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

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Received 4 February 1975

1. Introduction

We have shown in a previous paper [1] that the polar heads of the phospholipids manifest an *intrinsic preference* towards highly folded structures stabilized by a strong intramolecular hydrogen bond (ethanolamines) or electrostatic interaction (cholines) between the phosphate group and the cationic head. This type of structure is in fact observed in organic solvents [2]. In the present note we are studying *the effect of hydration* on the conformation of the polar head.

Beforehand we relate, however, some complementary results on the free model molecule of fig.1. In reference [1], the computations have been performed for this compound by the SCF ab initio procedure using Gaussian orbitals in the STO 3G basis set. In view of an extension of computations to larger systems, the calculations have been repeated by the PCILO method [3,4]. In this case they have been performed both for ethanolamine phosphate (EP) (fig.1, R = H) and for choline phosphate (CP) (fig.1, R = CH_3). The geometrical input data were taken from the crystal X-ray structure of GPE and GPC, respectively [5,6].

The PCILO conformational energy maps, with respect to the torsion angles α_4 and α_5 [1,7] are presented in figs. 2 and 3. (We have modified in the present note the presentation of the results by plotting the variables from 0 to 360° instead of from −180 to +180°, as was done in [1], in order to make them easily comparable with computations on phospholipids carried out by other authors).

Interestingly, fig.2 while confirming the intrinsic preference of EP for a folded, intramolecularly

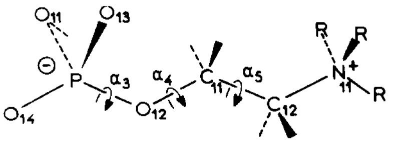

Fig.1. Choline phosphate (R = CH_3), CP, and ethanolamine phosphate (R=H), EP. Torsion angles, α_3, α_4, α_5.

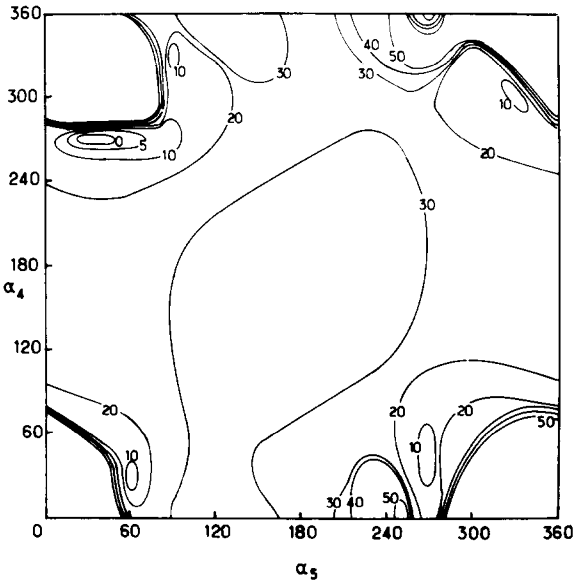

Fig.2. PCILO conformational energy map for EP. Isoenergy curves in kcal/mol with respect to the global energy minimum taken as energy zero.

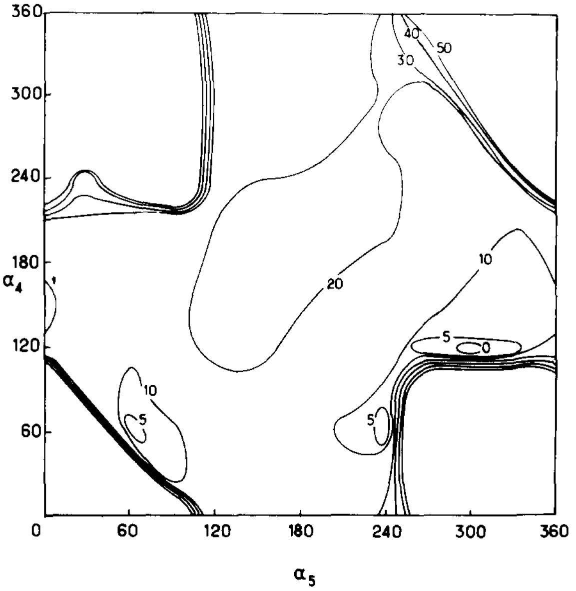

Fig.3. PCILO conformational energy map for CP. Isoenergy curves in kcal/mol with respect to the global energy minimum taken as energy zero.

