Potential Energy Calculations on Phospholipids. Preferred Conformations with Intramolecular Stacking and Mutually Tilted Hydrocarbon Chain Planes†

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ABSTRACT: The preferred conformations of phospholipids have been obtained by nonbonded potential energy calculations. The results indicate that intramolecular chain stacking not only stabilizes but also restricts the favored conformations of phospholipids to a limited number of possibilities. The glycerol "backbone" of the phospholipid exhibits two orientations leading to two conformations (A and B) where the β chain folds onto the γ chain to initiate chain stacking. It is found in both the conformations that although the hydrocarbon chain axes are parallel, the planes containing the carbon atoms beyond the ester group of the β and γ chains intersect at a dihedral angle of 72° in conformation A and 57° in conformation B. This tilt or inclination of the two hydrocarbon chain planes results to optimize the interactions between the two fatty acid tails and may also be important in the intermolecular packing of phospholipids in membranes. The α chain of the phospholipid, however, can exhibit several conformations as a result of different energetically likely conformations for the phosphodiester and choline moieties. These different conformations may be influenced by the local metal ions and membrane-associated molecules.

Phospholipids and proteins constitute the major components of biological membranes. The phospholipids are known to occur in various proportions ranging from approximately 25% in the inner mitochondrial membranes to 80% in myelin. However, they retain the basic structural features and presumably play the same functional role in all the membranes. Consideration of biological membranes at the molecular level requires a detailed knowledge of the preferred conformations of phospholipids. Furthermore, conformational details of these "monomers" are of considerable importance in developing a reasonable model for the "macromolecular" structure of the membrane complex and in the understanding of lipid–protein interactions.

While X-ray structural data have been obtained on a few phospholipid constituents and their analogs (see review by Sundaralingam, 1972a), such data on phospholipids themselves are not available to date and thus information regarding their structures and molecular conformations is meager. The present study has therefore been undertaken to determine the preferred conformations for phospholipids from nonbonded energy considerations. In general, the typical short range intramolecular nonbonded interactions play an important role in determining the conformations of molecules. In phospholipids, these interactions appear to be the major factor in producing the energetically favored conformations. Similar studies on phospholipids are also being carried out by other workers (Vanderkooi, 1972a,b; S. P. Gupta and G. Govil, 1972, private communication).

Theoretical Methods

The schematic representation of the phospholipid molecule along with the notations to specify rotations about the various bonds are shown in Figure 1. The notations and the conventions used here are given in Table I and they are the same as suggested by Sundaralingam (1972a). The glycerol group which may be regarded as the "backbone" of phospholipids has been fixed in the L configuration found in biological systems. The polar head group, typically a choline phosphate (ethanolamine phosphate or serine phosphate), is designated the α chain and is attached to the C1 atom of the glycerol moiety. Long fatty acid chains make up the β and γ chains of the phospholipid and are esterified at the C2 and C3 carbon atoms of the glycerol, respectively. These chains generally contain 16–22 carbons with the β chain often being unsaturated.

Structural parameters consisting of bond lengths and angles used in obtaining the coordinates of different components of phospholipids have been taken from the compiled data of Sundaralingam (1972a). Normal values are assumed for the bond lengths (1.52 Å) and bond angles (112°) involving the carbon atoms of the β and γ chains. All hydrogens are fixed tetrahedrally with bond lengths of 1.1 Å.

Potential Energy Function. Potential energy calculations have been successfully employed in the analysis of conformations of polypeptides, polysaccharides, and nucleic acids. Different types of potential functions have been used by different workers in the quantitative estimation of the nonbonded interaction energy of a conformation. In the present study, the Lennard-Jones potential function of the type given in eq 1 has been used. The constant A has been evaluated

$$V_{\mathrm{nb}}(r) = -A/r^6 + B/r^{12} \qquad (1)$$

knowing the atomic properties of the interacting pair of atoms (Scott and Scheraga, 1966). The constant B has been obtained by minimizing the potential function at a value of r equal to the sum of the van der Waals radii of the interacting pair of atoms plus 0.2 Å (Brant *et al.*, 1967).

Results and Discussion

As is seen in Figure 1, there are quite a large number of

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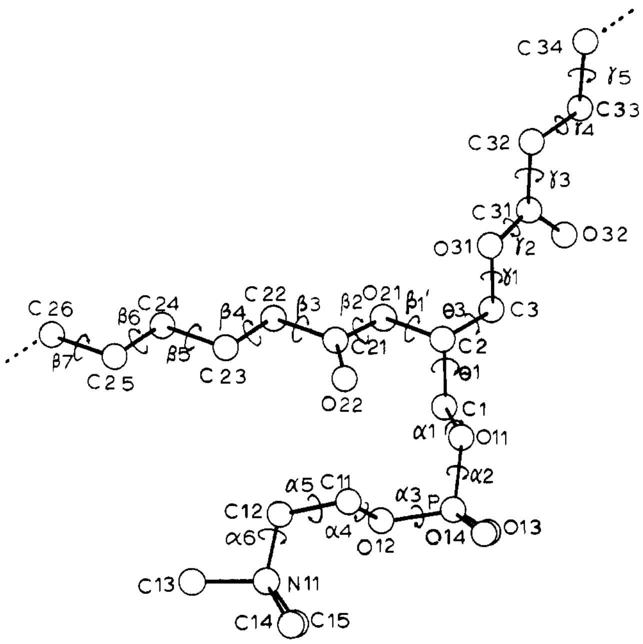

FIGURE 1: Schematic representation of the phospholipid molecule showing the atom numbering scheme and torsion angle notations (see Table I). Hydrogen atoms are omitted for clarity.

