CONFORMATIONS OF THE PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE POLAR GROUPS DETERMINED BY NMR SPECTROSCOPY

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1. Introduction

It is generally accepted that the matrix of cellular membranes is a bimolecular phospholipid leaflet. To this basic structure, proteins, cholesterol and other molecules are inserted in such a way as to confer to the bilayer the specific properties of a particular membrane. So far, only the structure of the aliphatic chains of phospholipids have been extensively studied, but the conformation of their polar head remains unexplored. Among the different classes of phospholipids, the phosphatidylcholine and the phosphatidylethanolamine have the same glycerol backbone and differ solely by the substitution of the choline $\overset{+}{N}(CH_3)_3$ by the $\overset{+}{N}H_3$ group. Our task was to determine the possible influence of this substitution on the conformation of the polar end, i.e. the respective orientation of the nitrogen and oxygen atom of the phosphate group. In fact four possibilities can exist in solution (fig. 2): the *trans* conformation $\varphi_1 = 180°$, the *gauche* ones $\varphi_1 = \pm 60°$, or the groups can jump quickly from one conformation to another. For simplicity we shall call this latter possibility "free rotation".

We have shown that the phosphocholine moiety is in *gauche* conformations like the acetylcholine [1], while the ethanolamine group is freely rotating. Likewise the methylene of the glycerol linked to the phosphate rotate but the last one is blocked.

2. Materials and methods

Phosphocholine, dipalmitoyl and dimyristoylphosphatidylcholine were purchased from Fluka and N.B.C.; egg phosphatidylcholine was extracted from hen egg yolk according to the method of Singleton et al. [2]. Glycerophosphorylcholine was prepared by the method of Brockerhoff et al. [3], phosphoethanolamine is a gift from Prof. Neuzil. The NMR spectra were recorded on a HA 100 Varian spectrometer. According to the molecules, solvents were D_2O or CD_3OD and internal references DSS or TMS.

3. Results

The PMR signals corresponding to the α and β methylene groups (see figs. 1 to 3) are in some cases partially overlapped by part of the glycerol spectrum. Both methylenes are coupled to each other and to the nitrogen and phosphorus nuclei. In the case of phosphoethanolamine in D_2O, fast exchange occurs between H and D on the ammonium group, and their coupling with the α methylene is no longer observable.

3.1. *Interpretation of the α spectra*

It is of interest to note that the coupling constants of heteroatoms with α protons are very small, J_{N-CH_2} is less than 1 Hz [4] and $J_{P-O-C-CH_2}$ is expected to vary from about 0 to 1 Hz [5]. So in a first approximation the α signal will be a triplet if free rotation occurs, or the AA′ part of an AA′BB′ spectrum for either

trans or *gauches* conformations. It is clear that both classes of molecules differ remarkably from each other. The phosphoethanolamine (fig. 1) presents a triplet, $J_{CH_2-CH_2}$ = 5.1 Hz, slightly doubled by the phosphorus coupling $J_{P-O-C-CH_2}$ = 0.9 Hz. This result agrees with free rotation and is confirmed by spin–spin decoupling (fig. 1c). This means that the three conformers can exist with approximatively the same probability.

In contrast all the choline moieties present an α quintuplet (figs. 2 and 3). We have previously analysed this quintuplet [6] and shown that both phosphocholine and glycerophosphocholine are in the *gauche* conformations $\varphi_1 = \pm 60°$ in D_2O. The α spectra of L-dimyristoyl, D,L-dipalmitoyl, egg phosphatidylcholine and even lysophosphatidylcholine in CD_3OD have exactly the same characteristic parameters and all the α signals are quite well fitted by the unique theoretical spectrum shown in fig. 2. The straightforward conclusion is that $\varphi_1 = \pm 60°$ for the choline and either in D_2O or CD_3OD. This conformation is

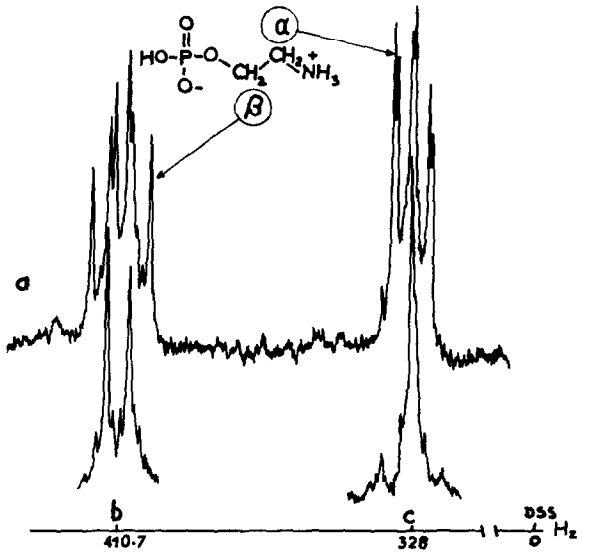

Fig. 1. a) Spectrum of the phosphoethanolamine in aqueous solution (D_2O). b) The β methylene region while decoupling α protons. c) The α methylene region while decoupling β protons.