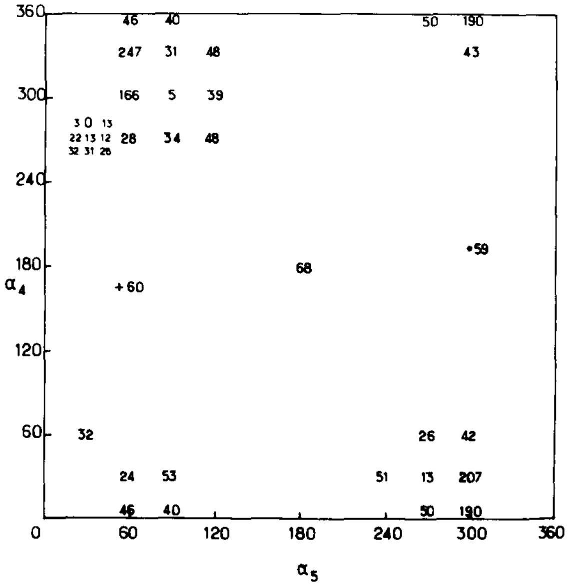

Fig.4. Ab initio STO 3G conformational energy map for EP. Isoenergy curves in kcal/mol with respect to the global energy minimum taken as energy zero.

hydrogen bonded conformation, indicates a global energy minimum ($\alpha_4 = 270°$, $\alpha_5 = 30°$) slightly displaced with respect to the global minimum of the ab initio computation as presented in fig.2 of [1] ($\alpha_4 = 300°$, $\alpha_5 = 90°$ in the notations of the present note). As the ab initio map has been computed, for reasons of economy, for selected values of α_4 and α_5 only, we have now extended it into the region of the PCILO global energy minimum, using small intervals of α_4 and α_5 in this vicinity. The results are presented in fig.4 and they essentially confirm the PCILO predictions. This is a remarkable case of an improvement of ab initio results by indications from the PCILO method.

The results for CP (fig.3) indicate a general analogy to those for EP. The conformationally allowed space, within the same isoenergy limit is somewhat more restricted owing to the increase of steric hindrance. Nevertheless the global energy minimum corresponds to a folded conformation similar to that found for EP: gauche (synclinal) with respect to α_5 (= 300°), somewhat more extended (anticlinal) with respect to α_4 (= 120°). The 'inversion' of the sign of the coordinates of the global minimum between figs. 2 and 3 (from the upper-left corner to the down-right corner of the map, respectively) is simply due to the difference in sign of the torsion angle α_3 in the crystallographic data of GPC ($\alpha_3 = 65°$) and GPE ($\alpha_3 = -81°$) which are used as input data.