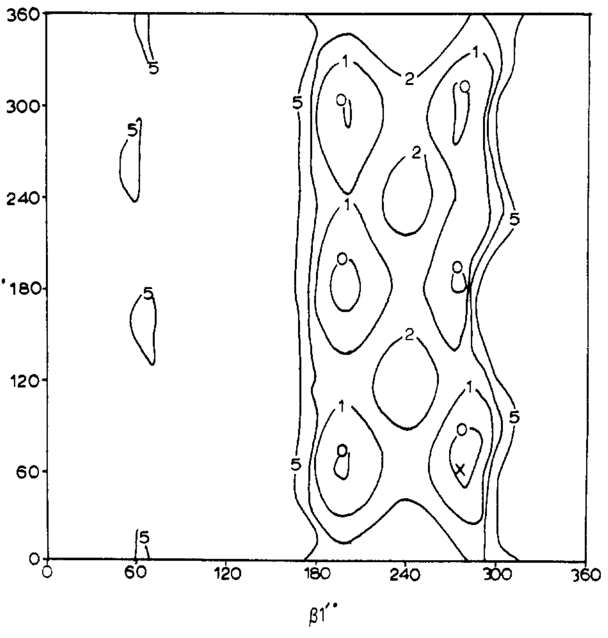

FIGURE 2: Contour map of the potential energy in kcal/mol obtained as a function of rotation angles $\theta 3$ and $\beta 1'$ with $\gamma 1 = \gamma 2 = \beta 2 = 180°$. The global minimum is marked by X.

single bonds about which rotations are possible and thus the conformational analysis of phospholipids is made considerably complicated. For this reason, the present calculations have been carried out in two stages. First the preferred conformations for the β and γ chains are established and later these calculations have been extended to the α chain. Nonbonded energy has been estimated as a function of a pair of rotation angles using eq 1 at intervals of 10° and the isoenergy contours thus obtained are shown at appropriate places in the discussion.

Conformation of the Glycerol Moiety. POTENTIAL ENERGY AS A FUNCTION OF $\beta 1'$ AND $\theta 3$. The glycerol portions of the phospholipids play a pivotal role in determining the overall conformation of the phospholipids in that the relative orientation of the α, β, and the γ chains are mainly governed by the conformation of this group. Therefore it is important to obtain first the preferred conformations of the glycerol moiety. Hence, potential energy has been computed as a function of the rotation angles $\beta 1'$ and $\theta 3$. In this calculation, those interactions which involve atoms beyond C22 in the β chain are not considered for the rotation $\beta 1'$. Similarly only the atoms through C32 are included for the rotation $\theta 3$. The interactions of these groups of atoms with the atom C1 have also been taken into account. The torsion angles $\gamma 1$, $\gamma 2$, and $\beta 2$ are fixed in the trans conformation. The nonbonded energy contours thus obtained are shown in Figure 2. It is seen from Figure 2 that two minima are found for values of $\beta 1' = 200$ and 270° separated by a region of relatively high energy. Further at each of these values of $\beta 1'$, there are three minima at values of $\theta 3$ corresponding to the three staggered positions ($\theta 3 = 60$, 180, and 300°) of the primary ester oxygen atom O31 with respect to the atom C1. Thus there are essentially six possible conformational combinations for the glycerol moiety. The lowest minimum occurs at $\beta 1' = 270°$ and $\theta 3 = 60°$. The five other minima differ from this by less than 0.5 kcal/mol.

TABLE I: Definitions and Notations of Torsion Angles for Phospholipids.

Notation	Torsion Angle	Notation	Torsion Angle
$\theta 1$	O11–C1–C2–C3	$\beta 1$	C1–C2–O21–C21
$\theta 2$	O11–C1–C2–O21	$\beta 1'$	C3–C2–O21–C21
$\theta 3$	C1–C2–C3–O31	$\beta 2$	C2–O21–C21–C22
$\theta 4$	O21–C2–C3–O31	$\beta 3$	O21–C21–C22–C23
$\alpha 1$	C2–C1–O11–P	$\beta 4$	C21–C22–C23–C24
$\alpha 2$	C1–O11–P–O12	βn	C2(n − 3)–C2(n − 2)–C2(n − 1)–C2n
$\alpha 3$	O11–P–O12–C11	$\gamma 1$	C2–C3–O31–C31
$\alpha 4$	P–O12–C11–C12	$\gamma 2$	C3–O31–C31–C32
$\alpha 5$	O12–C11–C12–N	$\gamma 3$	O31–C31–C32–C33
$\alpha 6$	C11–C12–N–C13	$\gamma 4$	C31–C32–C33–C34
		γn	C3(n − 3)–C3(n − 2)–C3(n − 1)–C3n

It is found from consideration of molecular models that the two conformations corresponding to ($\beta 1'$, $\theta 3$) = (200°, 300°) and (270°, 300°) will place the two fatty acid chains on opposite sides of the glycerol group and thus these conformations preclude the possibility of intramolecular stacking between the β and the γ chains. Although such open conformations are possible, they are not expected to be important in membranes since they do not conform to bilayer structure. The remaining four possible conformations show a tendency for stabilization by intramolecular chain stacking with the two acyl ester chains running parallel to each other. Hence only these are considered in further calculations.

