torsional angles	Total
PE 7 → PE 703	$\theta_{11}\alpha_{11}\theta_{31}\theta_{41}\alpha_{63}\theta_{65}\theta_{71}\theta_{75}$	8
PE 703 → PE 713	$\theta_{11}\theta_{11}\alpha_{12}\alpha_{13}\theta_{31}\theta_{41}\theta_{51}\theta_{71}\alpha_{72}\alpha_{75}$	10
PE 713 → PE 723	$\theta_{11}\alpha_{11}\theta_{31}\theta_{41}\alpha_{45}\theta_{51}\alpha_{55}\theta_{61}\alpha_{65}\theta_{71}\alpha_{75}$	11
PE 723 → PE 733	$\theta_{11}\alpha_{11}\theta_{21}\theta_{31}\theta_{41}\alpha_{45}\alpha_{55}\theta_{61}\alpha_{65}\alpha_{71}\alpha_{75}$	10
PE 733 → PE 743	$\theta_{11}\alpha_{11}\alpha_{15}\alpha_{25}\theta_{31}\alpha_{35}\theta_{41}\alpha_{45}\theta_{52}\alpha_{52}\alpha_{55}\theta_{61}\alpha_{65}\theta_{71}\alpha_{71}\alpha_{75}$	16

the hydration of the whole headgroup which includes the second hydrophilic group, namely, the ammonium moiety. We consider the trihydrated state which corresponds to the first hydration shell. The energy value has been calculated by Pullman and Armbruster [15,16] and corrected as already described ($\Delta F_{n\,\mathrm{hydr}} = 21.4$ kcal · mol^{-1}).

In analogy to the hydration of the phosphate group, we obtain a qualitative picture of the perturbations induced for the headgroup conformation by considering the number of torsional angles which have to been modified to accomodate the water molecules. Table IV gives the total number of torsional angles which vary, and their type. We start from the reference state PE7 ($n_{H_2O} = 0$) and consider first the hydration of the ammonium moiety which corresponds to state PE7 03 (three water molecules on $-NH_3^+$ and zero on PO^{-4}). The subsequent water molecules are bonded to the phosphate moiety. From Table IV it appears that the θ_{i1} angles of the glycerol determine the position of the headgroups relative one to another. Starting from an identical value of θ_{i1} (180°) for the seven molecules we observe an important dispersion in function of the hydration (0°, 220°, 80°, 200°, 140°, 310°, 300°, 160°, 340°, 180°, 320°, 40°, 60°, 170°). There seems to exist a certain mobility of rotation of the headgroups around this C_2-C_3 bond. Pearson and Pascher [28] have shown by X-ray diffraction studies that this is really observed in the case of dimyristoylphosphatidylcholine [14] in the dihydrated state. The elementary cell contains two molecules, A and B, with different values for the θ_1 angle (58° and 169°, respectively).

The energy curves (Fig. 5) give a more quantitative picture of the hydration process. The form of these curves is, as before, characteristic for the potentials used (6-12-1; Lennard-Jones + coulombic interactions). The energy minima rise with the degree of hydration and it is quite difficult to introduce a fourth water molecule on the phosphate group when the ammonium moiety is fully hydrated.

It is now possible to compute total free energy curves in the same manner as before. We obtain curves as a function of the mean intermolecular interchain distance $\langle d \rangle$ and of the hydration state

