Reprinted from the Journal of the American Chemical Society, **1983,** *105*, 2478.

Phospholipids Chiral at Phosphorus. 4. Could Membranes Be Chiral at Phosphorus?[1]

Ming-Daw Tsai,* Ru-Tai Jiang, and Karol Bruzik

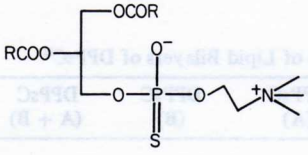
Department of Chemistry, The Ohio State University
Columbus, Ohio 43210
Received September 2, 1982

We present results of model study that suggest that phospholipid membranes could be chiral at phosphorus and the configuration of phosphorus could be important in the structure and properties of membranes.

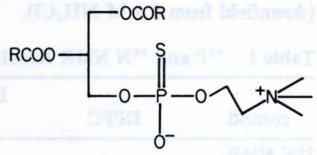
The prochiral phosphorus center of phospholipids could in principle exist in four possible states in the liquid crystalline phase: (I) achiral, $^{\delta-}O\cdots P\cdots O^{\delta-}$; (II) chiral, O═P—O⁻ (with the negative charge partially or fully localized); (III) chiral, ⁻O—P═O; (IV) racemic, as a mixture of II and III. This fundamental problem has never been considered in the models for membrane structures and for protein–lipid interactions, although there is increasing evidence for the involvement of the phosphate head group in protein–lipid interactions,[2] and the conformations of head group of phospholipids have been studied recently.[3]

The problem is even more intriguing when considered with the recent report of Arnett and Gold[4] that the chiral C-2 center of dipalmitoyl phosphatidylcholine (DPPC) cannot be recognized by (*R*)-*N*-(α-methylbenzyl)stearamide (NMBS), but L-DPPC is able to recognize the chiral center of NMBS. Although they provided no explanation, a very logical one is that DPPC has another chiral recognition site other than C-2. Could this be the prochiral phosphorus center?

The model compounds used for states II–IV of DPPC are the isomer A (**1**), isomer B (**2**), and mixture (A + B) (**3**), respectively



1, DPPsC (A)



2, DPPsC (B)

($R = C_{15}H_{31}$), of 1,2-dipalmitoyl-*sn*-glycero-3-thiophosphorylcholine (DPPsC).[5] It should be noted that in **1** and **2** the absolute configuration at phosphorus is still unknown, and the localization of the negative charge at oxygen has no experimental proof. However, on the basis of the work in the sulfur analogues of nucleotides,[6] **1** and **2** should be good models for II and III.

To compare the properties of **1**–**3** in the liquid crystalline phase, we chose to measure the quadrupolar splitting $\Delta\nu_Q$ in ^{14}N NMR[7] and the chemical shift anisotropy $\Delta\sigma$ in ^{31}P NMR,[8] both of which are sensitive to the structural and motional properties of the phosphate head group. Figure 1 shows the ^{14}N NMR spectra (single-pulse experiment)[9] of the unsonicated aqueous dispersion

(1) Supported by grants from NSF (PCM 8140443) and from NIH (GM30327). The NMR spectrometers used were funded by NIH GM 27431 and NSF CHE 7910019. Part 3: Bruzik, K.; Jiang, R.-T.; Tsai, M.-D. *Biochemistry*, in press. Abbreviations used: NMR, nuclear magnetic resonance; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine; DPPsC, 1,2-dipalmitoyl-*sn*-glycero-3-thiophosphorylcholine; NMBS, *N*-(α-methylbenzyl)-stearamide.

(2) (a) Yeagle, P. L. *Acc. Chem. Res.* **1978**, *11*, 321–327. (b) Rajan, S.; Kang, S.-Y.; Gutowsky, H. S.; Oldfield, E. *J. Biol. Chem.* **1981**, *256*, 1160–1166.

(3) (a) Seelig, J.; Gally, H.-U.; Wohlgemuth, R. *Biochim. Biophys. Acta* **1977**, *467*, 109–119. (b) Skarjune, R.; Oldfield, E. *Biochemistry* **1979**, *18*, 5903–5909.

(4) Arnett, E. M.; Gold, J. M. *J. Am. Chem. Soc.* **1982**, *104*, 636–639.

(5) Bruzik, K.; Gupte, S. M.; Tsai, M.-D. *J. Am. Chem. Soc.* **1982**, *104*, 4682–4685.

(6) (a) Knowles, J. R. *Ann. Rev. Biochem.* **1980**, *49*, 877–919. (b) Frey, P. A. *Tetrahedron* **1982**, *38*, 1541–1567. (c) Eckstein, F.; Romaniuk, P. J.; Connolly, B. A. *Methods Enzymol.* **1982**, *87*, 197–212. (d) Tsai, M.-D. *Methods Enzymol.* **1982**, *87*, 235–279.