Conformation of the Acyl Ester Groups. POTENTIAL ENERGY AS A FUNCTION OF $\beta 1'$ AND $\gamma 1'$. Energy variation in the $\beta 1'$–$\gamma 1$ surface has been studied as a function of $\theta 3$ to predict the preferred conformations of the two acyl ester groups attached

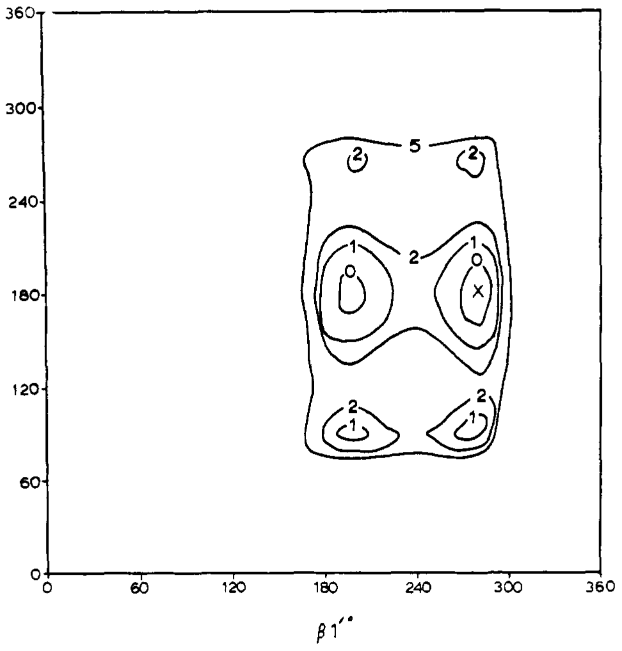

FIGURE 3: Contour map of the potential energy in kcal/mol obtained as a function of rotation angles $\gamma 1$ and $\beta 1'$ with $\beta 2 = \gamma 2 = 180°$ and $\theta 3 = 60°$. The global minimum is marked by X.

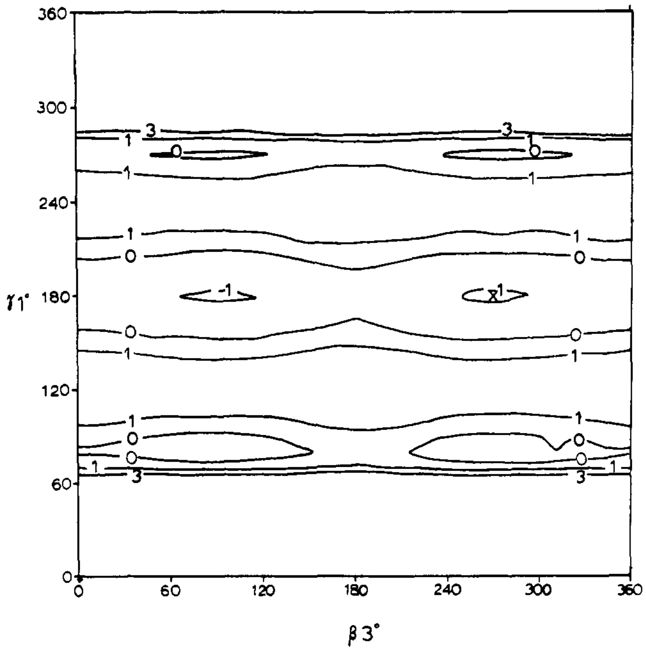

FIGURE 4: Contour map of the potential energy in kcal/mol obtained as a function of rotation angles $\gamma 1$ and $\beta 3$ with $\beta 2 = \gamma 2 = \theta 3 = 180°$ and $\beta 1' = 200°$. The global minimum is marked by X.

to the glycerol residue. Here, too, only those nonbonded pairs of interactions that depend on the rotations $\beta 1'$ and $\gamma 1$ are taken into consideration. Figure 3 shows the energy map obtained with $\theta 3$ fixed at one of the favored rotation angles (60°). It is seen that again two sets of minima are found for $\beta 1'$ at 200 and 270° with the global minimum occurring at ($\beta 1'$, $\gamma 1$) = (270°, 180°). The relative energy difference between this minimum and the one occurring at ($\beta 1'$, $\gamma 1$) = (200°, 180°) is only about 0.3 kcal/mol and hence these two conformations are considered to be possible. The other minima are higher in energy than the global minimum by approximately 0.8–2 kcal/mol. When the potential energy calculations are performed with $\theta 3$ fixed in the other favored conformation (180°), results essentially similar to above are found for the ($\beta 1'$, $\gamma 1$) map. This indicates that the most preferred conformation ($\gamma 1$) about the primary acyl ester linkage (C3–O31) is in the trans region and there are essentially two conformations for $\beta 1'$ (irrespective of whether the value of $\theta 3$ is 60 or 180°) in the region corresponding to the values of 200 and 270°, the latter being slightly more favored.

The phospholipid conformations where intramolecular chain stacking is possible are expected to be more stable due to hydrophobic interactions between the fatty acid tails. Molecular model studies (Sundaralingam, 1972a) have shown that there are essentially two possibilities for initiating the intramolecular stacking between the two fatty acid chains, namely, the γ chain remains in the trans conformation while the β chain folds to meet it or *vice versa*. In the present study, the former possibility is considered. (The latter possibility is currently being examined and will be reported later.)

