QUANTUM-MECHANICAL STUDIES ON THE CONFORMATION OF PHOSPHOLIPIDS. THE CONFORMATIONAL PROPERTIES OF THE POLAR HEAD

Bernard PULLMAN and Hélène BERTHOD

Institut de Biologie Physico-Chimique, Fondation Edmond de Rothschild, 13, rue P. et M. Curie, 75005 – Paris, France

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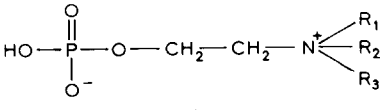
1. Introduction

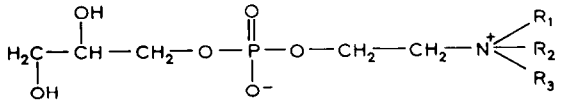
The polar head group of phospholipids may be considered as represented by choline phosphate (CP) (Ia) and ethanolamine phosphate (EP) (Ib) or, from a broader point of view, by L-α-glycerylphosphorylcholine (GPC) (IIa) and L-α-glycerylphosphorylethanolamine (GPE) (IIb). We shall denote the principal torsion angles of these structures by the notations proposed by Sundaralingam (fig. 1) and shall use also his conventions for measuring these angles [1]: The torsion angle about bond j (α_j) is considered positive for a right-handed rotation; when looking along the bond j, the far bond j + 1 rotates clockwise relative to the near bond j – 1. The angles are measured from O° to 360°, the O° value corresponding to the cis-planar arrangement of bonds j + 1 and j – 1.



I

(a) $R_1 = R_2 = R_3 = CH_3$: choline phosphate (CP)

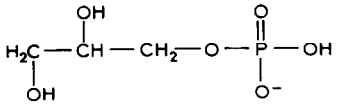
(b) $R_1 = R_2 = R_3 = H$: ethanolamine phosphate (EP)



II

(a) $R_1 = R_2 = R_3 = CH_3$: L-α-glycerylphosphorylcholine (GPC)

(b) $R_1 = R_2 = R_3 = H$: L-α-glycerylphosphorylethanolamine (GPE)



III

Following the general opinion explicitly expressed in ref. [1] the major flexibility to conformational changes in phospholipids occurs around the α-chain. It appears also that membrane permeability may be strongly correlated with the conformational changes in the α-chain. Essential in this respect are the torsion angles α_4 and α_5, defined more explicitly in the terminology of the bonded atoms [2] as $\alpha_4(P{-}O^{12}{-}C^{11}{-}C^{12})$ and $\alpha_5(O^{12}{-}C^{11}{-}C^{12}{-}N^{+})$.

Crystallographic X-ray studies on EP [3], GPE monohydrate [4,5], GPC [6], GPC cadmium chloride trihydrate [7] and on related systems e.g. serine phosphate etc. (for a summary of results see ref. [1] indicate that in all these compounds α_4 assumes a value around 180° (with an appreciable spreading from 140° to 220°), while α_5 is localized in the vicinity of

Scheme 1

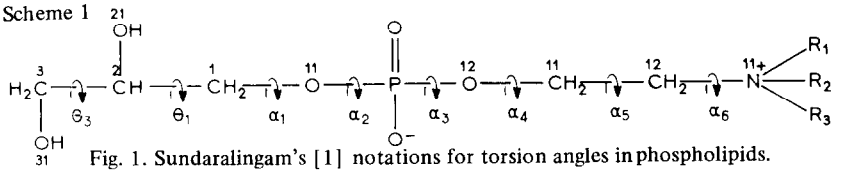


Fig. 1. Sundaralingam's [1] notations for torsion angles in phospholipids.

60° and 300°. The conformations with respect to these two torsion angles can thus be described as *trans–gauche.*

The gauche conformation about α_5 is also found in solution by RMN studies on CP [8] and on dipalmitoyl-lecithin [9]. On the other hand EP seems to exist in solution as a mixture of gauche and trans conformers with respect to this torsion angle [10].