2. The methodology for the study of the solvated molecules

This methodology belongs to the supermolecular approach as developed recently in our laboratory by A. Pullman et al. [8–10] and applied to conformational problems in particular in the field of fundamental pharmacological compounds [11–13]. It consists of fixing water molecules in the most favorable hydration sites of the compound studied and calculating the conformational energy map of the new 'supermolecule'. The most favorable hydration sites are determined by ab initio studies on model compounds. The construction of the conformational map of the new 'supermolecule' representing the hydrated species is carried out by the PCILO method since the compounds are then generally too large for an ab initio computation. In the polar heads of phospholipids there are obviously two principal hydration regions: the cationic $-N^+R_3$ end and the phosphate group. Information about the preferred hydration sites of these two groupings has been obtained from studies on the hydration of ammonium and alkylammonia [14] and dimethylphosphate [15], respectively. It indicates the possibility of fixing three water molecules at the $-N^+H_3$ terminal group, through strong hydrogen bonds (≈ 28 kcal/mol in the STO 3G basis set) along the N^+-H axes. The energy of binding of water molecules about the $-N^+(CH_3)_3$ terminal group is appreciably reduced with respect to that around the $-N^+H_3$ group, although the attraction still exceeds slightly that between water molecules. Up to five or even six water molecules may be fixed, strongly bound (≈ 17–20 kcal/mol in the same STO 3G basis set) around the phosphate group, in its intrinsically preferred g^-g^-conformation about the α_2 and α_3 torsion angles [1] which was maintained in this study. For an optimal first shell hydration with five water molecules, three are located in the planes $O_{13}PO_{14}$, $O_{11}PO_{13}$ and $O_{12}PO_{14}$ in a bridged position with respect to the phosphate oxygens involved and two in the planes $O_{11}PO_{14}$ and $O_{12}PO_{13}$ involving only one hydrogen bond with the second water hydrogen turned 'outside' (fig.2c, conformation E'_{12}, E'_{34} of [15]). For a given reduced number of water molecules different energetically nearly equivalent modes of hydration of the phosphate are possible. In a reduced hydration with 2–3 water molecules two somewhat more strongly (–29, –27 kcal/mol) bound water molecules are located in the plane $O_{13}PO_{14}$ and involve one hydrogen bond to the phosphate oxygen with the second of the water hydrogens turned 'outside', (conformation E_{13} of [15]) with eventually the third water molecule bridging O_{13} and O_{14}. The computed values of the hydration energies as indicated above are certainly overestimated due to the use of a relatively small basis set (STO 3G). Thus e.g. the experimental energy of $N^+-H\ldots H_2O$ interaction in ammonium is 17 kcal/mol [16]. Nevertheless they correspond to relatively very strong hydrogen bonds and the relative order of their values is most probably correctly reproduced.

The experimental information about the hydration of the polar head of phospholipids is relatively abundant [17–25] and although it does not lead to a unique scheme and does not fix precisely the preferred sites of hydration, it indicates a number for the 'bound' water molecules which altogether is comparable with that suggested by the theoretical studies. Depending upon the experimental conditions and techniques utilized the number of water molecules in the primary hydration shell (most strongly bound) varies at the polar head of phosphatidylcholine (which was more abundantly studied that phosphatidylethanolamine, more difficult to hydrate possibly because of intermolecular interactions) from 2 to 6. A secondary shell of 4–8 molecules of water, less strongly bound than the previous ones, is sometimes distinguished. (For more details see [15]. As discussed in papers [8–15] it is not assumed that the entire solution behavior of molecules will be explained by the reduced treatment considered here. It is, however, expected that this approach may yield a reasonable indication of the direction and magnitude of changes in conformational preferences of the isolated molecule as it enters aqueous solution and that from that point of view the inclusion of the essential water molecules of the first hydration shell should be particularly significant, especially in cases in which these molecules are very strongly bound to polar groups of the substrate (as it is the case in the polar head of phospholipids). This expectation was fully satisfied by the comparison of theory with experiments in the groups of compounds studied so far [11–13]. It is therefore expected that the procedure should give significant results in the case of phospholipids also.

3. Results and discussion

3.1. *Conformation of hydrated EP*

Two conformational energy maps have been constructed for hydrated EP. The first (fig.5) corresponds to the overall fixation of six water molecules upon the skeleton of EP: three at the cationic head along the N^+-H bonds and three at the phosphate group in the planes $O_{13}PO_{14}$, $O_{11}PO_{13}$ and $O_{12}PO_{14}$ in bridged positions with respect to the oxygens. The results indicate (fig.5) that the conformationally allowed space has somewhat decreased with respect to that of free EP; the global energy minimum still corresponds to a gauche conformation with respect to α_5 (= 60°) but it became somewhat more extended with respect to α_4 (= 240°).

