Structural analysis of phosphatidyl choline lipids and glycerol precursors

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Abstract: The structures and stabilities of the glycerol (G) and glycerol 3-phosphate (G3P) isomers have been calculated in the gas phase, using an ab initio density functional theory (DFT) method. The different conformational structures are shown to be at the origin of the various phospholipid conformers, except, obviously, for the alkyl chain torsions. The G3P conformations have been examined taking into account the experimental structures of the complexation sites in the glycerol kinase (GK) and glycerol 3-phosphate acyl transferase (G3PAT) enzymes, which correspond to the first and second steps of the "de novo" phospholipid biogenesis, respectively. The conformational analysis of the glycerophosphate skeleton is shown to determine most of the structural characteristics of the phosphatidyl choline lipids, which differ by the length of their diacyl chains, i.e., dilauroyl (DL), dimyristoyl (DM), and dipalmitoyl (DP) phosphatidylcholines (PC). Higher energy conformers with kinks in the acyl chains have been found, in preparation for molecular dynamics studies of the chain melting phase transformation.

Key words: glycerol, glycerol 3-phosphate, phosphatidylcholines, DLPC, DMPC, DPPC, conformational analysis, density functional theory, DFT-D.

Résumé : Les structures et stabilités relatives du glycérol (G) et du glycérol 3-phosphate (G3P) ont été calculées en phase gaseuze avec une méthode ab initio, basée sur la théorie de la fonctionnelle de la densité (TFD). A l'exception des formes gauches des chaînes alkyles, les différentes conformations de ces molécules sont effectivement à l'origine des conformères possibles des phospholipides. Les conformations de G3P ont été analysées en considérant les structures expérimentales des sites de complexation des enzymes glycérol kinase (GK) et glycérol 3-phosphate acyl transférase (G3PAT) qui correspondent, respectivement, aux deux premières étapes de la biosynthèse « de novo » des phospholipides. L'analyse conformationnelle du squelette glycérophosphate montre qu'il détermine la plupart des caractéristiques structurales des lipides phosphatidylcholines qui diffèrent par la longueur de leurs chaînes alkyles, c.-à-d. dilauroyl (DL), dimyristoyl (DM) et dipalmitoyl (DP) phosphatidylcholines (PC). Des conformères d'énergie plus élevée, présentant des formes gauches dans les chaînes alkyles, ont été étudiés en relation avec les simulations par dynamique moléculaire des transformations structurales des chaînes, au cours de la transition principale de phase.

Mots-clés : glycérol, glycérol 3-phosphate, phosphatidylcholine, DLPC, DMPC, DPPC, analyse conformationnelle, théorie de la fonctionnelle de la densité, TFD-D.

Introduction

Phospholipids and proteins are the main components of biological membranes, separating the cell and its various constituents from their environment. Cell membranes also have different functional roles, including protein synthesis, ion pumps and channels, receptor molecules, energy production and storage, and so forth. This versatility is due to the large range of phospholipid and protein structures, which can be involved in the membrane bilayers. The necessary fluid plasticity of these bilayers is obtained by the regulation of the lipid composition in the membrane. This regulation is accomplished by the "de novo" synthesis of the lipids in the various compartments of the cells of all living organisms.[1]

The synthesis protocol, in particular for the glycerophospholipids studied in this work, is a long and complex pathway, which starts with the enzymatic phosphorylation of the glycerol molecule and proceeds by three further successive enzymatic reactions.[2,3] The phospholipid molecule is thus built step by step through different enzyme-catalyzed reactions. The location in the enzymes of the different substrate-binding sites and their selectivity have not been identified unambiguously.[4–8] The structures of the phospholipid molecules synthesized and transferred to the membranes have thus not been determined, and there is no information concerning the existence of possible different conformers.

The presence of such conformers within phospholipid bilayers in their liquid-crystalline state has, however, been