POTENTIAL ENERGY AS A FUNCTION OF $\beta 3$ AND $\gamma 1$ WITH $\theta 3 = 180°$. With the above idea in view, the folding of the β chain has been achieved by varying the torsion angles $\beta 3$ and $\beta 4$ in conjunction with $\gamma 1$ to produce hydrophobic stacking. The other torsion angles ($\gamma 2$, $\gamma 3$, $\gamma 4$) in the γ chain are fixed in the trans conformation. Conformational energies have then been computed as a function of $\beta 3$ and $\gamma 1$ by fixing the torsion angles $\beta 1'$ and $\theta 3$ at their energetically favored conformations. This has been carried out for all combinations of the favored rotation angles of $\beta 1'$ and $\theta 3$. The occurrence of the planar ester groups in both the β and γ chains constrains the torsion angles $\beta 2$ and $\gamma 2$ to predominantly trans conformations which considerably simplify the conformational analysis. As mentioned eariler, all the atoms through C32 have been considered for $\gamma 1$ rotation. For $\beta 3$ rotation, atoms only up to C23 are taken into account because any unfavorable interactions introduced beyond this point could be relieved by rotation about the subsequent bonds. The energy variation in the $\beta 3$–$\gamma 1$ plane obtained with $\beta 1' = 270°$ is shown in Figure 4. It can be seen from figure that the lowest minimum again occurs at $\gamma 1 = 180°$ for $\beta 3 = 270$ and 90°. Two more regions of low energy occur for $\gamma 1$ near 90 and 270° and these are of higher energy by 0.5–1 kcal/mol, respectively. Similar conformational features are observed when the above calculations are repeated for $\beta 1' = 200°$.

In the same manner, when the above calculations are performed with $\theta 3$ fixed at the other preferred value (60°) and the torsion angle $\beta 1'$ set first at 200° and then at 270°, results essentially similar to that discussed above are obtained. These results again suggest that for all combinations of $\theta 3$ and $\beta 1'$, the trans conformation for the primary acyl ester group corresponding to the value of $\beta 1 \simeq 180°$ is energetically the most preferred. On the other hand, two equally favored conformations are found for $\beta 3$ corresponding to the values of 90 and 270°. It may be mentioned that the energy minimum obtained with $\beta 1'$ fixed at 270° is of slightly lower energy (though not significantly) than that obtained with $\beta 1' = 200°$ as noticed in previous cases.

The folding of the β chain may be regarded as complete with the $\beta 4$ rotation since, beyond this, all the conformational angles ($\beta 5$, $\beta 6$, etc.) are expected to take up essentially the trans conformation and hence rotations about the subsequent bonds do not produce any significant alterations in the stacking pattern. Hence, the $\beta 4$–$\gamma 1$ combination of rotations are

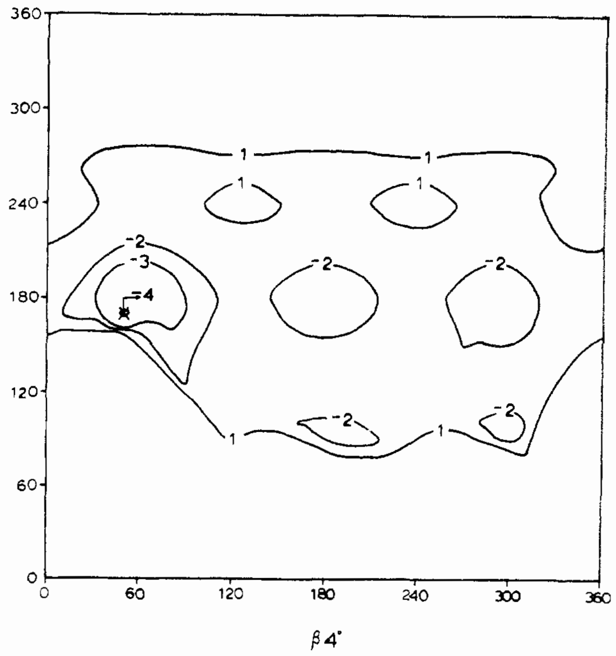

FIGURE 5: Contour map of the potential energy in kcal/mol obtained as a function of rotation angles $\gamma 1$ and $\beta 4$ with $\gamma 2 = \gamma 3 = \gamma 4 = 180°$, $\beta 5 = \beta 6 = 180°$, $\theta 3 = 60°$, and $\beta 1' = \beta 3 = 270°$. The global minimum is marked by X.

of extreme importance in optimizing the chain stacking interactions between the two fatty acid chains. In the following calculation, atoms through C26 and C34 of the β and γ chains, respectively, have been considered with the terminal atoms replaced by methyl groups. The torsion angles $\gamma 2$ through $\gamma 4$ and $\gamma 5$ and $\gamma 6$ are fixed in the trans conformation. Interestingly, it has been found from the results discussed above and from careful molecular model building studies that only the conformation corresponding to $\beta 3 = 270°$ is able to produce chain stacking interactions for all the four combinations of $\beta 1'$ and $\theta 3$. The other preferred conformation of $\beta 3 = 90°$ will take the β chain away from the γ chain and hence this conformation is not important from the point of view of stacking. Hence in subsequent calculations only the conformation corresponding to $\beta 3 = 270°$ has been considered. However, the influence of $\beta 1'$ and $\theta 3$ on the $\beta 4$–$\gamma 1$ energy plane has been studied in detail.