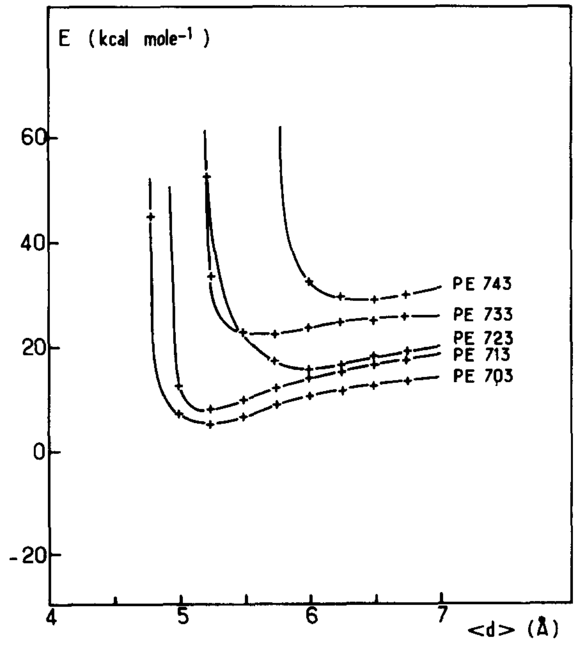

Fig. 5. Interaction energy curves of seven phosphatidylethanolamine molecules in function of the degree of hydration (n_{H_2O} varies from 0 to 4) of the phosphate; the ammonium group has three water molecules of hydration ($\epsilon = 20$).

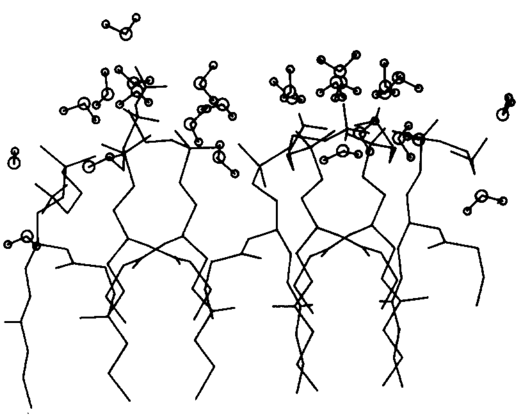

Fig. 6. Schematic representation of seven phosphatidylethanolamine molecules in the hydration state 13 (one water molecule on the phosphate and three water molecules on the ammonium).

(n_{H_2O}). In addition, we have considered phospholipids with different chain lengths (C_{12}, C_{14}, C_{16}) to compare with the experimental values of the transition temperature.

To begin with, we consider the dipalmitoylphosphatidylethanolamine (C_{16}) at $t = 25°C$. The enveloping curve reveals the existence of two energy wells, one corresponding to the gel phase (approx. 5.10 Å) and the second to the liquid crystal phase (approx. 5.90 Å). The energy difference of ΔF is about 2 kcal · mol^{-1}. The hydration state varies along the curve F_{tot} from the state 1/3 (Figs. 6 and 7a) to the state 2/3. The number of water molecules which it is possible to bind to the phosphate group is less than before, due to the hydration of the ammonium group. It is also well known that the ethanolamine headgroups are not so easy to hydrate as the choline group [33]. Again, at the transition there could be an increase in bonded water molecules, as suggested by some experimental results [23,25].

If we compare only the hydration of the phosphate moiety, the energy difference between the two wells (Fig. 3a) has diminished by 2 kcal · mol^{-1}, which constitutes an interesting trend.

The increase in temperature ($t = 60°C$) reduces the energy difference ΔF between the two wells to about 1 kcal · mol^{-1} (Fig. 7b). At $t = 100°C$ we