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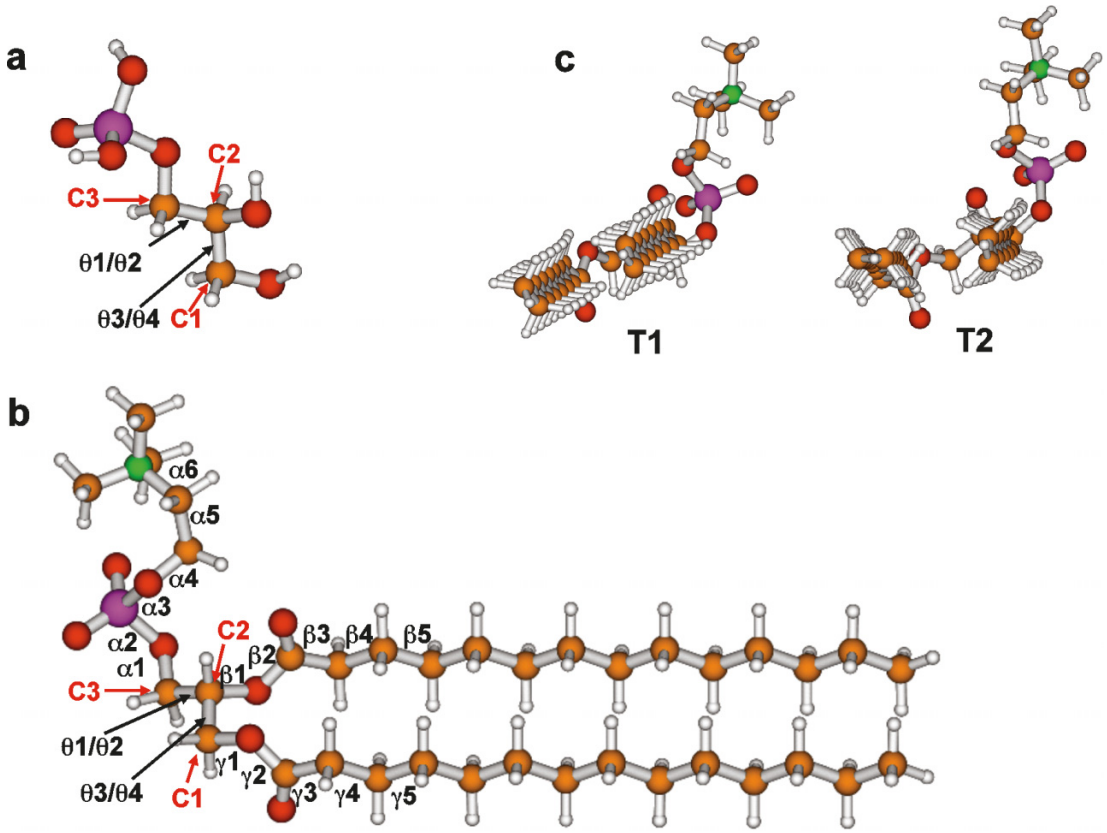
Fig. 1. Nomenclature of the atoms and torsion angles: (a) the glycerol molecule; (b) the DMPC molecule; (c) the DMPC molecule with parallel (T1) and perpendicular (T2) alkyl chains.



suggested from NMR experiments, that have also provided some structural information on the overall structure of the lipids.[9,10] X-ray diffraction data demonstrate the possible presence of two structurally different molecules in the solid-crystalline state of a few phospholipids.[11,12] In a previous theoretical work on the dimyristoyl phosphatidylcholine (DMPC) molecule, we have shown that several conformational structures can exist (minima on the potential-energy surface), a number of them having comparable energies.[13] The dynamics of the individual DMPC has been studied for a series of temperatures, and the nature of lipid melting has been elucidated.[14] The existence in the membranes of different lipid conformers, even iso-energetic ones, depends, of course, on the barriers for their in situ interconversion. One may also infer that the synthesis pathway itself selects the lipid conformations within the specific enzymatic catalytic sites at one or at several steps of the synthesis.

In this work, we present the structures and stabilities of the glycerol (G) and glycerol 3-phosphate (G3P) isomers, calculated in the gas phase, using an ab initio density functional theory (DFT) method. We show that their different conformational structures are at the origin of the various phospholipid conformers, except, obviously, for the alkyl chain torsions. The G3P conformations are then examined taking into account the experimental structures found for the complexation sites in the glycerol kinase (GK) and glycerol 3-phosphate acyl transferase (G3PAT) enzymes, which correspond to the first and second steps of the "de novo" phospholipid biogenesis, respectively. Finally, the conformational analysis of the glycerophosphate skeleton is shown to determine most of the structural characteristics of the phosphatidyl choline lipids, which differ by the length of their diacyl chains, i.e., DMPC, dilauroyl (DL), and dipalmitoyl (DP) phosphatidylcholines (PC).