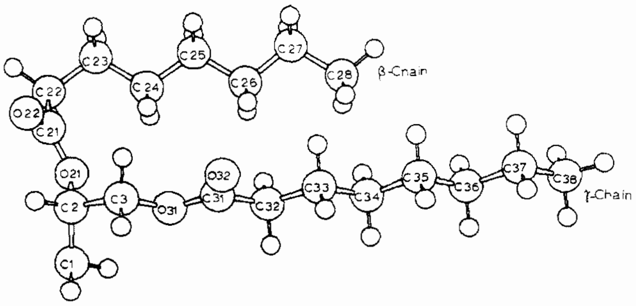

FIGURE 6: Perspective view of the conformation A (model 1) showing the stacking between β and γ chains. The dihedral angle between the chain planes is 72°. Atoms beyond C26 and C34 in the β and γ chain, respectively, are not taken into account in the calculation but they are shown in figures to stress that with longer chains these conformations would be further stabilized or lead to skew conformations as the case may be (see Figure 7). α chain is not shown for clarity.

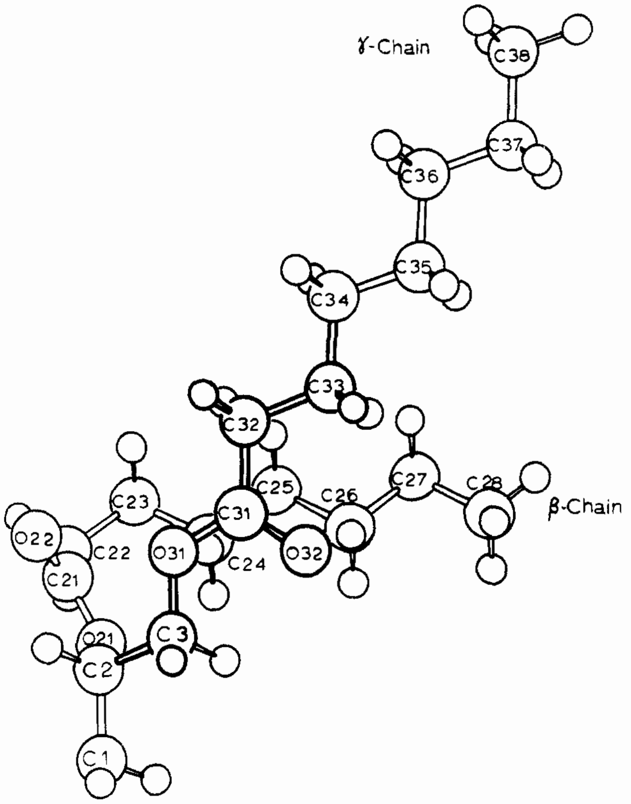

FIGURE 7: Perspective view of a typical skew (nonstacked) conformation. α chain is now shown for clarity.

POTENTIAL ENERGY AS A FUNCTION OF $\beta 4$ AND $\gamma 1$ WITH $\theta 3 = 60°$. Figure 5 shows the energy variation in the $\beta 4$–$\gamma 1$ surface obtained for $\beta 1' = 270°$ with $\theta 3$ fixed at one of the preferred rotation angles (60°). It is seen from the figure that the lowest minimum (which we refer to as A) occurs at ($\beta 4$, $\gamma 1$) = (50°, 170°). In this conformation (Figure 6), the axes of the two fatty acid chains are found to run almost parallel to each other. The potential energy minimum corresponding to this conformation is due mainly to the hydrophobic interactions of the methylene groups of the hydrocarbon chains. It is noteworthy that the planes containing the carbon atoms beyond the ester groups of the γ chain and the β chain intersect at a dihedral angle of 72°. This "tilt" of the hydrocarbon chain planes results to optimize the interactions between the fatty acid tails.

The other minima which arise as a result of favorable interactions between the portions of the fatty acid tails proximal to the glycerol in the $\beta 4$–$\gamma 1$ energy map are of higher energy than the lowest minimum by more than 2 kcal/mol. These minima will lead to skew conformations as shown in Figure 7, with increase in length of the fatty acid chains. On the other hand, the conformation corresponding to the global minimum would be further stabilized with increase in the lengths of the β and γ chains (Figure 6).

With the $\beta 1'$ torsion angle fixed at the other preferred value (200°), no satisfactory model for chain stacking emerges. The conformations corresponding to the minima in the $\beta 4$–$\gamma 1$ energy plane are again found to lead to the skew conformations of the type mentioned above. The various torsion angles corresponding to the conformation A are given in Table II.

TABLE II: Preferred Conformations for the Phospholipid Tails.

Conformation	$\theta 3°$	$\theta 4°$	$\beta 1'°$	$\beta 3°$	$\beta 4°$	$\beta 5°$	$\beta 6°$	$\gamma 1°$	$\gamma 2°$	$\gamma 3°$	$\gamma 4°$	$\phi^{\circ a}$
A	60	300	270	270	50	180	180	170	180	180	180	72
B	180	60	270	270	290	180	180	190	180	180	180	57

[a] ϕ = the dihedral angle between the hydrocarbon chain planes.