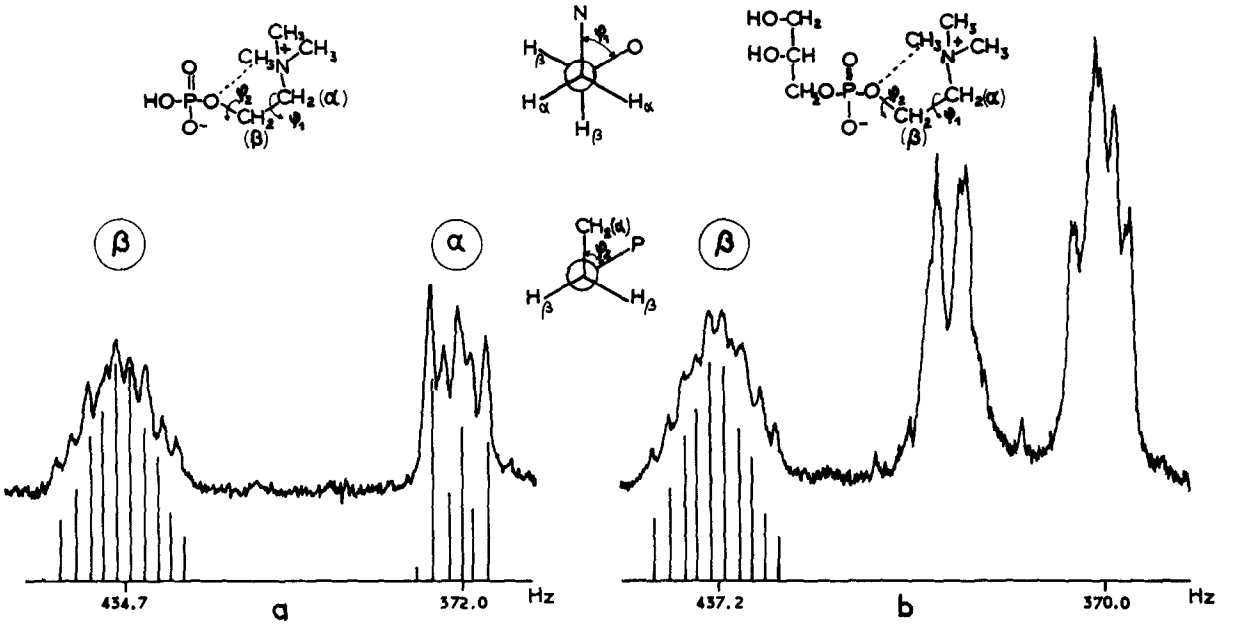

Fig. 2. Experimental spectra for α and β methylene protons of phosphocholine (a) and glycerophosphorylcholine (b) in D_2O (DSS reference). Calculated spectra for a gauche conformation from [6].