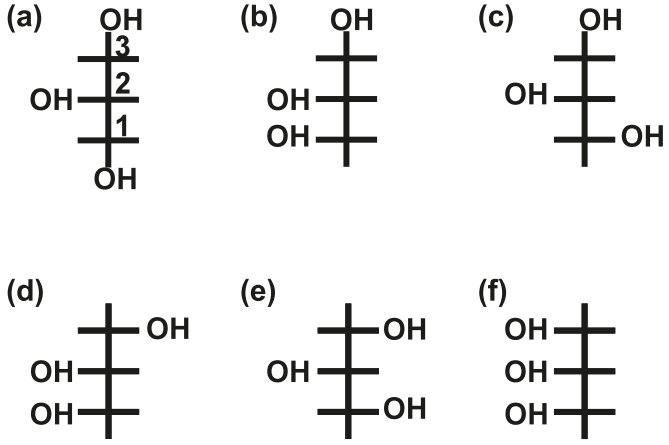
Computational methods

Models

The carbon numbering (C1, C2, and C3), illustrated in Figs. 1 and 2, refers to the standard biochemical nomenclature defined for the glycerol molecule: if the hydroxyl group on C2 is oriented to the left, C1 is the carbon below and C3 the carbon above. When the hydroxyl on C3 is phosphorylated, the glycerol 3-phosphate molecule formed is chiral. Its in vivo synthesis produces, following the stereospecific numbering (sn) system, the sn-glycerol 3-phosphate. The diacyl phosphatydilcholine lipids have been generally characterized as a head group or α chain that is connected through one CH_2 group (C3 carbon) to two glycerolipid β and γ chains, as shown in Fig. 1b. The nomenclature defining the characteristic lipid torsion angles is illustrated in Fig. 1.

In addition, for a better understanding of the torsion angles, we have chosen to partition the molecule into four building blocks, labelled as head (H), neck (N), body (B), and tails. The head is built from the $N(CH_3)_3$–CH_2–CH_2–PO_4 atoms and is described by the α1 to α6 torsion angles. The neck includes the glycerol C3–C2–C1 fragment, characterized by the θ1 and θ2 angles, and defines the orientation of the head with respect to the carbonyl groups and the tails. The body is formed by the next two O–CO–CH_2–CH_2

Fig. 2. The glycerol conformers with the standard nomenclature: C2, secondary carbon; C3, carbon where phosphorylation will occur.



groups of the β and γ chains and is characterized by the θ3, θ4, β1 to β4, and γ1 to γ4 torsion angles. The tails consist of the two β and γ alkane chains and are defined by the β_n and γ_n torsions with $n \geq 5$. Following the calculations for the DMPC molecule,[13] for the search of the ground-state conformers, only trans β and γ angles ($n \geq 5$) have been considered for the starting geometries.

The DLPC and DPPC lipid molecules differ from DMPC only in their hydrocarbon chain lengths, namely, 12, 14, and 16 carbons for DLPC, DMPC, and DPPC, respectively.

Methods

The calculations were performed in the framework of density functional theory (DFT), using a linear combination of Gaussian orbitals, as implemented in the deMon2k program.[15] All calculations were performed using the revised PBE exchange functional (revPBE)[16] and the LYP[17] correlation functional, augmented by a damped empirical correction for dispersion-like interactions,[18] referred to as DFT-D. This DFT-D approach is necessary to account for the stabilizing interaction between the alkyl chains.[19]

DFT-optimized double-zeta plus valence polarization (DZVP) basis sets[20] were employed for all atoms, associated with automatically generated functions up to l = 2 for fitting the density.[15] The exchange-correlation potential was numerically integrated on an adaptive grid.[21] The grid accuracy was set to 10^{-5} in all calculations. The Coulomb energy was calculated by the variational fitting procedure proposed by Dunlap, Connolly, and Sabin.[22,23] A quasi-Newton method in internal redundant coordinates with analytical energy gradients was used for the structure optimization.[24] The convergence was based on the Cartesian gradient and displacement vectors with a threshold of 10^{-4} and 10^{-3} au, respectively.

Results and discussion

Conformational analysis of the glycerol and glycerol 3-phosphate molecules

The in vivo phosphatidyl choline lipid synthesis involves first the phosphorylation of glycerol in the GK enzyme, at the expense of ATP (adenosine triphosphate). The product formed is exclusively the G3P molecule (phosphate group at C3), which is further esterified at the free primary alcohol with one fatty acid molecule by an acyl transferase enzyme using an acyl carrier protein (ACP) or an acyl coenzyme A (CoA) as substrate. A second esterification at the secondary carbon leads further to a phosphatidate or phosphatidic acid including two fatty acid chains. The phosphatidate phosphatase enzyme, which regulates the glycerolipid synthesis, catalyzes its hydrolysis into diacylglycerol (or diglyceride). The reaction of this molecule with the cytidine diphosphate (CDP)-choline (via a transferase enzyme) leads finally to a phosphatidyl choline lipid.[25]

Table 1. Relative energies (ΔE in kcal/mol), dipole moment (D), and characteristic torsion angles (θ in degrees) of the different glycerol isomers (without internal hydrogen bonds).

