Biochimica et Biophysica Acta, 1023 (1990) 143–146
Elsevier

BBAMEM 70647

BBA Report

^{13}C CP-MAS study of the gel phases of 1,2-dipalmitoylphosphatidylcholine

Karol S. Bruzik, Grzegorz M. Salamonczyk and Beata Sobon

Center of Molecular and Macromolecular Studies, Polish Academy of Sciences, Lodz (Poland)

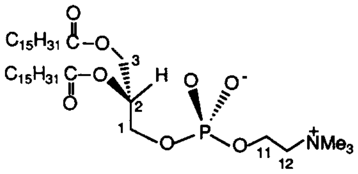
(Received 11 July 1989)
(Revised manuscript received 29 December 1989)

Key words: Dipalmitoylphosphatidylcholine; NMR, ^{13}C CP-MAS; CP-MAS NMR; Phospholipid bilayer; Phospholipid conformation

The multilamellar liposomes of the racemic 1,2-dipalmitoylphosphatidylcholine (DL-DPPC) existing in the various gel phases were investigated by means of the cross-polarization/magic angle spinning (CP-MAS) ^{13}C-NMR. The intensity and the width of the ^{13}C-NMR signals were found to depend to a large extent on temperature. In the metastable gel phase (L_β) signals from all carbon atoms are apparent, with the signals arising from the glycerol backbone significantly broader than those of the choline function. The signal from C-2 of the glycerol backbone undergoes additional broadening between 298 K and 307 K, and at 307 K its coalescence is observed. In the $P_{\beta'}$ phase all carbon atoms give rise to relatively sharp separate resonance lines. In the liquid crystalline phase (L_α) the signals from the choline function and from the terminal methyl groups of hydrocarbon are not observed in the spectrum. The recorded spectral changes are explained in terms of the rate of motional processes occurring in the lipid bilayer in different phases.

The properties of the model phospholipid membranes have been extensitvely studied for two decades using the variety of biophysical methods including NMR spectroscopy [1–5]. The most widely used techniques of NMR have been ^{31}P-[1-3], ^{2}H-[4,5] and ^{14}N-NMR [6] spectroscopy of oriented and unoriented bilayers. The application of the ^{13}C-NMR spectroscopy for the elucidation of the bilayers has been limited to small unilamellar vesicles, where rapid molecular tumbling averages out the ^{13}C chemical shielding anisotropy (σ_{csa}) and ^{1}H-^{13}C dipolar couplings, resulting in the relatively well resolved spectra albeit of low sensitivity [7]. Another approach requires selectively ^{13}C-labeled samples to measure σ_{csa} of a given ^{13}C carbon nucleus [1].

The cross-polarization/magic angle sample spinning (CP-MAS), commonly used in studies of solids [8], was applied earlier for studies of model phospholipid membranes by Haberkorn et al. [9], who reported spectra of dimirystoyl- (DMPC) and dipalmitoylphosphatidylcholine (**1**, DPPC), and by Sefcik et al. [10], who studied protein-reconstituted phospholipid membranes. The application of MAS technique alone to studies of PC-cholesterol systems has been recently reported [11]. The advantage of MAS relies upon its ability to resolve all resonance lines airsing from carbon atoms of the phospholipid polar head group. We now report CP-MAS ^{13}C-NMR spectra of solid DPPC and of the multilamellar dispersions of DPPC existing in the various phases.



1

DL-α-Dipalmitoylphosphatidylcholine (DL-DPPC) was purchased from Sigma and was further purified by column chromatography on silica gel using chloroform/methanol/water (65 : 35 : 4, v/v) as the eluting solvent. DPPC thus obtained was further purified by precipitation from ethanol solution with low residue acetone. L-α-Dipalmitoylphosphatidylcholine (L-DPPC) was synthesized as described earlier [12] and purified as above. Dry phospholipid (150 mg) was hydrated by adding water (150 μl) and forcing the semisolid through the narrow constriction made in the glass tubing. The DSC trace of the DPPC dispersions was consistent with the literature results [13].

Correspondence: K.S. Bruzik, Center of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-362 Lodz, Poland.