POTENTIAL ENERGY AS A FUNCTION OF $\beta 4$ AND $\gamma 1$ WITH $\theta 3 = 180°$. The potential energy variation obtained with $\beta 1'$ fixed at 270° shows the lowest minimum (hereafter referred to as B) at ($\beta 4$, $\gamma 1$) = (290°, 190°). It is found that this conformation (see Table II) provides good stacking between the β and γ chains (Figure 8). Interestingly, it is again noticed that though the hydrocarbon chain axes are parallel, the chain planes are inclined, *i.e.*, the planes containing the carbon atoms of the fatty acid chains beyond the ester group intersect at a dihedral angle of 57°. Another noteworthy feature in this conformation is that the hydrogen atoms of the β chain fall almost in between those of the adjacent carbon atoms of the γ chain (Figure 8). The other minima in the potential energy surface correspond to nonstacked conformations of the skew type mentioned above and are also of higher energy than the global minimum.

When the calculations are repeated for $\beta 1' = 200°$ with $\theta 3$ fixed at 180°, only one conformation (C) corresponding to the values of ($\beta 4$, $\gamma 1$) = (60°, 180°) is found to lead to a structure where there is a fairly good stacking between the two chains. This conformation (C) may probably assume importance with increase in the hydrocarbon chain lengths. It is interesting that in contrast to the conformation (B) (Figure 8), the hydrogens of the β chain point nearly directly at those of the γ chain in this conformation (C). This clearly accounts for the relatively higher instability (1 kcal/mol) of the latter conformation (C) compared to the former (B).

As discussed earlier in this section, the minima other than the global minimum represent interaction between portions of the fatty acid tails proximal to the glycerol residue (Figure 7). But when the longer chains are considered, these conformations lead to skew-type structures and will undoubtedly decrease in importance since the conformation corresponding to the global minimum will be greatly stabilized by enhanced hydrophobic interactions.

Conformation of the α Chain. The α chain may be regarded as a phospholipid "side chain" attached to the glycerol residue and comprising a phosphodiester and a choline or ethanolamine or serine moiety. It is essential to know the conformations of these constituents in order to elucidate the conformation of the α chain relative to the β and γ chains.

CONFORMATION OF THE PHOSPHODIESTER MOIETY. It is known from studies on the constituents of phospholipids (Sundaralingam, 1969, 1972a) that the most preferred conformations ($\alpha 2$ and $\alpha 3$) around the two P–O bonds are the double gauche conformations (g^+, g^+) = (60°, 60°) and (g^-, g^-) = (300°, 300°). The other possible conformations which have slightly higher energy than these conformations are (g^+, t), (t, g^+), (t, g^-) and (g^-, t). The remaining three staggered conformations (g^+, g^-), (g^-, g^+) and (t, t) possess considerably higher energies due to unfavorable steric or electronic interactions (Sundaralingam, 1969, 1972b). The above information has been utilized in the investigation of the α chain conformation.

CONFORMATION OF THE CHOLINE MOIETY. Potential energy has been computed as a function of rotation angles $\alpha 4$ and $\alpha 5$. In this calculation, the phosphate oxygens O11, O13, and O14 and methyl groups attached to the quaternary nitrogen are kept in an orientation such that any two oxygens or the methyl groups are staggered with respect to the atom C11. The atoms beyond O11 are not considered in the calculation. The potential energy map thus obtained indicates that there is a strong preference for the trans conformation for $\alpha 4$ whereas all the three staggered conformations are possible for $\alpha 5$. However, all the observed conformations in phosphorylcholine and related compounds are in the ($\pm$)-gauche conformation for $\alpha 5$ (Sundaralingam, 1972a). This is mainly due to the influence of the electrostatic interactions between the positively charged ammonium (or quaternary ammonium) and the negatively charged phosphate groups (Sundaralingam, 1968; Pullman *et al.*, 1971).

It therefore appears that the energetically most preferred conformational combinations for the phosphodiester and choline moieties are (g^-, g^-)-g^+ (referred to as conformation 1) and (g^+, g^+)-g^- (referred to as conformtion 2) corresponding to the rotational angles $\alpha 2$, $\alpha 3$, and $\alpha 5$, respectively. The other combinations such as (g^+, g^+)-g^+ and (g^-, g^-)-g^- appear to be less favored. The preferred rotation about the C11–C12 bond ($\alpha 4$) is centered around the trans conformation. The energetically probable conformational angles for the phosphorylcholine moiety are given in Table III.

RELATIVE CONFORMATION OF THE α, β, AND γ CHAINS. The relative conformations of the α chain with respect to the two stacked conformations (A and B) of the β and γ chains (Table II and Figures 6 and 8) have been studied by fixing the phosphorylcholine in its energetically favored conformations and estimating the potential energies as a function of the rotation angles $\theta 1$ and $\alpha 1$ about the bonds C1–C2 and C1–O11. The equienergy contour map thus obtained for conformation A2 (Table III) of the β and γ chains is shown in Figure 9. For rotation $\theta 1$, only atoms through C22 and C32 of the β and γ chains, respectively, have been considered, while for rotation $\alpha 1$ the entire phosphorylcholine group is included. It is seen from Figure 9 that three

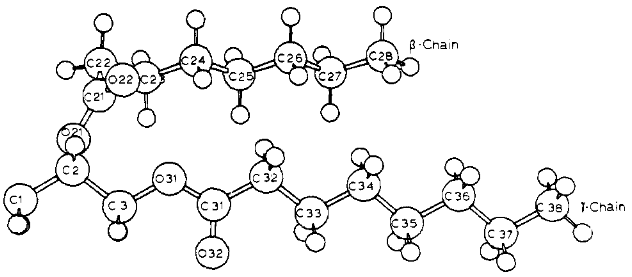

FIGURE 8: Perspective view of the conformation B (model 2) showing the stacking between β and γ chains. The dihedral angle between the chain planes is 57°. α chain is not shown for clarity.