Name	ΔE	Dipole moment (D)	θ1/θ2[a]	θ3/θ4[a]
a	0.6	3.4	178/59	180/–60
b	0.0	3.9	68/–55	182/–54
c	2.5	2.0	184/59	60/180
d	1.7	1.5	74/–50	61/185
e	2.7	1.9	–68/170	58/182
f	0.0	3.3	76/–50	–50/76

[a]The θ angles are defined in Fig. 1.

The G3P molecule provides, in some sense, the precursor of the neck–body skeleton (Fig. 1) that is common to all glycerophospholipids. The catalytic reaction forming G3P occurs at a site of the GK enzyme, which forms a complex with Mg-ATP and the G molecule. Structural information on this site has been provided by several X-ray studies[4,26,27] and molecular dynamics simulations.[28,29]

Chemically, the G molecule is achiral, since the two –CH_2OH groups are equivalent. The total number of combinations of the three OH relative positions reduces to six, taking into account mirror-image structures. Figure 2 illustrates these isomers.

The GK active site for glycerol phosphorylation is a deep cleft where ATP and glycerol are stabilized. The X-ray structures of GK show that the glycerol molecule forms hydrogen bonds with several residues.[4,27] Therefore, it is reasonable to assume that, during the glycerophosphate synthesis, external hydrogen bonds rather than internal ones are favoured. The calculated relative stabilities of the G isomers without internal hydrogen bonds are displayed in Table 1, along with the dipole-moment values and characteristic torsion angles, as defined in Fig. 1. These torsion angles are also characteristic of the final phospholipid, once the phosphocholine head and acyl chains have been bound. It is interesting to note that three glycerol structures are competitive at the lowest energy, i.e., the a, b, and f isomers (Fig. 2), which correspond very clearly to the largest dipole moments. The calculated ΔE values are only indicative of the possible conformers in the GK channel, since they do not take into account the interaction of glycerol with the surrounding residues. It is interesting to note that two types of "necks" (180/60 and 60/–60) and "bodies" (180/–60 and –60/60) are represented among the a, b, and f isomers.

The crystallization of GK leads to tetrameric structures, which are inactive forms of the enzyme.[26] Among the mono-

mers, a "closed" and an "open" form were distinguished.[27] The glycerol conduction to the GK enzyme-binding site could be facilitated by its hydrogen bonding with the surrounding GK residues more than with water molecules, due to the deepness of the GK cleft that argues for poor accessibility to the solvent.[27] The processing of glycerol from the cleft opening to the catalytic site could be helped by fluctuations of its large dipole moment interacting with the electric field along the cleft. MD simulations have shown that the protein attracts a substrate with a strong dipole moment, requiring its change of orientation at some positions in the cleft.[28] Binding the glycerol at the catalytic site is performed through tight hydrogen bonds to the closest residues (Asp245, Arg83, and Tyr135). All of these features would be in favour of promoting the a, b, and f isomers in the GK site.

X-ray structures of GK (pdb access 1glf[26] and 1glj[27]) also contain glycerol molecules. The structures of the glycerol molecule inside the tetramers are different in the "closed" and the "open" forms, with neck and body torsion angles very comparable to the conformer a for pdb.1glf ("closed" GK structure) and to a deformed f conformer (torsions of about 100°–120° instead of 60°–70°) for pdb.1glj ("open" GK structure).