(7) Rothgeb, T. M.; Oldfield, E. *J. Biol. Chem.* **1981**, *256*, 6004–6009.

(8) (a) Seelig, J. *Biochim. Biophys. Acta* **1978**, *515*, 105–140. (b) Griffin, R. G. *Methods Enzymol.* **1981**, *72*, 108–174.

0002-7863/83/1505-2478$01.50/0

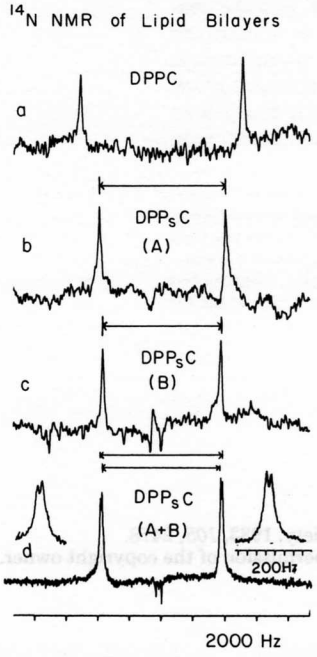


Figure 1. ^{14}N NMR spectra (at 21.7 MHz, Bruker WM-300) showing the quadrupolar splitting of DPPC (a), DPPsC (A) (b), DPPsC (B) (c), and DPPsC (A + B) (d) dispersed in excess water (H_2O/D_2O = 3/1). The weight percents of lipids are 12% (a–c) and 20% (d). Spectral parameters: spectral width, 50 KHz; acquisition time, 41 ms (except d, 82 ms); 45° pulse, broad-band ^{1}H decoupling (2.5 W); probe temperature, 46 °C, ^{2}H locked, spinning; line broadening, 50 Hz (except d, 10 Hz). Chemical shifts are 25.9 ppm for DPPC and 24.0 ppm for DPPsC (downfield from 5.4 M NH_4Cl).

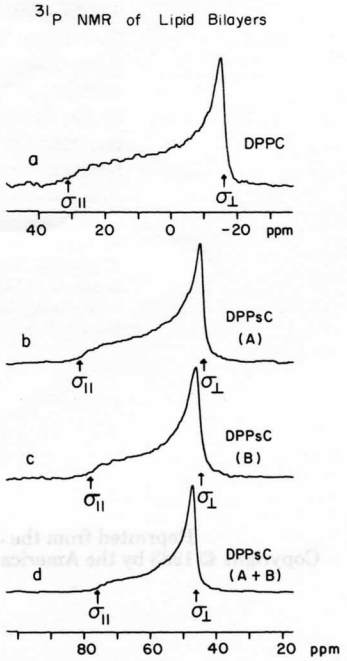


Figure 2. ^{1}H decoupled ^{31}P NMR spectra (at 81 MHz, Bruker WP-200) of DPPC (a), DPPsC (A) (b), DPPsC (B) (c), and DPPsC (A + B) (d) dispersed in excess water (H_2O/D_2O = 3/1). The weight percent of lipids is 12%. Spectral parameters: spectral width, 25 KHz; acquisition time, 0.164 s; pulse width, 15 μs (90° pulse, 20 μs), decoupler power, 3 W (further increase in the decoupler power did not change the line shape), probe temperature 46 °C; ^{2}H locked, spinning, line broadening, 50 Hz. Chemical shifts are referenced to external 1 M H_3PO_4 in D_2O.

Table I. ^{31}P and ^{14}N NMR Results of Lipid Bilayers of DPPsC[a]

compd	DPPC	DPPsC (A)	DPPsC (B)	DPPsC (A + B)
^{14}N NMR				
$\Delta\nu_Q$, KHz	10.26 ± 0.1	7.9 ± 0.1	7.5 ± 0.1	7.5 ± 0.1 7.7 ± 0.1
^{31}P NMR[b]				
$\sigma_\perp$, ppm	−16.3 ± 0.2	43.8 ± 0.4	44.7 ± 0.4	46.1 ± 0.2
$\sigma_\parallel$, ppm	30.4 ± 1.0	81.0 ± 1.0	79.0 ± 1.0	77.0 ± 1.0
$\Delta\sigma$, ppm	46.7 ± 1.2	37.2 ± 1.4	34.3 ± 1.4	30.9 ± 1.2

[a] The data are obtained from Figures 1 and 2 and at least three other independent sets of experiments (except for DPPC) at 46 °C. In each experiment the sample came from an independent synthesis or chromatography, and the weight percent lipid varies from 10% to 20%. The errors are estimated from the accuracy of the measurements and from the deviation in the four sets of data. The actual sample temperature was between 46 and 50 °C. Separately, we have shown that the $\Delta\nu_Q$ and $\Delta\sigma$ are constant within experimental error in the range of the probe temperature from 45 to 52 °C. [b] In all cases the $\sigma_\perp$ and the $\sigma_\parallel$ are measured at the half-height of the upfield shoulder and the downfield shoulder, respectively. The values of the peak tops are 44.7 ± 0.2 (A), 46.3 ± 0.3 (B), and 47.6 ± 0.3 (A + B).

