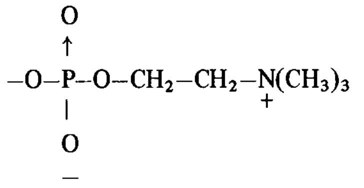
CONFORMATION AND SOLVATATION STUDY OF PHOSPHATIDYLETHANOLAMINE BY NMR

H. RICHARD, J. DUFOURCQ and C. LUSSAN
Centre de Recherches Paul Pascal, Domaine Universitaire, 33405 – Talence, France

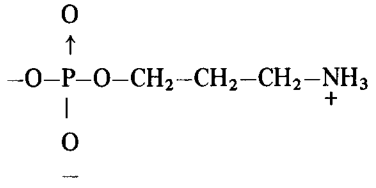
Received 21 May 1974

1. Introduction

The phosphatidylethanolamine (PE) and the phosphatidylcholine or lecithin (PC) are major components of biological membranes. They only differ from each other by their polar head. The former being the phosphocholine and the latter the phosphoethanolamine.



$$-O-\underset{\underset{\textstyle O^-}{|}}{\overset{\overset{\textstyle O}{\uparrow}}{P}}-O-CH_2-CH_2-\underset{+}{N}(CH_3)_3$$



$$-O-\underset{\underset{\textstyle O^-}{|}}{\overset{\overset{\textstyle O}{\uparrow}}{P}}-O-CH_2-CH_2-CH_2-\underset{+}{N}H_3$$

According to the Sundaralingam notation [1] the phosphatidylethanolamine is represented by scheme shown on fig. 1.

2. Materials and methods

The synthetic L-α phosphatidelethanolamine and phosphoethanolamine are respectively from Sigma and Calbiochem. They were used without further purification. Solvents are CD_3OD, mixtures of $CDCl_3-CD_3OD$ or D_2O.

The proton NMR spectra have been done on a BRUKER WH 270 a Varian XL 100 or a Varian HA 100.

3. Experimental results

3.1. *NMR spectrum of the PE at 270 MHz*

The fig. 1a shows the proton NMR spectra of the PE at 270 MHz. The assignements indicated in the spectrum have been done using the following characteristics: chemical shifts, coupling constants, homonuclear decoupling. The PMR signals of $C_{(2)}H$, $C_{(3)}H_2$, $C_{(1)}H_2$ of the glycerol, CH_2OCO, CH_2-CH_2-OCO and $(CH_2)_n$ of the aliphatic chains are identical in both PE and PC [2,3].

On fig. 1b, we can see that the $C_{12}H_2$ spectrum differs from a classical triplet so corresponds to a blocked conformation around the $C_{11}-C_{12}$ bond.

Unfortunately the $C_{11}H_2$ line is generally overlapped either by the OH signal at 270 MHz or by the C_1H_2 line from glycerol at 100 MHz.

3.2. *Phosphoethanolamine spectrum*

The fig. 2a shows the spectrum obtained at 100 MHz. We have previously proposed from this spectrum [2] that there is a free rotation around the $C_{12}-C_{11}$ bond. Taking in account that in the whole PE the conformation around the same bond is blocked we have done a more careful analysis of the phosphoethanolamine spectrum.

The $C_{(12)}H_2$ and $C_{(11)}H_2$ are an AA'XX' system with:

North-Holland Publishing Company – Amsterdam

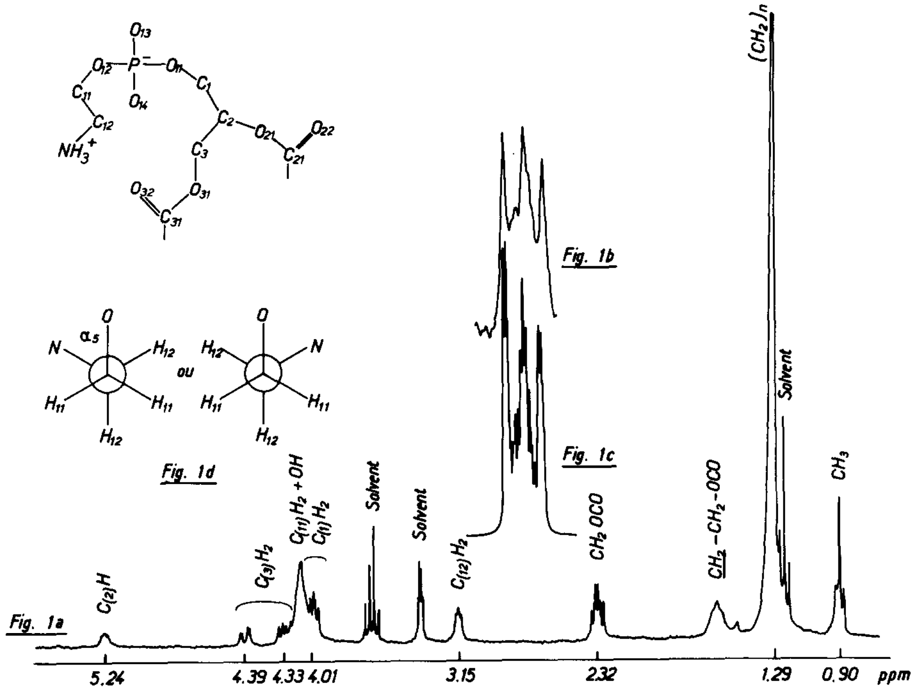


Fig. 1: a) 270 MHz NMR spectra of phosphatidylethanolamine (PE) in $CDCl_3 + CD_3OD$ (4 : < –1) solution; b) 100 MHz (F.T.) spectra of the $C_{(12)}H_2$ from PE; c) theoretical spectrum of the $C_{12}H_2$ of phosphoethanolamine.

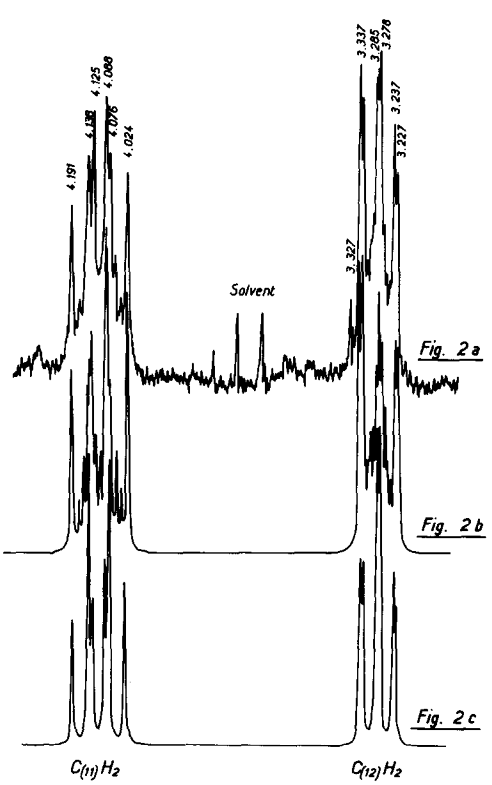


Fig. 2: a) 100 MHz spectrum of the phosphoethanolamine in D_2O; b) theoretical spectrum of the phosphoethanolamine with the hypothesis of a blocked conformation; c) theoretical spectrum of the phosphoethanolamine with the hypothesis of a free rotation.

$N = 11$ [4]
$|L| = 5$
$M = 1.5$ $JP{-}O{-}C_{11}H_2 = 6.4$ Hz
$K >> L$ $JP{-}O{-}C_{12}H_2 = 0.9$ Hz

From these values according to the L sign we can calculate the protons coupling constants:

$L < 0$ trans conformation
$Jt = 8$ Hz $Jg = 3$ Hz $J = 4.7$ Hz
$L > 0$ gauche conformation
$Jt = 13$ Hz $Jg = 3$ Hz $J = 6.3$ Hz

From the Abraham and Pachler rule [4] we found $j = 5.8$ Hz. The calculated spectrum corresponding to the different conformational possibilities are shown on fig. 2b and 2c; it is clear that the 2b spectra agree much better with the experimental one. So we conclude that the phosphoethanolamine itself is also in a gauche conformation.

3.3. *$C_{12}H_2$ and $C_{11}H_2$ spectrum of PE*

With the same parameters we can also interpret the PE $C_{11}H_2$ line. This is shown on fig. 1b–c where we can see again a good agreement between the experimental and theoretical spectrum. So in PE the $C_{11}-C_{12}$ bond is blocked in the gauche conformation.