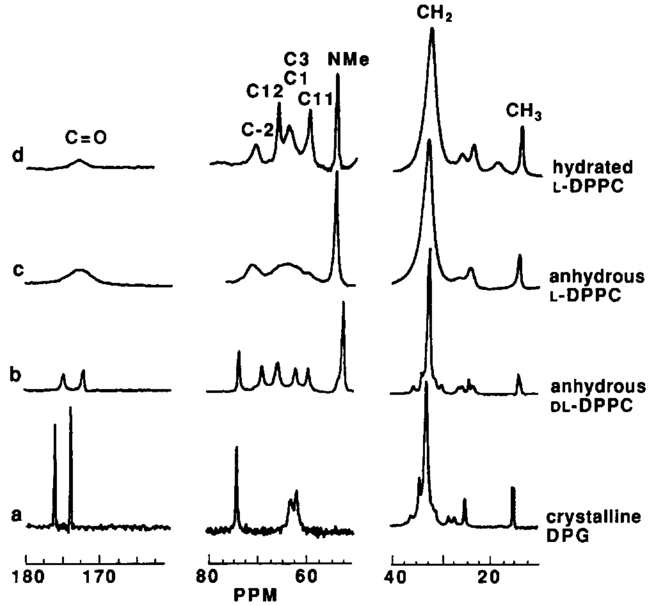


Fig. 1. 75.47 MHz ^{13}C CP-MAS NMR spectra of anhydrous and hydrated lipids: (a) crystalline DPG, 295 K; (b) anhydrous DL-DPPC, 295 K; (c) anhydrous L-DPPC, 295 K; (d) hydrated L-DPPC in the gel phase, 302 K. Spectral parameters: sweep width 16 kHz, ^1H $\pi/2$ pulse 5.5 μs, contact time 1 ms, acqusition time 52 ms, scan repetition 4 s. Typically, 2000 scans of 1670 data points each were zero filled to 8K, and exponentially multiplied prior to Fourier transform, line broadening factor, 20. In case of trace d the acquisition time was minimized to 20 ms and scan repetition was increased to 10 s in order to minimize the heat absorption by the sample. Vertical expansions of left, middle and right parts of the spectra are 3.8, 5 and 1, respectively.

^{13}C-NMR spectra were obtained with Bruker MSL-300 NMR spectrometer at 75.47 MHz using the cross-polarization pulse sequence with magic angle spinning and high power proton decoupling. Throughout the measurements both rf fields at proton and carbon frequencies were maintained at approximately constant level approx. 1.3 mT for ^1H and 5.6 mT for ^{13}C. The temperature inside the rotor was calibrated by ^1H-NMR spectrum of methanol sample. Solid phospholipid samples were rotated at 3.5 kHz speed and the hydrated samples at approx. 1 kHz. The assignment of resonance lines was based on the comparison of the CP-MAS spectra with the solution spectra reported earlier [14]. The chemical shifts were referenced indirectly to carbonyl carbon of glycine as 176.06 ppm.

The spectra of solid and anhydrous racemic DL-DPPC, solid L-DPPC and of crystalline L-α-dipalmitoylglycerol (DPG) are shown in Fig. 1. The chemical shifts of ^{13}C signals are listed in Table I. The most striking feature apparent from these data is the difference of the chemical shifts of carbon atom C-2 of the glycerol moiety in studied samples. In DL-DPPC and DPG the position of this signal is shifted 2.5 ppm downfield related to anhydrous L-DPPC and to hydrated sample of DL-DPPC. The line width of the signals originating from the polar headgroup carbons in the spectrum of L-DPPC is also much greater than it is in other cases (trace 1c). The increase in the temperature had no narrowing effect on the lines in this spec-