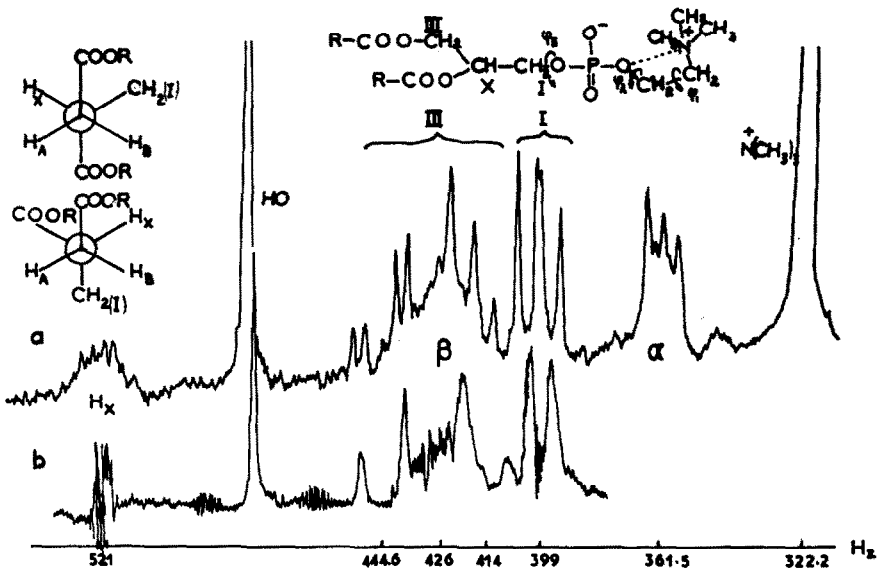

Fig. 3. a) Spectrum of the L-dimyristoyl phosphatidyl choline in DC_3OD (TMS reference). b) Spectrum recorded in the presence of an irradiation centred at the –CH– resonance. The D,L-dipalmitoyl and egg phosphatidylcholines give the same spectra but only the α signal is identical for the lysolecithin.

rather stable since the α spectrum does not change from 20° up to 90° for phosphocholine and glycerophosphorylcholine in D_2O, and from 20° up to 51° for synthetic or egg phosphatidylcholines in CD_3OD.

3.2. *Interpretation of the β spectra*

When assignable the β spectrum appears to be more complex because the coupling constants due to heteroatoms are larger. J_{N-C-CH_2} vary from 1 to 2.6 Hz when φ_1 changes [1, 7] and the extreme values for J_{P-O-CH_2} when φ_2 is varied are J_t = 28 Hz and J_g = 1 Hz [9, 10]. A fairly good fit is reached between experimental and calculated β spectra of the phosphocholine or glycerophosphorylcholine, when using the proton coupling constants of the α spectrum: J_g = 2.7 Hz and J_t = 11.1 Hz and J_{N-C-CH_2} = 2.6 Hz, J_{P-O-CH_2} = 7.0 Hz (fig. 2). The value of 2.6 Hz is consistent with $\varphi_1 = \pm 60°$ [1]. For φ_2 it is difficult to conclude, however the expected value for free rotation around the O–C_β being 10 Hz, the smaller value observed suggests that $\varphi_2 = 180°$ is slightly favoured.

The β spectrum of the phosphoethanolamine is easily interpretable by free rotation: the proton triplet, $J_{CH_2-CH_2}$ = 5.1 Hz, is doubled by the phosphorus coupling J_{P-O-CH_2} = 6.4 Hz, this is confirmed by spin–spin decoupling (fig. 1b).

3.3. *Interpretation of the glycerol spectrum*

The assignments of the glycerol proton signals are known [10]. Toward the high fields we observe in the following order (fig. 3) the –CH– signal, the $-CH_2-$ (III) one superimposed to the β methylene, and a quadruplet due to the $-CH_2-$ (I). The two methylenes are coupled to the –CH– proton, moreover $-CH_2-$ (I) is coupled to the phosphorus. Free rotation would lead to a quadruplet for (I) and a doublet for (III). Consequently methylene (I) is free to rotate and decoupling (fig. 3b) gives J_{P-O-CH_2} = 7.0 Hz, and $J_{CH_2-CH_2}$ = 5.7 Hz. About this latter value, the comment for φ_2 is valuable for φ_3. It is quite obvious that methylene (III) spectrum is an ABX one with $|J_{AB}|$ = 12.5 Hz, J_{AX} = 3.25 Hz, J_{BX} = 7.3 Hz. As J_{AX} and J_{BX} are significantly different, we can exclude the free rotation and the symmetrical structure in which H_X is between H_A and H_B. The remaining possible conformations are those shown in fig. 3 indiscernable for the NMR spectroscopy.

4. Discussion

The above findings clearly show a difference about the conformational possibilities of two phospholipid polar groups. It is of interest to compare these results with those of Sundaralingam [11] who reported that in the solid state both glycerophosphorylcholine and phosphoethanolamine are in *gauche* conformations. We have found that these structures are preserved in solution for the choline moiety, but not for the ethanolamine one. The explanation of this discrepancy will be somewhat difficult, but it can arise either from a different intramolecular energy of interaction and a different hydratation of the ammonium groups. As for the phosphatidylcholine glycerol backbone, the blocking of the methylene (III) is certainly due to the steric hindrance of the two aliphatic chains. That the above polar head structures are the actual ones existing within the organized systems, such as phospholipid bilayers or even biological membranes, is still a speculation. However, as the choline moiety remains *gauche* either in solid state or in solution, it is highly probable that this conformation exists at the water–phospholipid leaflet interface. But the conformations of the ethanolamine end must be a function of different parameters, mainly the degree of hydratation and the transition temperature of the organized system.

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