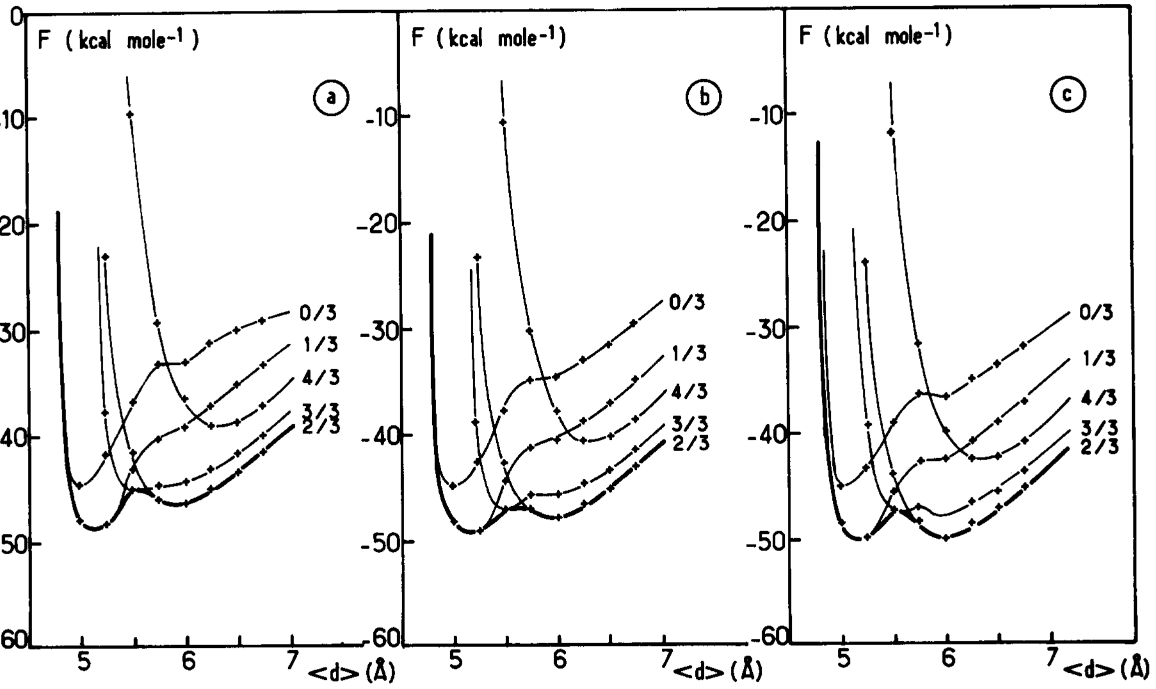

Fig. 7. Free energy curves for a dipalmitoylphosphatidylethanolamine molecules (C_{16}) asa function of the degree of hydration of the phosphate (n_{H_2O} varies from 0 to 4) and of the intermolecular interchain distance; the ammonium is in the trihydrated state ($\epsilon = 20$). (a) $t = 25°C$; (b) $t = 60°C$; (c) $t = 100°C$; ———, enveloping curve.

observe $\Delta F = 0$, which corresponds to the theoretcal phase transition (Fig. 7c). We are, of course, quite far from the experimental value determined for the dipalmitoylphosphatidylethanolamine, which is approx. 63°C. But if we consider the approximation of this model, especially as concerns the translational term, the result is fairly satisfying. Another point concerns the glycerol group, which we consider in a rigid conformation during these calculations to reduce the computing time. In fact, we have shown [34] that the intramolecular interchain distance can follow the intermolecular interchain distance during the transition phenomenon.

Another simplification concerns the headgroup conformations. We do not consider the conformational modifications which could occur when the distance between the molecules varies. This would necessitate an energy minimization for each free energy value, F, taking into account the continuous reorganisation of the polar heads. This would introduce an entropy term for the headgroup which we have considered negligible in our model.

In fact, we have used a relative static approach to investigate the hydration of phospholipid molecules. A more dynamic model (including statistical mechanical techniques) should give other information and could improve the results. This would, or course, require longer computational time.

With the shortening of the aliphatic chains, the experimental transition temperatures decrease. The same trend is observed with calculated values. On the curves it is visible (Figs. 8 and 9) that for the dimyristoyl- (C_{14}) and dilauroylphosphatidylethanolamine (C_{12}) the liquid crystal well has a lower free energy than the gel phase well. We can estimate the transition temperatures to be approx. 70°C for the C_{14} chains (approx. 50°C experimentally) and approx. 40°C for the C_{12} chains (approx. 30°C experimentally).

There remains a difference between calculated and experimental values, but with regard to the relative crude model we have improved the results obtained with the chain contribution only [6].