of DPPC (**1a**), DPPsC (A) (**1b**), DPPsC (B) (**1c**), and DPPsC (A + B) (**1d**). The $\Delta\nu_Q$ measured from Figure 1 are listed in Table I. Figure 2 shows the ^{31}P NMR spectra of the same samples. The magnitudes of $\sigma_\parallel$, $\sigma_\perp$, and $\Delta\sigma$ obtained from Figure 2 are also listed in Table I. The result of the ^{31}P NMR of DPPsC (A + B) is consistent with that of Vasilenko et al.[10]

The data (Figures 1 and 2 and Table I) indicate several important points: (1) The model phospholipids **1–3**, which are chiral at phosphorus, are capable of forming lipid bilayers that give ^{31}P line shapes and ^{14}N quadrupolar splittings characteristic of natural membranes. (2) Both $\Delta\nu_Q$ and $\Delta\sigma$ have reduced to ca. 70 ± 5% for DPPsC relative to DPPC. (3) **1–3** give small yet reproducible differences in the values of $\Delta\nu_Q$, $\sigma_\parallel$, $\sigma_\perp$, and $\Delta\sigma$. In ^{31}P NMR, the $\Delta\sigma$ falls in the order A > B > (A + B). In ^{14}N NMR, the $\Delta\nu_Q$ of isomer A is larger than that of isomer B by 0.4 ± 0.1 KHz, whereas the mixture A + B shows a splitting of 0.2 ± 0.1 KHz. The differences in both $\Delta\sigma$ and $\Delta\nu_Q$ have been shown not to be caused by small variations in sample preparation, weight percent lipid, and sample temperature.

The difference between the properties of DPPsC (A), DPPsC (B), and DPPsC (A + B) in the liquid crystalline phase is significant since in solution isomers A and B show only a small difference (<0.05 ppm) in ^{31}P chemical shifts[5] and show no detectable difference in ^{14}N NMR. In CH_3OD, the mixture DPPsC (A + B) gave a sharp ^{14}N NMR signal (half-width <3 Hz), which could not be resolved into two peaks. In $CDCl_3$, a broader signal (half-width 11 Hz) was observed due to micelle formation, but there was no detectable difference in both chemical shift and line width between DPPsC (A + B) and DPPsC (A).[11]

The mixture DPPsC (A + B) apparently forms a lipid bilayer that has different properties from those of isomer A, isomer B, or their additives. Therefore, the configuration of phosphorus seems important in determining the membrane properties, at least in the phosphate group and choline chain region. Work is in progress in our laboratory to examine other physical properties of **1–3** carefully.[12]

In conclusion, our results suggest that it should be possible for natural membranes to exist in one of the four states I–IV. In other

(9) Due to the limit in sample quantity and in the capability of our spectrometer, we were unable to perform quadrupole echo experiments. The single-pulse experiments gave distorted line shapes, but the $\Delta\nu_Q$ should be accurate and were reproducible within ±0.1 KHz. The samples were prepared by vortexing at 50–60 °C.

(10) Vasilenko, I.; de Kruijff, B.; Verkleij, A. J. *Biochim. Biophys. Acta* **1982**, *685*, 144–152.

(11) The solution ^{14}N NMR was measured with 100 mg of samples in 4.5 mL of solvents at 45 °C, with broad-band ^{1}H decoupling. Chemical shifts are 24.41 ppm in CH_3OD and 23.41 ppm in $CDCl_3$ (downfield from an external 5.4 M NH_4Cl solution in 15% D_2O).

(12) We have submitted crystal to measure the gel → liquid crystal transition temperature (T_c) by differential scanning calorimeter (Du Pont 1090 thermal analyzer). The T_c observed are 44.7 °C for DPPsC (A), 44.0 are for DPPsC (B), and 44.5 °C for DPPsC (A + B) (0.6 mg in 20 μL of potassium phosphate buffer, pH 7.12). Whether the difference is real or is due to experimental error remains to be further investigated.

words, membranes *could be* chiral at phosphorus, and the configuration at phosphorus could be important in membrane structures. It should not be presumed that the phosphorus is achiral (as in state I) in membranes without experimental proof. In the interaction of phospholipids with membrane proteins or enzymes, it is not impossible that the phosphate group may function as a chiral recognition site.[13]

(13) Although this concept may be difficult to be envisioned in membranes, we have already found that the phosphate group of phospholipids in the form of micelles functions as a chiral recognition site in the catalysis of phospholipase A_2. This enzyme hydrolyzes the C-2 carboxylic ester, which is five bonds away from the phosphorus center. However, it specifically takes the isomer B of DPPsC as a substrate.[5]