As it was shown in [15] that up to five or six water

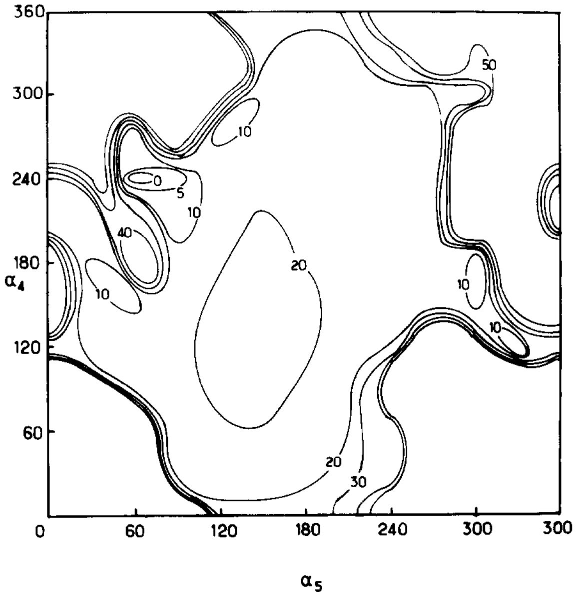

Fig.5. PCILO conformational energy map of hydrated EP ($EP + 6H_2O$, for details see text). Isoenergy curves in kcal/mol with respect to the global energy minimum taken as energy zero.

molecules may be strongly bound to the phosphate, a second map was constructed for hydrated PE, involving five water molecules at the phosphate (the three previous ones plus two in the planes $O_{11}PO_{14}$ and $O_{12}PO_{13}$ with one of their hydrogen turned outside (fig.1, conformations E'_{12} and E'_{34} of [15]), and three on the cationic head, thus eight altogether. The results are presented in fig.6 which shows the conservation of the gauche conformation with respect to α_5 (= 60°C) and a further elongation of the structure with respect to α_4 now equal to 150°.

3.2. *Conformation of hydrated CP*

Because the energy of binding of water molecules to a $-N^+(CH_3)_3$ cationic head is considerably reduced with respect to its value for N^+H_3 [14] we did not consider the presence of 'bound' water at the cationic end of CP and have limited our study to the successive fixation of 3,4 or 5 water molecules at the phosphate group alone.

Figs. 7 and 8 represent the results obtained for the tri- and pentahydrate of CP, respectively (the results for the tetrahydrate are very similar to those for the

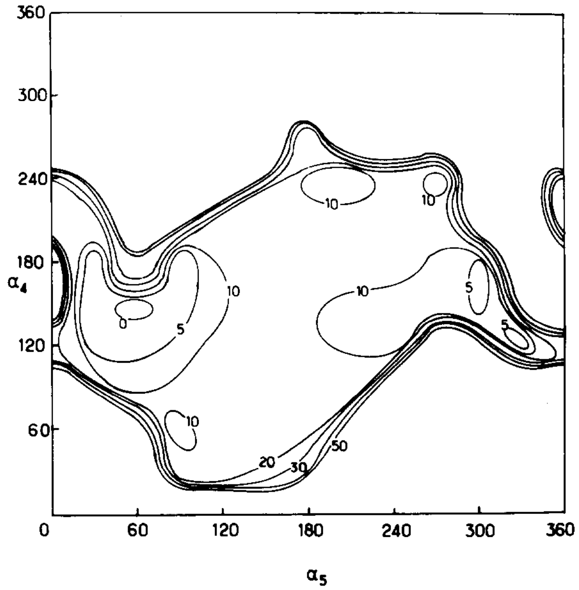

Fig.6. PCILO conformational energy map of hydrated EP ($EP + 8H_2O$, for details see text). Isoenergy curves in kcal/mol with respect to the global energy minimum taken as energy zero.

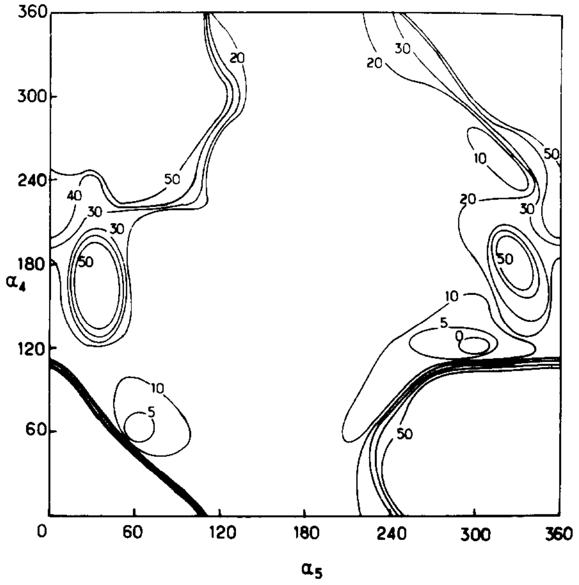

Fig.7. PCILO conformational energy map of hydrated CP ($CP + 3H_2O$, for details see text). Isoenergy curves in kcal/mol with respect to the global energy minimum taken as energy zero.