At the end of this work we have tried to define the parameters of the energy curve which reproduces the headgroup interaction. This curve is obtained as the difference between F_{tot} and the sum of F_{chain} and F_{transl} which gives E_{head} (Fig. 10). The best fit results from the use of a Morse-type function:

$$E_{\mathrm{head}} = E_0(1 - e^{-n\Delta\langle d\rangle})^2 - E_0$$

where E_0 corresponds to the energy minimum, n is

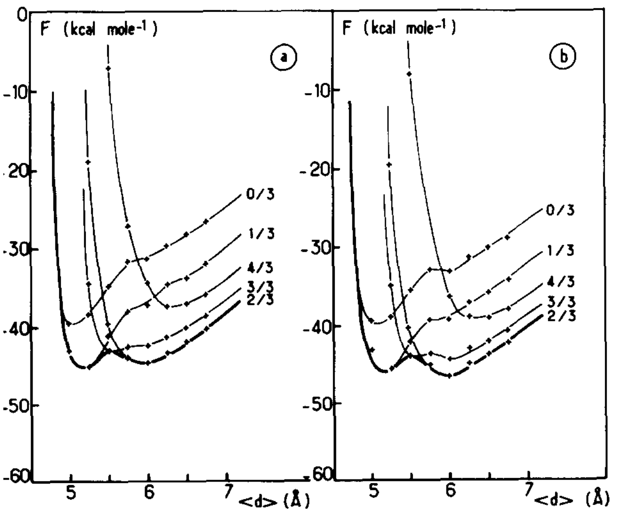

Fig. 8. Free energy curves for a dimyristoylphosphatidylethanolamine molecules (C_{14}). (a) $t = 60$°C; (b) $t = 100$°C; ———, enveloping curve.

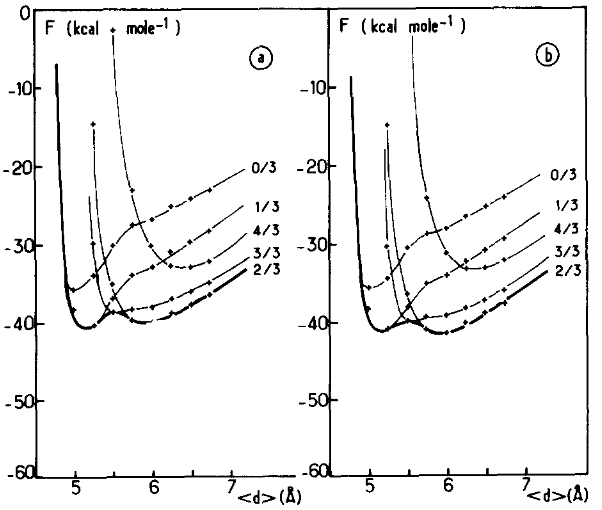

Fig. 9. Free energy curves for a dilauroylphosphatidylethanolamine molecule (C_{12}). (a) $t = 60$°C; (b) $t = 100$°C; ———, enveloping curve.

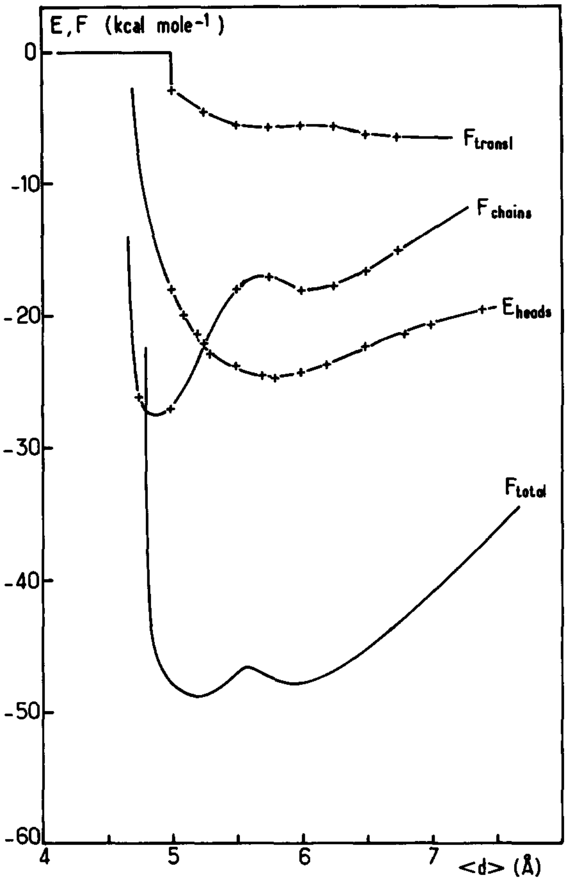

Fig. 10. Components of the total free energy (F_{tot}) with the contribution E_{heads} ($\epsilon = 20$) for the dipalmitoylphosphatidylethanolamine ($t = 60°C$).