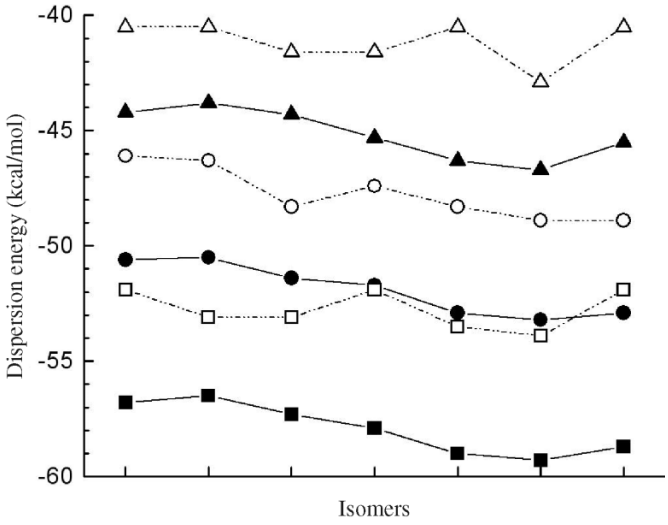
The phosphorylation of one $-CH_2OH$ group of glycerol leads to a chiral C2 carbon in the sn-glycerol-3 phosphate. Starting from each of the six glycerol isomers allows one to generate the possible glycerol phosphate isomers. There are, in fact, nine topologically different structures, due to the lowering of the molecular symmetry. The presence of the phosphate group generates also additional rotational isomers, taking into account the rotation of the $-PO_3H_2$ group around the C3–O bond. Variations of the C2–C3–O–P torsion angle, α1, when sterically possible, leads to more rotational isomers. It must be noted that most of these rotations generate a steric strain due to the P=O or P–OH groups being confined too close to the atoms of the free primary CH_2OH group. In the same spirit as for the glycerol molecule, intramolecular hydrogen bonds have been avoided. After geometry optimization, 12 isomers were obtained with relative total energies within a range of 0 to about 4.0 kcal/mol. The five most stable structures (between 0 and 1.5 kcal/mol) are reported in Table 2. They are labelled by priming the letter of the original glycerol conformers. The isomers a′ and f′ are the counterparts of the a and f glycerol isomers mentioned above. They could thus both exist as products of the GK phosphorylation. These two isomers have different θ1/θ2 and θ3/θ4 values. It is thus not clear why the other isomers of equivalent energies, differing by either θ1/θ2 or θ3/θ4, could be much less stabilized through their interaction with the GK residues. It seems possible that the glycerol 3-phosphate, already before binding the acyl chains and the phosphatidyl choline head, has some structures with comparable energies.

The site and the mechanism of acylation of the G3P primary alcohol have not yet been completely elucidated. However, the X-ray structure of the squash G3PAT enzyme has been reported, and a presumptive G3P binding site is identified.[6,7] A fatty acid binding site is also proposed, in which the reactive thio ester group of the acyl-coA is located in the vicinity of the C1 alcohol of G3P. Thus, at present, there are no experimental data, which specifically contradict the possibility of finding several different conformers for the precursors of phospholipids.

Table 2. Relative energies and characteristic torsion angles of the glycero-3-phosphate most stable isomers. The primed labels correspond to the non-primed labels used for glycerol (Fig. 1).

Isomers	ΔE (kcal/mol)	θ1/θ2	θ3/θ4	α1
a′	0.7	180/60	180/–60	180
b′	0.8	70/-60	180/–60	180
e_1'	0.8	60/185	70/–60	90
e_2'	0.8	50/180	–70/180	70
f′	0.0	60/–70	–70/60	–100

Fig. 3. Damped empirical dispersion energies for the DLPC (triangles), DMPC (circles), and DPPC (squares) ground-state conformers. The conformers are ordered from left to the right as follows: $H1N1B_{A1}$, $H1N3B_{A1}$, $H2N3B_{A1}$, $H1N1B_{A2}$, $H1N2B_{A2}$, $H2N2B_{A2}$, and $H2N2B_{B1}$. The filled symbols are for T1 isomers and the open symbols are for T2 isomers.



Structural analysis of the DLPC, DMPC, and DPPC molecules

Ground-state conformers with all trans chains

As in the case of the DMPC molecule, the DFT-D energy optimization for the DLPC and DPPC isomers led to 14 conformers in the range between 0 and 2 kcal/mol. The 14 lowest isoenergetic conformers are subsequently grouped into two sets of structures, differing by the mutual orientations of the carbon skeletons of the β and γ alkyl chains: in the first group of seven lowest energy structures, these two carbon skeletons are in two parallel planes (T1), and in the second half of the minimum-energy conformers, the two carbon skeletons are in two perpendicular planes (T2) (Fig. 1*c*). For a given T1 or T2 orientation of the hydrocarbon chains, the ground-state conformers arise from the following combinations between two heads (H1, H2), three necks (N1, N2, and N3) and three bodies (B_{A1}, B_{A2}, and B_{B1}): $H1N1B_{A1}$, $H1N3B_{A1}$, $H2N3B_{A1}$, $H1N1B_{A2}$, $H1N2B_{A2}$, $H2N2B_{A2}$, and $H1N1B_{B1}$.

These conformational structures are described in detail for the DMPC molecules in ref. 13. Their averaged α, β, and γ

Table 3. Relative energies in kcal/mol with respect to all-trans conformers of the DPPC, DMPC, and DLPC gauche isomers (H1N1B_{A2}T2) at various positions of the chain.