TABLE I

^{13}C-NMR chemical shifts of lipid samples

Carbon atom	DPG	DL-DPPC	Hydrated DL-DPPC [a]	L-DPPC [b]	Hydrated L-DPPC [a]	Hydrated L-DPPC [a,e]
C = O	175.8 173.6	175.0 172.3	172–175	172.5	173.6	173.7
C-1	61.6 [d]	62.5 [d]	64.3	broad	64.1	67.6
C-2	73.9	74.0	71.3 [c]	71 [c]	70.9	71.2
C-3	63.0	69.3 [d]	64.3 [c]	broad	64.1 [f]	63.7
C-11		59.9	59.9	broad	59.9	59.8
C-12		66.1	66.4	broad	66.3	66.4
NMe	52.9	54.5	54.0	54.0	54.4	54.5
$(CH_2)_n$	32.7	32.7	32.9	32.7	32.7	30.8
2'-CH_2	34.0	34.1	[f]			34.4
3'-CH_2	28.5 26.7	26.4 25.8	26.3	26.2	26.3	25.4
15'-CH_2	24.8	24.5 23.9	23.8	24.1	23.8	23.0
CH_3	15.1 14.7	14.5	14.3	14.1	14.1	14.1

[a] DPPC in L_β phase at 302 K.

[b] One broad line from C-1, C-3 and C-11 was observed.

[c] Broad line centered about this value.

[d] Tentative assignment.

[e] Data from Ref. 9. The chemical shift of the hydrocarbon $(CH_2)_n$ (30.8 ppm) indicates that the lipid sample was in the liquid crystalline state and not in the gel phase as stated.

[f] Broad overlapped line.

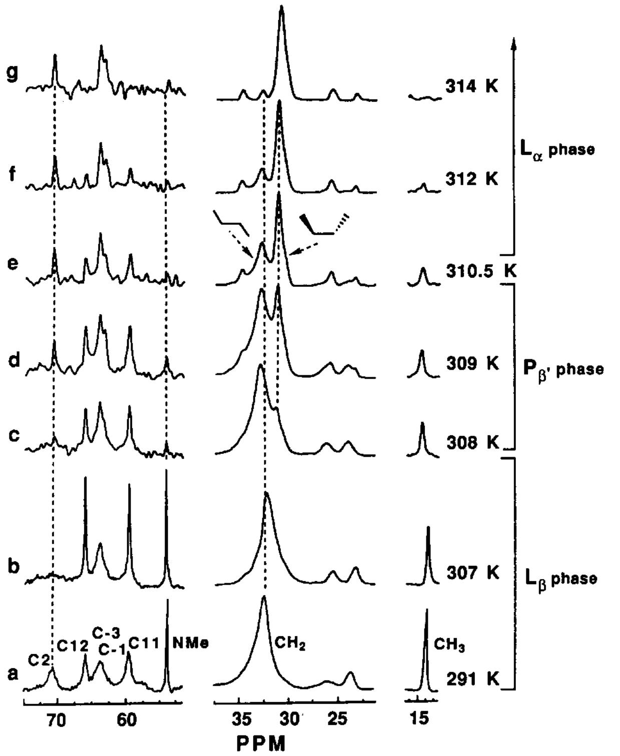


Fig. 2. 75.47 MHz ^{13}C CP-MAS NMR spectra of multilamellar liposomes of DL-DPPC at indicated temperatures and phases. Spectral parameters are as in Fig. 1. Vertical expansions of left, middle and right parts of the spectra are 10, 1, 1.6, respectively. Phase transition temperatures of the studied sample (DSC): T_m 314 K, $\Delta T_{1/2}$ 1.5 deg, midpoint of pretransition T_p 309 K.

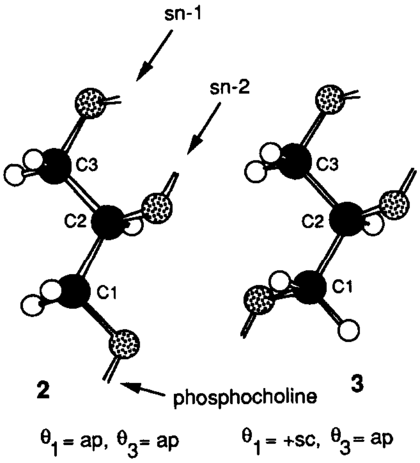
trum (data not shown). Differences in the purification protocol and precipitation of the phospholipid had little or no effect on the spectrum. In the spectra of DL-DPPC and DPG carbonyl carbon atoms give rise to separate signals, while in the spectra of solid L-DPPC and of aqueous dispersions of both DL-DPPC and L-DPPC only one broad line is observed. The phosphocholine group gives signals with quite constant chemical shifts regardless of the sample condition and DPPC configuration. There is an uncertainty in the assignment of the signals from C-1 and C-3 of the glycerol. In dispersed DL-DPPC and L-DPPC situation is similar to small micelles, while in solid DL-DPPC the chemical shifts are quite different and therefore the assignment is not obvious. Haberkorn et al. [9] have also reported different chemical shifts for C-1 and C-3 from those currently observed.