an adjustable parameter and $\Delta\langle d \rangle = \langle d \rangle - \langle d_0 \rangle$ ($\langle d_0 \rangle$, position of the energy minimum).

We obtain the following values: $E_0 = 25$ kcal · mol^{-1}; $\langle d_0 \rangle = 5.9$ Å; $n = 0.52$ Å^{-1}, which gives

$$E = 25(1 - e^{0.52\Delta\langle d \rangle})^2 - 25$$

with E in kcal · mol^{-1} and $\Delta\langle d \rangle$ in Å.

This type of macroscopic representation for the interactions between headgroups could be used for other phospholipid systems. It implies, of course, that for choline or serine heads the values E_0, n and $\langle d_0 \rangle$ would be different.

It is interesting to compare the commonly used headgroup energy curve of the type C/A and our calculated curve (Fig. 11). The first decreases monotonously as a function of the intermolecular interchain distance. This function acts as destabilizing factor at short distances and contributes to shift the gel energy minimum to higher values compared to the liquid crystal minimum. The addition of this term to the total energy function allows us to achieve good agreement between calculated end experimental transition temperatures [6]. But it is necessary to adjust parameter c to obtain the response. The C/A curve has a passive behaviour.

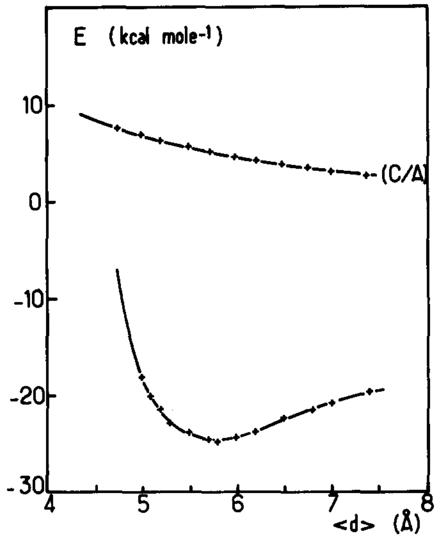

Fig. 11. Comparison of the headgroup contribution calculated by our method with the headgroup contribution of type C/A. (c is a parameter and A the surface per molecule; A and d are related through $A = \sqrt{3}/2\, d^2$).

We have tried to use a 'microscopic' approach to evaluate the interactions between headgroups and it seems that these interactions are more likely of 'active' nature because of the existence of an energy minimum at about 5.9 Å (Fig. 11).

Conclusion

In this work we have used the supermolecular approach which describes the organization of the solvent molecules around the solute in term of hydration sites.

It is thus possible to investigate the influence of the degree of hydration of the ethanolamine headgroup on the well-known phospholipid phase transition. Our model combines a classical theoretical conformational analysis method for the description of the polar heads with a statistical mechanical method for the treatment of the aliphatic chains. In this way we can describe, at the level of atom-atom interactions, a whole phospholipid molecule and evaluate the variation in its free energy as a function of both temperature and degree of hydration.

During the gel to liquid crystal transition the number of water molecules which are bonded to the headgroups increases. In a recent review article, Hauser et al. [35] proposed that this could happen at the phase transition to maintain the existence of a lamellar phase after the transition had occurred.

However, our molecular description is far from the complexity of the biological membranes and an improvement could be the inclusion of other membrane components such as cholesterol or proteins. A first stage could be to study the interactions between small peptides and phospholipids, a field in which many experimental results have been obtained in our laboratory and elsewhere.

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