Kinks position *n*	DPPC (16C)	DMPC (14C)	DLPC (12C)	Tetradecane $C_{14}H_{30}$
***n* = 16**				
60	0.72			
300	0.80	—	—	
Average	0.76			
***n* = 14**				
60	0.85	0.75		0.65
300	1.10	0.66	—	
Average	0.98	0.70		
***n* = 12**				
60	1.09	0.84	0.88	0.46
300	1.29	1.10	0.61	
Average	1.19	0.97	0.74	
***n* = 10**				
60	1.21	1.00	0.75	0.45
300	1.22	1.18	0.63	
Average	1.21	1.09	0.69	
***n* = 8**				
60	1.40	1.11	0.87	0.45
300	0.89	0.92	0.58	
Average	1.15	1.01	0.72	
***n* = 6**				
60	1.02	0.87	0.73	0.46
300	0.24	0.20	0.30	
Average	0.63	0.53	0.51	

angles and their mutual combinations leading to those ground-state isomers are not affected by the chain length, e.g., the same arrangement of α, β, and γ chains is obtained for the DLPC, DMPC, and DPPC molecules. As a consequence, the characteristic geometries of the head, body, and neck conformations display very similar structural parameters. The displacement of the polar head with respect to the β or γ alkyl chains in the various isomers is characterized by the distances of the closest C=O oxygen to the choline N atom (N···C=O) and to the non-ester phosphate O atom (P=O···O=C). These distances do not vary with the chain length.

The 14 isomers have the same relative energy stability for the DLPC, DMPC, and DPPC molecules. The computed damped empirical dispersion energies, E_{disp}, plotted in Fig. 3, indicates an increase of approximately 3.1 kcal/mol per CH_2 unit for the lipids with 12, 14, and 16 carbons in the alkyl chains in their T1 and T2 arrangements. This value is somewhat smaller than the E_{disp} increase of 3.8 kcal/mol per CH_2 in the case of *n*-alkane dimers (from butane to dodecane) in their most stable arrangement.[19] The averaged DFT-D empirical dispersion in the T1 isomers is higher than the average value for the T2 isomers. This difference increases slightly with the chain length, e.g., E_{disp} of T1 isomers is higher by 3.8, 4.0, and 5.2 kcal/mol for the DLPC, DMPC, and DPPC molecules, respectively. The E_{disp} difference for both tail arrangements correlates with the difference between the β and γ hydrocarbon chain separation in T1 and T2. The relative dispersion energy in the case of isomers with parallel tails (T1) follows a very similar trend for the three lipid molecules as seen from Fig. 3. This regular behaviour is not so well-respected for the T2 isomers for a particular diacyl PC lipid. It indicates, thus, some influence of the chain length when T2 chains are coupled with a specific head and body conformation. However, this "discrepancy" is less than 3%–4% of the dispersion energy values.

Higher energy structures with gauche conformers in the alkyl chains

Gauche chain conformers are predominantly formed in phospholipid aggregates in their liquid phase. The relative energies of the possible gauche conformers with one kink per alkyl chain, optimized at $T = 0$ K, with respect to the all trans structures are presented in Table 3 for the DLPC, DMPC, and DPPC H1N1B_{A2}T2 isomers. For comparison, relative energies between gauche and trans isomers of tetradecane are reported in the same table. The torsions in Table 3 follow the nomenclature indicated in Fig. 1 for the β and γ chains. In contrast to tetradecane, the gauche conformers in DLPC, DMPC, and DPPC are not degenerate with, however, insignificant relative energy variations for three of the four possible gauche conformers in the DLPC molecule. Interestingly, comparable stability is obtained for the structures with a kink at the free end of the C12, C14, and C16 chains. The DPPC gauche conformers follow the behaviour of DMPC: the least stable structures are those with kinks in the middle of the hydrocarbon chains (at β_n, γ_n = 8, 10, 12 for DPPC and β_n, γ_n = 8, 10 for DMPC); the most stable conformation is the one with a torsion of 300° at β_n, γ_n = 6. The latter is obtained also for the DLPC molecules.

