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Static and magic angle spinning ^{31}P NMR spectroscopy of two natural plasma membranes

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Abstract Static and magic angle spinning ^{31}P NMR spectroscopy was used for the first time in natural plasma membranes from erythrocytes and skeletal muscle to study phospholipid arrangement and composition. Typical static powder-like spectra were obtained showing that phospholipids were in a bilayer arrangement. Magic angle spinning narrowed spectra into two components. The first one corresponded to phosphatidylcholine and the second one to the other phospholipids with intensities in agreement with the known phospholipid composition. These findings show that NMR data previously acquired using model membranes can be transposed to studies on phospholipids in their natural environment.

Key words: ^{31}P NMR spectroscopy; Magic angle spinning; Plasma membrane; Phospholipid composition; Bilayer arrangement

1. Introduction

For over two decades, a substantial amount of knowledge, largely acquired with NMR methods, has been accumulated on the structural and dynamic properties of phospholipids in model membranes [1–12]. Conversely, very few studies have been undertaken using phospholipids in their natural plasma membrane environment [13–16]. As the protein-membrane interactions occurring in the membrane environment raise a major challenge for understanding the wide range of cellular processes (for review see [12]), new investigation methods are needed to analyse structurally intact natural membranes. The aim of the present study was to determine whether ^{31}P magic angle spinning (MAS) NMR can provide useful insights.

Previous static ^{31}P NMR spectroscopy studies have shown that artificial phospholipid mixtures in aqueous environment have a polymorphic phase behaviour [1–3]. Depending on the liposome composition and temperature, large multilamellar vesicles (MLV) present distinctive phospholipid ^{31}P NMR spectra with a chemical shift anisotropy (CSA) in the 30–40 ppm range characteristic of bilayer or hexagonal (HII) phase [4,5]. Very often, the addition of drugs [6–8] or proteins [9–11] to the phospholipid mixture induces NMR-detectable modifications, in particular the appearance of an additional central spectral component around the isotropic chemical shift; this component is attributed to the presence of small vesicles or micellar structures.

Very few static studies have been undertaken on natural membranes [13–16]; spectra obtained are characteristic of the bilayer configuration and often have a narrow component as observed in phospholipid-protein systems [9–11]. However, these studies were principally performed on organelle membranes which have a significantly lower cholesterol content than plasma membranes. In addition, it is not known whether such composite spectra are induced by the long isolation procedure and/or depend on the nature of the membrane. Except for the human erythrocyte membranes [5], there are, to our knowledge, no studies reporting phospholipid spectra of natural plasma membranes.

In artificial phospholipid mixtures arranged in MLV, information about individual phospholipids is not directly available since the CSA of ^{31}P is not averaged by vesicle tumbling. CSA averaging can be partially achieved by MAS of the sample and the broad line can be resolved into its principal molecular components [17]. This technique has been largely used in the field of solid materials [18], but very few studies present results concerning individual phospholipids in artificial liposomes. Pinheiro et al. [11] and Traikia et al. [19] used this technique to resolve spectra of cardiolipin (CL), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) and PC, PE and phosphatidylglycerol (PG) mixtures respectively. However, for natural membranes, to our knowledge, there are no studies using the MAS technique to resolve individual phospholipids and it is not known if the presence of cholesterol and proteins could modify phospholipid mobility, line shape or chemical shift.

In this work, we analysed isolated human erythrocyte membranes and rabbit skeletal muscle sarcolemma to determine (i) whether their different phospholipid and cholesterol compositions [20–24] give different static ^{31}P NMR spectra and (ii) whether specific information on each of the five principal phospholipid species can be obtained with MAS ^{31}P NMR spectroscopy.

2. Materials and methods

2.1. Isolation of erythrocyte membranes

Heparinised human blood was obtained from the Regional Blood Bank (ETSBE, Rennes). All procedures were carried out at 4°C. Erythrocytes from 30 ml of total blood were obtained by centrifugation at $1000\times g$ for 15 min. After three washes with 0.9% NaCl, erythrocytes were lysed for 30 min in 2 volumes of acetic acid 0.1%. The lysate was centrifuged at $10\,000\times g$ for 15 min and the pellet was washed three times in cold water. The final pellet of erythrocyte membranes (EM) was used for NMR studies.

2.2. Isolation of rabbit skeletal muscle sarcolemma

New Zealand white rabbits were killed by i.v. pentobarbital injec-

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tion. Approximately 300 g of back and hind leg skeletal muscles were excised and weighed. The isolation method described by Ohlendieck et al. [25] was used and all steps were carried out at 4°C. Briefly, muscles were homogenised in a Waring blender three times for 30 s in buffer A (20 mM sodium pyrophosphate, 20 mM sodium phosphate, 1 mM $MgCl_2$, 0.303 M sucrose, 0.5 mM EDTA, pH 7.0) in the presence of protease inhibitors (76.8 nM aprotinin, 1.1 μM leupeptin, 0.7 μM pepstatin A, 0.83 mM benzamidine, 1 mM iodoacetamide and 0.23 mM phenylmethylsulphonyl fluoride).

The homogenate was centrifuged twice at $14\,000\times g$ and $17\,000\times g$. The final supernatant was incubated for 30 min with KCl to 0.6 M final concentration and centrifuged at $130\,000\times g$ for 35 min. The pellet was suspended in buffer B (0.303 M sucrose, 20 mM Tris-maleate, pH 7.0) added with 0.6 M KCl, layered on the top of a sucrose gradient (0.878 M sucrose, 0.6 M KCl, 20 mM Tris-maleate, pH 7.0) and centrifuged for 17 h at $104\,000\times g$. Sarcolemma membranes recovered at the 0.303 M/0.878 M sucrose interface were diluted in buffer B and centrifuged at $104\,000\times gg$ for 4 h. The pellet (skeletal muscle sarcolemma, SMS) was used for the NMR studies.