Parallel, theoretical computations carried out by the empirical potential functions procedure [11] and by the molecular orbital Extended Hückel Theory [12] indicated that the most stable conformations of these molecular systems should correspond to $\alpha_4 = 180°$ and $\alpha_5 = 60°$, 180° and 300°. These theoretical results agree satisfactorily with the above quoted experimental ones and this situation leads to the impression that the conformations observed in the solid state or in the water for the polar head of phospholipids in their constituents (and presumed to occur also in membranes) correspond to their *intrinsically* most stable conformation.

In this note we present evidence that this is not so.

2. The methodology

The method used in our computations is the ab initio self-consistent field procedure with an STO 3G basis set, employing the program Gaussian 70 [13,14]. The method has been used recently with striking success for the study of the conformational properties of a large number of fundamental biological and pharmacological compounds: acetylcholine [15,16], histamine [17], serotonin and bufotenine [18], a series of phenethylamines and phenethanolamines [19], GABA [20] etc.

The computations of the conformational energies have been performed for EP with the geometrical input data (bond lengths and valence angles) taken from the crystallographic results on the corresponding part of GPE [5]. Because of the high cost of the corresponding part of GPE [5]. Because of the high cost of the computations by the ab initio method we did not construct the complete α_4–α_5 conformational energy map but computed only the energies of the a priori most representative conformations and of the crystallographically observed ones. The remaining torsion angles have been kept at $\alpha_2 = -81°$, $\alpha_3 = -81°$, $\alpha_6 = 165°$, which are their values in GPE.

3. Results and discussion

The results obtained are presented in fig. 2 which indicates the energies of a number of conformations of EP as a function of the torsion angles α_4 and α_5. The global energy minimum representing the intrinsically most stable conformation corresponds to $\alpha_4 = -60°$ $\alpha_5 = 90°$. It represents a seven-membered hydrogen-bonded ring structure, depicted in fig. 3, with the H-bond between an $N_{11}^{+}H$ of the cationic head and O^{-} of the phosphate group.

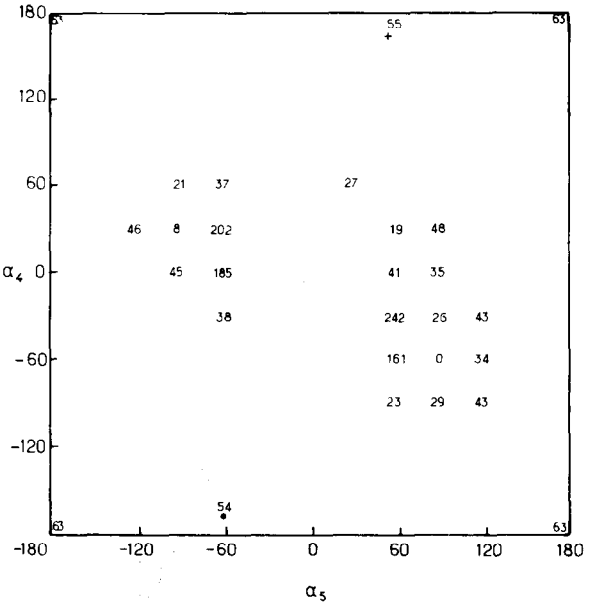


Fig. 2. Energy of conformations of EP in kcal/mole with respect to the global energy minimum taken as energy zero. Experimental conformations: • (3), + (5).

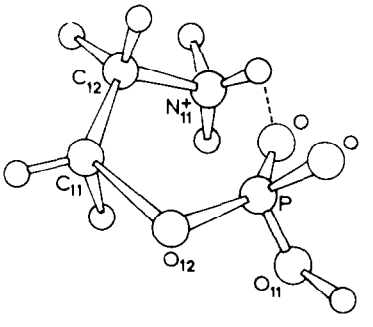


Fig. 3. The preferred conformation of the polar head.

A closely related seven-membered H bonded ring corresponds to $\alpha_4 = 30°$, $\alpha_5 = -90°$ at 8 kcal/mole above the former. The two rings are not equivalent in the computations, in spite of their apparent equivalence from chemical formulae because of the unsymmetrical character of the geometrical input data, taken from crystallographic results.

These rings are closely similar to the C_7 ring found as the most stable conformation in a large number of dipeptides [21].