TABLE III: Preferred Conformations of the Phosphorylcholine Moiety.

Conformation[a]	$\alpha2°$	$\alpha3°$	$\alpha4°$	$\alpha5°$	$\alpha6°$
A1	300	300	180	60	180
A2	60	60	180	300	180
B1	300	300	180	60	180
B2	60	60	180	300	180

[a] A and B refer to the conformations of the lipid tails given in Table II. The numerals (1 and 2) which follow the letters A and B denote the two predominant conformations of the phosphorylcholine moiety.

minima occur corresponding to the three staggered conformations of $\theta1$ with $\alpha1$ being trans, the lowest minimum occurring near ($\theta1$, $\alpha1$) = (g^+, t). The low energy of this conformation is found to result from favorable interaction of the ethylene portion of the choline group with the γ chain. In the presence of a highly polar solvent (water in the case of membranes), such conformations are not expected to occur due to the more favorable interaction of the charged group with the solvent dipole. Further this conformation would interfere with the intermolecular packing of the phospholipids in the membrane. From these considerations, it is believed that such conformations may not be important in general membrane structure. Other conformations corresponding to the minima occurring in the regions (t, t) and (g^-, t) for ($\theta1$, $\alpha1$) place the charged choline phosphate in an extended conformation with respect to the lipid tails allowing for better solvent interaction (Figure 10).

When the above calculations are repeated for conformation B1 (Table III), minima are again found at ($\theta1$, $\alpha1$) $\simeq$ (g^+, t), (t, t), and (g^-, t). Essentially similar results have been obtained when the calculations are carried out for conformations A1 and B2 (Table III). Thus, it is seen that rotation about the C1–O11 bond ($\alpha1$) favors the trans conformational range. In fact, the torsion angles $\alpha1$ found for the six known phospholipid constituents fall in this range (Sundaralingam, 1972a). On the other hand, $\theta1$ can assume all three staggered conformations depending on the conformation of the glycerol moiety.

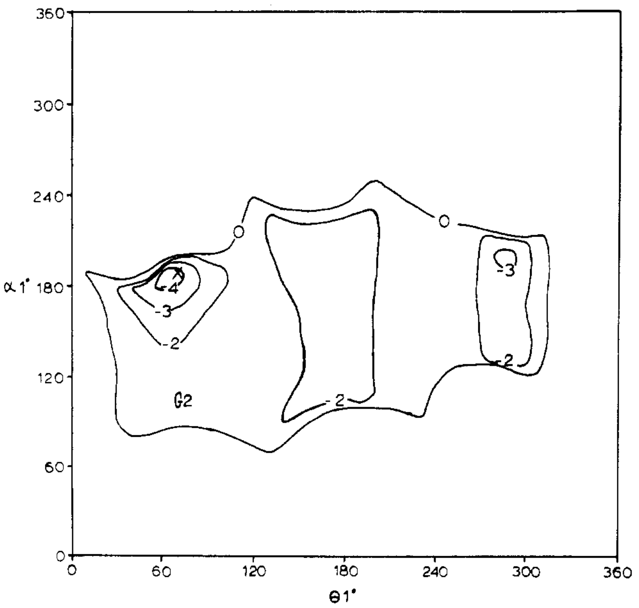

FIGURE 9: Contour map of the potential energy in kcal/mol obtained as a function of rotation angles $\theta1$ and $\alpha1$ with remainder of the molecule fixed in conformation B1 (Table II).

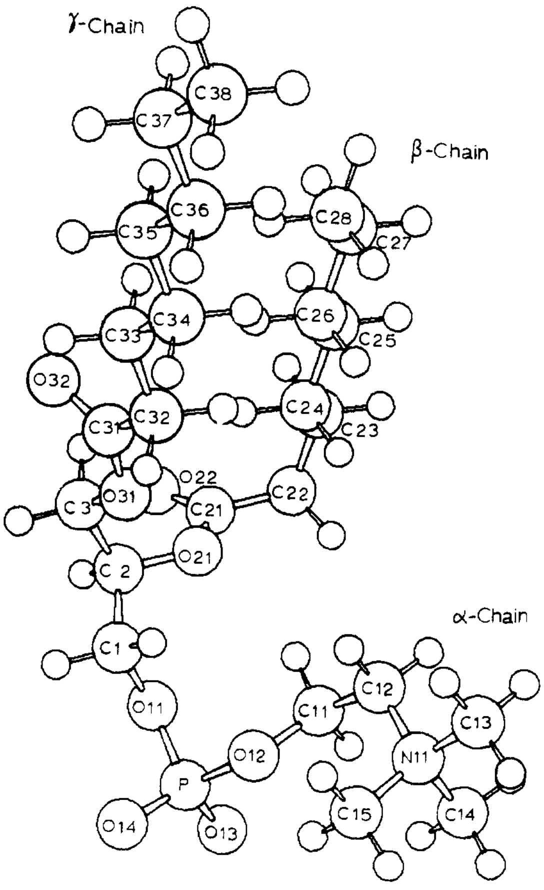

FIGURE 10: Perspective view of the conformation B1 (Table II) with the α chain. This represents one of the preferred conformations of the α chain with respect to the β and γ chains.