Partial spectra of multilamellar liposomes of DL-DPPC are given in Fig. 2. In L_β phase, at the temperature 291 K, all carbon atoms of the polar headgroup contributed their resonance lines (resonances from C-3 and C-1 overlap), however, all lines were slightly broadened except for the NMe function of the choline (Fig. 2a,b). The increase in the temperature to 307 K had narrowing effect on the lines of C-11 and C-12, while it caused broadening of the line arising from C-2, such that it could not be observed. Further heating of the sample to 308 K resulted in the dramatic decline in the intensity of the signals of the choline group (NMe, C-11, C-12) implying the onset of the pretransition. In addition a sharp resonance line of low intensity from carbon atom C-2 started emerging. The broad line at 32.5 ppm from methylene groups of the hydrocarbon core was split to give another line at 30.8 ppm suggesting the formation of the significant population of the synclinical rotational isomers in the hydrocarbon chain characteristic of the melted hydrocarbon [15,16].

At the temperature 309 K all lines related to carbon atoms of the glycerophosphocholine were visible, although the intensity of the lines arising from the choline methyl groups was low. The intensity of the line at 71.5 ppm (C-2) was positively correlated with intensity of the line at 30.8 ppm (Fig. 2, c–e). The resonance line from C-2 was sharp and those from C-3 and C-1 were resolved due to their small line-widths. The interior hydrocarbon CH_2 groups gave rise to a stronger upfield signal, indicating the progress in melting of the hydrocarbon. This change was accompanied by the appearance of the peak at 23.1 ppm at the cost of the signal at 23.8 ppm. Further increase in the temperature (to 310.5 K) resulted mainly in the decrease of the intensities of the lines from all choline carbon atoms and of the terminal methyl group (to a lesser extent). The NMe function signal has become completely suppressed. At the temperature 312 K only signals from the glycerol function and from the melted hydrocarbon could still be seen. The increase in the temperature up to 314 K had only minor effect on the observed spectra, except for the decrease in the total signal receptivity.

Three principal effects of the state of the bilayer phase on ^{13}C-NMR spectra were observed: (i) change of the chemical shift of the lines, (ii) change in the line width; (iii) change of the line intensity.

(i) The chemical shift. The comparison of the spectra of solid DL-DPPC, L-DPPC and DPG reveals significant differences in the chemical shifts of signals. Differences in the chemical shift of C-2 may be regarded as resulting from the conformational changes among the lipids studied. It becomes clear, that the conformation of DL-DPPC in the solid state differs from its conformation in the hydrated bilayers and from the conformation of L-DPPC (whether solid or hydrated). The differences most likely concern the existence of two different conformations (2 and 3) about the C1-C2 bond (θ_1 torsional angle) since in all reported single crystal structures of double chain phospholipids only antiperiplanar conformation about C2-C3 bond has been found, while the conformation about C1-C2 varies from one lipid to another. In the case of L-DMPC two molecules with different θ_1 angles have been observed in the crystalline lattice [17]. Our observation underlines the uncertainty encountered, when the phospholipid conformation de-



termined in the solid crystalline state is compared with its conformation in the bilayer.

The upfield shift of the ^{13}C-NMR signal of the hydrocarbon chain (Fig. 2d–g) is caused by a *gauche* γ-effect, due to an increase in the population of synclinical conformers about CC-CC bond, characteristic ot the hydrocarbon chain melting [15,16].

(ii) The line width. Changes in the line width noted for C-2 carbon in the metastable gel phase are consistent with the explanation given by Lyerla et al. [18], who attributed the increase in the line width at certain temperatures to the ineffectiveness of the proton decoupling in the 'intermediate correlation' time regime, when the correlation time for molecular motion coincides with the modulation period of the decoupling field (45 kHz in this case). Line narrowing at a threshold temperature of pretransition is in agreement with the notion, that the transition from L_β to $P_{\beta'}$ phase enhances the rate of a headgroup rotation.