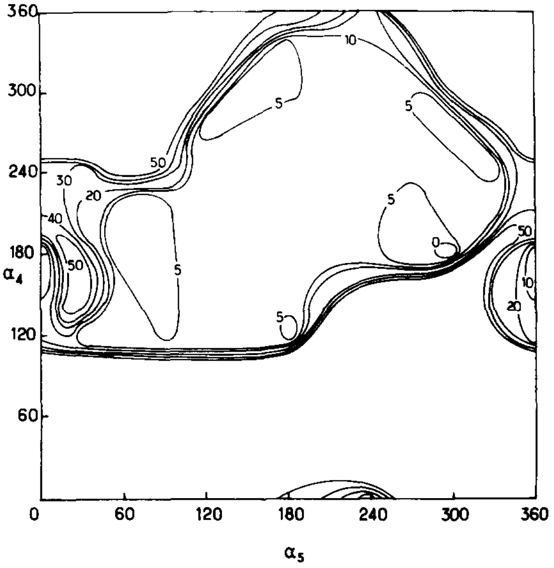

Fig.8. PCILO conformational energy map of hydrated CP (CP + $5H_2O$,. for details see text). Isoenergy curves in kcal/mol with respect to the global energy minimum taken as energy zero.

trihydrate). The water molecules are fixed in the same positions as in the corresponding study on EP.

The results show the presence of a global energy minimum at $\alpha_5 = 300°$, $\alpha_4 = 120°$ in fig.7 and at $\alpha_5 = 300°$, $\alpha_4 = 180°$ in fig.8. The preferred conformation of hydrated CP corresponds thus consistently to a gauche arrangement with respect to α_5 (as in the free molecule) and five water molecules are needed in the first hydration shell around the phosphate to produce an extension about α_4 which reaches then 180°.

Altogether these results seem thus to indicate that the effect of water upon the conformation of the polar head of phospholipids consists essentially of extending the structure through the evolution of the torsion angle α_4 towards the value 180° (trans), leaving nevertheless the torsion angle α_5 at the gauche arrangement (60° or 300°). The trans conformation about α_4 occurs essentially for strong hydration of CP. In EP the conformation remains in-between gauche and trans with respect to this torsion angle.

This evolution of α_4 and α_5 brings the results closer to the situation expected to be found in membranes ($\alpha_4 = 180°$, $\alpha_5 = 60$ or $300°$). It must, however, be clearly realized that these conformations are the result of environmental forces and not of an intrinsic tendancy of the polar head.

In relation to the theoretical prediction that the value of α_4 of the solvated polar head of EP should correspond rather to an anticlinal than to an antiperiplanar arrangement, it may be interesting to draw attention to the recent result of the X-ray crystallographic study of 1,2-dilauroyl-DL-phosphatidyletholamine which is in fact the first crystal study of a phospholipid [26]. This compound exists in the crystal in a conformation corresponding to $\alpha_4 = 101°$, $\alpha_5 = 77°$. (α_3 is positive in this crystal, equal to 64°, and the situation is therefore similar to that represented in fig.3). The value of α_4 in the crystal environment, closer to that which the PCILO computations predict for the free rather than for the hydrated polar heads, is at least an indication of the complexity of the problems related to this torsion angle which seems to have been overbrooked by the empirical [27] or EHT [28] computations. In this case it is moreover influenced by the intermolecular hydrogen bonding occurring in the crystal between the cationic and anionic parts of the polar heads on adjacent molecules.

Finally, it may be remarked that our results indicate that no difference should be expected between EP and EC with respect to the conformation about the α_5 torsion angle, neither in the free nor in the solvated molecules. In this respect it is gratifying to record that contrary to previous statements [29,30] more recents NMR studies [31] confirm this analogy.

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