Conclusions

The most significant result of this study is that the number of possible conformations for the phospholipids is considerably restricted. The conformation of the glycerol moiety mainly determines the relative orientations of the two fatty acid tails and is instrumental in promoting stacking interactions between them. There are essentially two favored conformations for the torsion angles $\theta3$ and $\beta1'$ of the glycerol "backbone" in the regions 60° and 180° and 200° and 270°, respectively, which generate stacking between β and γ chains. The torsion angle $\beta4$ is correlated with the conformational changes in $\theta3$, assuming the values of 60 and 300° when $\theta3$ takes the values of 60 and 180°, respectively. Thus, two major conformations (A and B) are found for the diacylglycerol portion of the molecule. In these conformations, the two hydrocarbon chain planes, comprising carbon atoms beyond the ester group, are mutually tilted and they intersect at dihedral angles of 72 and 57°, respectively, while the planes through the atoms of the acetate group of the two chains are approximately perpendicular to each other.

It is noteworthy that similar conformational features are observed in the recently reported X-ray crystal structure of the sulfolipid, glycerol 1,2-bis(11-bromoundecanoate) 3-(*p*-toluenesulfonate)[1] (Watts *et al.*, 1972). In this structure, the chain axes are approximately parallel with their hydrocarbon chain planes intersecting at a dihedral angle of 79° which is strikingly similar to conformation A (72°). This tilt or inclination of the chain planes results to optimize the hydrophobic interactions between the hydrocarbon tails and may possibly be important for the packing of phospholipids in membranes. Further, it is seen that electron spin resonance experiments utilizing spin-labeled phospholipids (Libertini *et al.*, 1969; Hubbell and McConnell, 1969), and low-angle X-ray diffraction (Levine and Wilkins, 1971) and birefringence (Schmitt and Bear, 1937) studies on oriented lipid bilayers, membrane dispersions and nerve fiber membranes yield results which indicate that though there is considerable thermal motion in the fatty acid tails, as well as possible bending of these chains (McConnell and McFarland, 1972), the hydrocarbon tails are extended and approximately perpendicular to the plane of the membrane with their axes nearly parallel. It might be expected then from the present analysis that the two hydrocarbon chains of the phospholipids are held together by stacking interactions with their chain planes mutually tilted with respect to each other.

While the conformations found in this study are well defined, the energy minima for the torsion angles involved are sufficiently broad to allow for small fluctuations in the orientation of the β and γ chains without completely destroying intramolecular chain stacking. These small changes in torsion angles near the proximal end of the fatty acid ester would be amplified at the distal end to allow considerable motion of the terminal methyl groups (besides the rotational motions of the terminal methyl groups themselves). Thus, the proposed conformations for the phospholipids not only provide maximal intramolecular interactions but also explain the observed thermal fluidity (McConnell and McFarland, 1972) of the membrane phospholipids.

The nonstacked conformations of the β and γ chains corresponding to the gauche conformation ($\theta 3 = 300°$) about the C2–C3 bond of glycerol are probably not of much importance in membrane structures since they place the two hydrocarbon tails at approximately 180° with respect to each other. This is also true of the skew conformations where one chain runs in a direction approximately perpendicular to the other (Figure 7). Such types of conformation are obtained when the other energetically favored torsion angles (other than those leading to stacking interactions) are considered. While the skew conformations could pack regularly in a two-dimensional array with the polar head groups in the plane of the membrane, it is difficult to conceive of a three-dimensional packing scheme in which the polar groups lie on the surface of the membrane. Thus, while these conformations may play a role in membrane structure (particularly in regions containing intrinsic proteins), they may be regarded as less likely in view of the results of the physicochemical studies cited above and their inability to pack regularly. Hence, it is anticipated that conformations having intramolecular stacking will predominate and these conformations will be expected to be further stabilized by intermolecular stacking between the neighboring molecules.

The α chain possesses several preferred conformations as a result of different energetically likely conformations for the phosphodiester and the choline moieties. Further, the rotation $\theta 1$ which determines the relative orientations of the α and the stacked β and γ chains can adopt three staggered conformations depending on the conformation of the glycerol moiety (see text). These provide sufficient flexibility (which should not be confused with the thermal fluidity of the fatty acid chains and their terminal methyl groups) to the α chain to orient itself to optimize interactions of the head group with other molecules and membrane-associated ions and molecules (proteins, etc.).

The major phospholipids occurring in membranes are structurally quite similar, the main difference being in the substitution of phosphorylserine or phosphorylethanolamine in place of phosphorylcholine considered here. The conformations of the diacylglycerol portions of these molecules are not expected to differ markedly from those reported here since the α chains of the phospholipids do not interact (intramolecularly) to a significant extent with the β and γ chains.

The present analysis has provided information on the preferred conformations of the phospholipids in membranes. It is hoped that this knowledge will not only be helpful in the understanding of intermolecular packing of phospholipids in membranes and phospholipid bilayers but also in the interactions between phospholipids and membrane associated ions and molecules.

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[1] Note that in the numbering scheme of Figure 1, this would be glycerol 2,3-bis(11-bromoundecanoate) 1-(*p*-toluenesulfonate).