(iii) The line intensity. In CP-MAS experiments this parameter is governed, among other factors, by the cross polarization rate [8,9,19]. In fluid systems the cross polarization may be slow depending on the dynamics of the particular environment. In the case of the fluid bilayer, the cross polarization is inefficient resulting in a loss of signal receptivity [19,20]. As seen in the spectra in Fig. 2, the line intensity of the choline methyl group at pretransition is particularly low. This effect may be linked to an increase in the rate of the choline headgroup rotation at the onset of the pretransition [21,22]. The decrease in the line intensity is also observed for the terminal methyl function at the main transition.

In summary, it is concluded that ^{13}C CP-MAS NMR spectroscopy is of high value for the characterization of structure and of dynamics of the lipid bilayer phases.

The help of Mr. J. Krzywanski of this Laboratory in solving hardware related problems is gratefully acknowledged. This work was supported by Polish Academy of Sciences, Grant No. CPBP 01.13.3.16.

References

1 Griffin, R.G. (1981) Meth. Enzymol. 72, 108–174.
2 Smith, I.C.P. and Ekiel, I.H. (1984) in Phosphorus-31 NMR (Gorenstein, D.G., ed.), pp. 447–475, Academic Press, New York.
3 Seelig, J. (1978) Biochim. Biophys. Acta 515, 105–140.
4 Seelig, J. and Seelig, A. and Tamm, L. (1982) in Lipid-Protein Interactions (Jost, P., ed.), Vol. 2, pp. 127–148, J. Wiley, New York.
5 Davis, J.H. (1983) Biochim. Biophys. Acta 737, 117–172.
6 Rothgeb, T.M. and Oldfield, E. (1981) J. Biol. Chem. 256, 6004–6009.
7 Levine, Y.K., Birdsall, N.J.M., Lee, A.G. and Metcalfe, J.C. (1972) Biochemistry 11, 1416–1421.
8 Schaefer, J. and Stejskal, E.O. (1981) in Topics in Carbon-13 NMR Spectroscopy, Vol. 3, p. 283, J. Wiley, New York.
9 Haberkorn, R.A., Herzfeld, J. and Griffin, R.G. (1978) J. Am. Chem. Soc. 100, 1296–1298.
10 Sefcik, M.D., Schaefer, J., Stejskal, E.O., McKay, R.A., Ellena, J.F., Dodd, S.W. and Brown, M.F. (1983) Biochem. Biophys. Res. Commun. 114, 1048–1055.
11 Oldfield, E., Bowers, J.L. and Forbes, J. (1987) Biochemistry 26, 6919–6923.
12 Bruzik, K.S., Salamonczyk, G.M. and Stec, W.J. (1986) J. Org. Chem. 51, 2368–2370.
13 Boyanov, A.I., Tenchov, B.G., Koynova, R.D. and Koumanov, K.S. (1983) Biochim. Biophys. Acta 732, 711–713.
14 Burns, R.A. and Roberts, M.F. (1980) Biochemistry 19, 3100–1306.
15 Saito, H. (1986) Magn. Res. Chem. 24, 835–852.
16 Moller, M., Gronski, W., Cantow, H.-J. and Hocker, H. (1984) J. Am. Chem. Soc. 106, 5093–5099.
17 Hauser, H., Pascher, I., Pearson, R.H. and Sundell, S. (1981) Biochim. Biophys. Acta 650, 21–51.
18 Lyerla, J.R., Yannoni, C.S. and Fyfe, C.A. (1982) Acc. Chem. Res. 15, 208–216.
19 Yannoni, C.S. (1982) Acc. Chem. Res. 15, 201–208.
20 Yamamobe, T., Tsukuhara, M., Komoto, T., Watanabe, J., Ando, I., Uematsu, I., Degichi, K., Fujito, T. and Imanari, M. (1988) Macromolecules 21, 48–50.
21 Shepherd, J.C. and Buldt, G. (1978) Biochim. Biophys. Acta 514, 83; ibid., (1979) 558, 41–47.
22 Cevc, G. and Marsh, D. (1987) Phospholipid Bilayers. Physical Principles and Models, J. Wiley, New York.