Comparison of the G3P and phospholipid conformers

The precursor G3P values of θ1/θ2 and θ3/θ4, characterizing the "neck" and "body", respectively, correspond to those found for the complete phospholipid structures. Indeed, if one considers all the isomers of Table 2, the three phospholipid necks are found with the combinations 180/60 (N1), 60/–60 (N2), and 60/180 (N3). Since G3P has no acyl chains, only three types of body exist: two of them correspond to those found for the phospholipids: B_A and B_B, with the θ3/θ4 values of 180/60 and 60/–60, respectively. The value of –70/180, (e'_2 in Table 2), related to two opposite OH groups at the C1 and C2 carbons, i.e., acyl chains in opposite directions, would not lead to the formation of phospholipid bilayers. Actually, this configuration (opposite acyl chains) was reported in NMR studies for PC lipids at low concentration in water, i.e., isolated molecules or very small aggregates.[9] In the same work, the B_A and B_B conformers were proposed as the only two present in the PC lipid bilayers, related with specific ^{1}H NMR signals (rotamers A and B, respectively, in ref. 9).

We thus see that G3P already provides the basis of the conformations found for the final phospholipids, moreover corresponding to similar energies. The bonding of the β and then γ acyl chains and finally that of the head α chain will allow the possibility of two relative orientations for the carbonyl groups (splitting B_A into B_{A1} and B_{A2}) and for the alkyl chains (T1 and T2).

If the G3P isoenergetic structures are not specifically selected in the G3PAT enzyme at the binding site with the fatty acyl chain, more than one or a few different neck and body conformers could exist in the final phospholipids. Indeed, at the two next steps, i.e., dephosphorylation and addition of the phosphocholine, one could assume that the glycerol backbone and the acyl chains will keep their conformations. The existence of more than one phospholipid conformer in the "de novo" synthesis is thus not unreasonable.

Conclusion

The different conformational structures of the glycerol (G) and glycerol 3-phosphate (G3P) molecules have been shown to be at the origin of the various phospholipid conformers, except, obviously, for the alkyl chain torsions. Conformations lacking internal hydrogen bonds are imposed by the enzymes involved in the synthesis, and these persist through the various subsequent steps of the synthesis, adding the tails and the head. The conformational analysis of the glycerophosphate skeleton determines most of the structural characteristics of the phosphatidyl choline lipids, which differ by the length of their diacyl chains, i.e., DLPC, DMPC, and DPPC.

Higher energy conformers with kinks in the acyl chains have been found, in preparation for molecular dynamics studies of the chain melting phase transformation, which will be published elsewhere.[30] The comparison of their relative energies with respect to the acyl chain length shows that DLPC, the shortest lipid to form bilayers, may have a different thermodynamical behaviour at the melting transition from the other two lipids, displaying more similar gauche energies along the alkyl chains.