3.4. *Interpretation of the glycerol spectrum*

Since the glycerol spectrum of PE is almost identical to that of PC we can give the same interpretation as that we proposed previously for PC [2] i.e. the three conformers can exist around the C_1-C_2 bond and there is no free rotation around the C_2-C_3 bond. However Birdsall et al. [3] proposed a somewhat different interpretation and found different proportions for each one of the conformers especially around C_2-C_3 bond. It is difficult for the moment to choose between the two possibilities.

4. Effect of an addition of a polar solvent to a solution of PC in $CDCl_3$

It is necessary to have about 30 M CD_3OD/1 M PE to observe a downfield shift of the $C_{(11)}H_2$ and $C_{(12)}H_2$ shift up field. The maximum shift, 0.04 ppm, is obtained after addition of 120 M CD_3OD/1 M PE, fig. 3. The effect is rather small compared to that published elsewhere on PC 5 since 12 M CD_3OD/1 M PC gave a maximum shift also obtained with 6 M D_2O/1 M PC. The upfield shifts were those of $C_{(12)}H_2$ and $N(CH_3)_3$ but the poor resolution of the $C_{11}H_2$ signal prevented us to see the $C_{(11)}H_2$ and $C_{(1)}H_2$ downfield shift. This latter effect was recently observed by adding water to a solution of PC in CCl_4 [6].

5. Conclusion

The NMR spectra of phosphatidylethanolamine (PE) and phospnatidylcholine (PC) in organic solvents are almost identical. They differ only by the spectra of the $C_{(12)}H_2$ and $C_{(11)}H_2$ groups corresponding to the polar head moiety. A careful analysis shows that

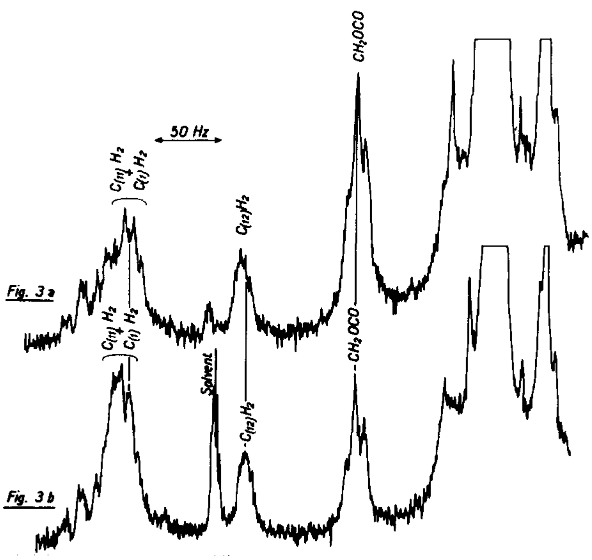


Fig. 3: a) Part of the spectrum of PE in $CDCl_3$; b) part of the spectrum of PE in a $CDCl_3 + CD_3OD$ solution 120 M CD_3OD/1 M PE.

this difference is due to different coupling constants occurring when the $\overset{+}{N}H_3$ is changed by a $N^+(CH_3)_3$ group. It results that in both cases the conformation of the polar head is gauche.

The behaviour of PE and PC in interaction with polar solvents is markedly different for PE. It is necessary to have about 10-fold increase of CD_3OD to get the same maximal solvatation effect compare to PC.

Acknowledgements

We are indebted to Doctor B. Clin for his advice in the interpretation of theoretical spectra.

References

[1] Sundaralingam, M. (1972) Ann. N.Y. Acad. Sci. 195, 324.
[2] Dufourcq, J. and Lussan, C. (1972) FEBS Letters 26, 235.
[3] Birdsall, N. J. M., Feeney, J., Lee, A. G., Levine, Y. K. and Metcalfe, J. C. (1972) J. Chem. Soc. 10, 1441.
[4] Abraham, R. J. and Pachler, K. G. R. (1963) Mol. Phys. 7, 165.
[5] Richard, H. and Lussan, C. (1973) Compt. Rend. 276, 1345.
[6] Shaw, Y. H., Kaw, L. S., Li, N. C. (1973) J. Magnet. Resonance 12, 209.