The completely extended form corresponding to $\alpha_4 = \alpha_5 = 180°$ is 63 kcal/mole above the preferred conformation. The crystallographic conformation at $\alpha_4 = 164°$ $\alpha_5 = 55°$ lies 55 kcal/mole above the intrinsically most stable one. Although it seems probable that these two values are somewhat overestimated because of the use of a relatively small basis set, the fundamental result indicating a strong intrinsic preference of free EP for a folded rather than extended conformation with respect to α_4 cannot be doubted. Under these circumstances the existence of an extended conformation in the solid state must be attributed to the action of crystal packing forces.

A striking confirmation of our viewpoint is being provided by the results of recent ^{31}P nuclear magnetic resonance studies on phospholipids, in organic solvents [22]. These studies lead to the conclusion that the hydrogen bonded seven member ring found by our computations to be the most stable arrangement exists in fact in phosphatidylethanolamine. An electrostatic interaction between the positively charged terminal trimethyl-amino group and the negatively charged phosphate oxygens is suggested also by these studies for the related phosphatidylcholine. It may be considered that the situation in organic solvents is the closest to that of the free molecule and confirms the intrinsic *preference* of the polar groups of phospholipids for a highly folded arrangement.

Starting from that result it may, on the other hand, be recognized that the influence of water will tend most probably to disrupt at least in part the intramolecularly hydrogen bonded ring and to produce more extended conformations. We have studied recently a somewhat similar case of Zwitterionic GABA and shown this to actually occur [23]. The above quoted results of Dufourcq and Lussan [8,10] on the difference in behaviour of phosphocholine and phosphoethanolamine moieties in solution, although concerned only with the torsion about α_5, are also interesting. The phosphocholine moiety is *gauche* in water, while the ethanolamine one is 'freely rotating' i.e. 'jumping quickly from the *gauche* to the *trans* one'. The authors find it difficult to ascertain whether this difference springs from a different intramolecular energy of interaction or a different hydration of the ammonium group. Following our viewpoint it must essentially be due to this last cause especially as it has been demonstrated recently that the hydration of the N^+H_3 group is much stronger than that of the $N^+(CH_3)_3$ group [24]. Explicit studies on the effect of water on the conformational properties of a number of pharmaceutical drugs of the arylethanolamine type with an N^+H_3 terminal group (histamine, serotonin etc) have shown that this solvent has the effect of producing an equilibrium mixture of *trans* and *gauche* forms in compounds which in the free state show a strong preference for a gauche conformation alone. [17,25]. On the other hand the absence of a strong hydration around the $N^+(CH_3)_3$ group reduces the influence of the solvent which is thus not expected to have any appreciable effect on the intrinsically preferred conformation. As a classic example one may quote in this respect acetylcholine which is predicted to have a preference for a *gauche* conformation and is *gauche* in the crystal and in water, or acetylthiocholine which is predicted to have a preference for a *trans* conformation and which is *trans* in the crystal and in water [26].

Finally we would like to mention that in compounds such as GPC or GPE there exists also the possibility of intramolecular hydrogen bonding in the glycerol part of the molecule, in particular between the OH group no. 21 of fig. 1 and the negative oxygens of the phosphate, leading again to a seven-membered hydrogen bonded ring. The existence of such an interaction has indeed been shown again by the above mentioned ^{31}P NMR studies on phosphatidylglycerol [22]. This is again a conformation quite different from that existing in the crystal [4–7]. Although we did not explore this problem completely we did compute by the ab initio procedure for the model compound III the energy of the seven membered hydrogen bonded ring structure, corresponding to the values of $\theta_1 = 150°$ $\alpha_1 = 30°$ for the two principal torsion angles involved and the energy associated with the crystallographic values of these angles in the related GPE molecule, which correspond to $\theta_1 = 166.8°$ and $\alpha_1 = 186.3°$.

The former appears to be 14 kcal/mole more stable than the latter.

Conclusion

It is thus evident that the polar heads of the phospholipids show an *intrinsic* preference toward highly folded structures with strong intramolecular hydrogen bonds. Their existence in the open form in the crystals and possibly in water must be attributed to the effect of the environmental forces. This situation must be kept in mind when establishing any energy balance which may imply conformational stabilities in processes involving these molecules.

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