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References

(1) Fagone, P.; Jackowski, S. *J. Lipid Res.* **2008**, *50* (Suppl.), S311–S316. doi:10.1194/jlr.R800049-JLR200. PMID:18952570.
(2) Kennedy, E. P. *J. Biol. Chem.* **1953**, *201*, 399.
(3) Thorner, J. W.; Paulus, H. *J. Biol. Chem.* **1973**, *248*, 3922.
(4) Mao, C.; Ozer, Z.; Zhou, M.; Uckun, F. M. *Biochem. Biophys. Res. Commun.* **1999**, *259* (3), 640–644. doi:10.1006/bbrc.1999.0816. PMID:10364471.
(5) Slabas, A. R.; Simon, W. R.; Schierer, T. P.; Kroon, J.; Fawcett, T.; Hayman, M.; Gilroy, J.; Nishida, I.; Murata, N.; Rafferty, J.; Turnbull, A.; Rice, D. *Biochem. Soc. Trans.* **2000**, *28* (6), 677–679. doi:10.1042/BST0280677. PMID:11171167.
(6) Slabas, A. R.; Kroon, J. T. M.; Scheirer, T. P.; Gilroy, J. S.; Hayman, M.; Rice, D. W.; Turnbull, A. P.; Rafferty, J. B.; Fawcett, T.; Simon, W. J. *J. Biol. Chem.* **2002**, *277* (46), 43918–43923. doi:10.1074/jbc.M206429200.
(7) Turnbull, A. P.; Rafferty, J. B.; Sedelnikova, S. E.; Slabas, A. R.; Schierer, T. P.; Kroon, J. T. M.; Simon, J. W.; Fawcett, T.; Nishida, I.; Murata, N.; Rice, D. W. *Structure* **2001**, *9* (5), 347–353. doi:10.1016/S0969-2126(01)00595-0. PMID:11377195.
(8) Agarwal, A. K.; Sukumaran, S.; Bartz, R.; Barnes, R. I.; Garg, A. J. *Endocrinology* **2007**, *193* (3), 445–457. doi:10.1677/JOE-07-0027.
(9) Hauser, H.; Pascher, I.; Sundell, S. *Biochemistry* **1988**, *27* (26), 9166–9174. doi:10.1021/bi00426a014. PMID:3242620.
(10) Hong, M.; Schmidt-Rohr, K.; Zimmermann, H. *Biochemistry* **1996**, *35* (25), 8335–8341. doi:10.1021/bi953083i.
(11) Pearson, R. H.; Pascher, I. *Nature* **1979**, *281* (5731), 499–501. doi:10.1038/281499a0. PMID:492310.
(12) Pascher, I.; Sundell, S.; Harlos, K.; Eibl, H. *Biochim. Biophys. Acta* **1987**, *896* (1), 77–88. doi:10.1016/0005-2736(87)90358-0. PMID:3790589.
(13) Krishnamurty, S.; Stefanov, M.; Mineva, T.; Bégu, S.; Devoisselle, J. M.; Goursot, A.; Zhu, R.; Salahub, D. R. *J. Phys. Chem. B* **2008**, *112* (42), 13433–13442. doi:10.1021/jp804934d. PMID:18817435.
(14) Krishnamurty, S.; Stefanov, M.; Mineva, T.; Bégu, S.; Devoisselle, J. M.; Goursot, A.; Zhu, R.; Salahub, D. R. *ChemPhysChem* **2008**, *9* (16), 2321–2324. doi:10.1002/cphc.200800511. PMID:18924197.
(15) Köster, A. M.; Calaminici, P.; Casida, M. E.; Flores-Moreno, R.; Geudtner, G.; Goursot, A.; Heine, T.; Ipatov, A.; Janetzko, F.; Martin del Campo, J.; Patchkovskii, S.; Reveles, J. U.; Salahub, D. R.; Vela, A. *The deMon developers*; Cinvestav, Mexico. 2006.
(16) Zhang, Y.; Yang, W. *Phys. Rev. Lett.* **1998**, *80* (4), 890. doi:10.1103/PhysRevLett.80.890.
(17) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B Condens. Matter Mater. Phys.* **1988**, *37*, 785.
(18) Zhang, Y.; Yang, W. *Phys. Rev. Lett.* **1998**, *80* (4), 890. doi:10.1103/PhysRevLett.80.890.
(19) Goursot, A.; Mineva, T.; Kevorkyants, R.; Talbi, D. *J. Chem. Theory Comput.* **2007**, *3* (3), 755–763. doi:10.1021/ct600373f.

(20) Godbout, N.; Salahub, D. R.; Andzelm, J.; Wimmer, E. *Can. J. Chem.* **1992**, *70* (2), 560–571. doi:10.1139/v92-079.
(21) Köster, A. M.; Flores-Moreno, R.; Reveles, J. U. *J. Chem. Phys.* **2004**, *121* (2), 681. doi:10.1063/1.1759323.
(22) Dunlap, B. I.; Connolly, J. W. D.; Sabin, J. R. *J. Chem. Phys.* **1979**, *71* (12), 4993. doi:10.1063/1.438313.
(23) Mintmire, W.; Dunlap, B. I. *Phys. Rev. A* **1982**, *25* (1), 88–95. doi:10.1103/PhysRevA.25.88.
(24) Reveles, J. U.; Köster, A. M. *CJ. Comput. Chem.* **2004**, *25*, 1109. doi:10.1002/jcc.20034.
(25) Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. In *Molecular biology of the cell*, 5th ed.; Garland Science, Taylor & Francis Group: New York, 2008.
(26) Feese, M. D.; Faber, H. R.; Bystrom, C. E.; Pettigrew, D. W.; Remington, S. J. *Structure* **1998**, *6* (11), 1407–1418. doi:10.1016/S0969-2126(98)00140-3. PMID:9817843.
(27) Bystrom, C. E.; Pettigrew, D. W.; Branchaud, B. P.; O'Brien, P.; Remington, S. J. *Biochemistry* **1999**, *38* (12), 3508–3518. doi:10.1021/bi982460z. PMID:10090737.
(28) Grayson, P.; Tajkhorshid, E.; Schulten, K. *Biophys. J.* **2003**, *85* (1), 36–48. doi:10.1016/S0006-3495(03)74452-X. PMID: 12829462.
(29) Schlick, T. *Biophys. J.* **2003**, *85* (1), 1–4. doi:10.1016/S0006-3495(03)74448-8. PMID:12829458.
(30) Mineva, T.; Krishnamurty, S.; Goursot, A.; Salahub, D. R. Unpublished results.