2.3. Biochemical assays on SMS

Protein concentration was determined by the biuret method with bovine serum albumin (Sigma) as standard. Acetylcholinesterase specific activity was determined spectrophotometrically at 412 nm and 30°C using acetylthiocholine as substrate [26].

2.4. Electron microscopic analysis of SMS and EM

Membranes were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2 and 0.05 M calcium chloride for 1 h at 4°C, then post-fixed in 2% osmium tetroxide in 0.2 M cacodylate buffer for 1 h at 4°C, dehydrated and embedded in Epon Araldite. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined under a JEOL JEM 100 CX II electron microscope (JEOL Europe, SA) at 80 kV.

2.5. ^{31}P NMR spectroscopy

^{31}P NMR spectra were obtained at 121.9 MHz on a Bruker ASX300 solid-state spectrometer (Bruker spectrospin, Wissembourg) operating at 7 T using a 4 mm diameter ceramic rotor (100 μl volume) at 25°C without proton decoupling. For non-spinning samples, acquisition parameters were 3 μs (90° pulse), dwell-time 5 μs, spectral width 100 kHz, data points 8K, acquisition time 40.97 ms, repetition delay 1 s, number of scans 4000. When samples were spun at 5000 Hz, dwell-time was changed to 10 μs, data point to 16K, scan number to 1000. Free induction decays were treated with exponential broadening of 100 Hz for non-spinning samples before Fourier transform. No line broadening was applied to spinning samples.

2.6. Quantification and data analysis

^{31}P chemical shift reference was 85% H_3PO_4 (ppm). Chemical shift anisotropy of the static spectrum was determined by the same investigator who determined the chemical shift anisotropy tensors ($\sigma_\perp$ and $\sigma_\parallel$ values) with WIN-FIT software (Bruker Spectrospin, Wissembourg). Quantification of peaks observed in MAS ^{31}P NMR spectrum was effected by line fitting using WIN-FIT. Line width was determined at peak half height.

Comparison of data was made by a one-way analysis of variance and Student's *t*-test. Values are reported as mean ± S.D.

3. Results

3.1. Preparations

Electron microscopy showed that EM were composed of membrane fragments, non-resealed as round vesicles (Fig. 1a) while SMS were composed of closed vesicles measuring approximately 0.2–0.3 μm in diameter (Fig. 1b). Acetylcholinesterase activity was 6-fold higher in SMS than in the first homogenate showing that the purity of the preparation was highly increased.

3.2. Static ^{31}P NMR spectroscopy

A typical ^{31}P NMR powder pattern spectrum of phospholipids was obtained for both membrane preparations with an intense high field peak corresponding to the $\sigma_\perp$ component and a low field shoulder corresponding to the $\sigma_\parallel$ component (Fig. 2). CSA, defined as the $\sigma_\parallel$-$\sigma_\perp$ distance, was 5 ppm larger for EM than for SMS resulting from a significantly different $\sigma_\parallel$ value for EM and SMS (Table 1). The $\sigma_\perp$ was the same in both preparations at around −12 ppm. In the SMS preparation, there were three additional sharp peaks at 3.64 ± 0.05, 2.2 ± 0.17 and -7.1 ± 0.23 ppm, corresponding roughly to 1%, 5% and 2% of the total area.

3.3. Magic angle spinning ^{31}P NMR spectroscopy

Spinning at the magic angle at 5000 Hz narrowed spectra into two resonances, peak 1 at low field and peak 2 at high field (Fig. 3).

Chemical shifts of the peaks were +0.025 and −0.072 ppm for peak 1 of EM and SMS respectively and −0.51 and −0.63 ppm for peak 2 of EM and SMS respectively (Table 2). The chemical shift difference between the two peaks was significantly larger in SMS at 0.56 ± 0.01 ppm than for EM at 0.53 ± 0.02 ppm. The line width of the two peaks was slightly larger in SMS than in EM, but the difference was more significant for peak 2 (Table 2).

During acquisition, the repetition delay was set at 1 s in order to avoid excessive experimental time with a biological

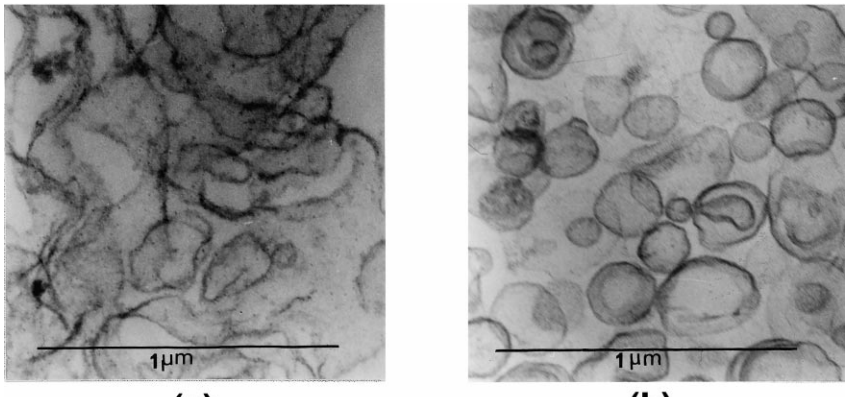

Fig. 1. Electron microscopy from (a) EM and (b) SMS. ×43 200.

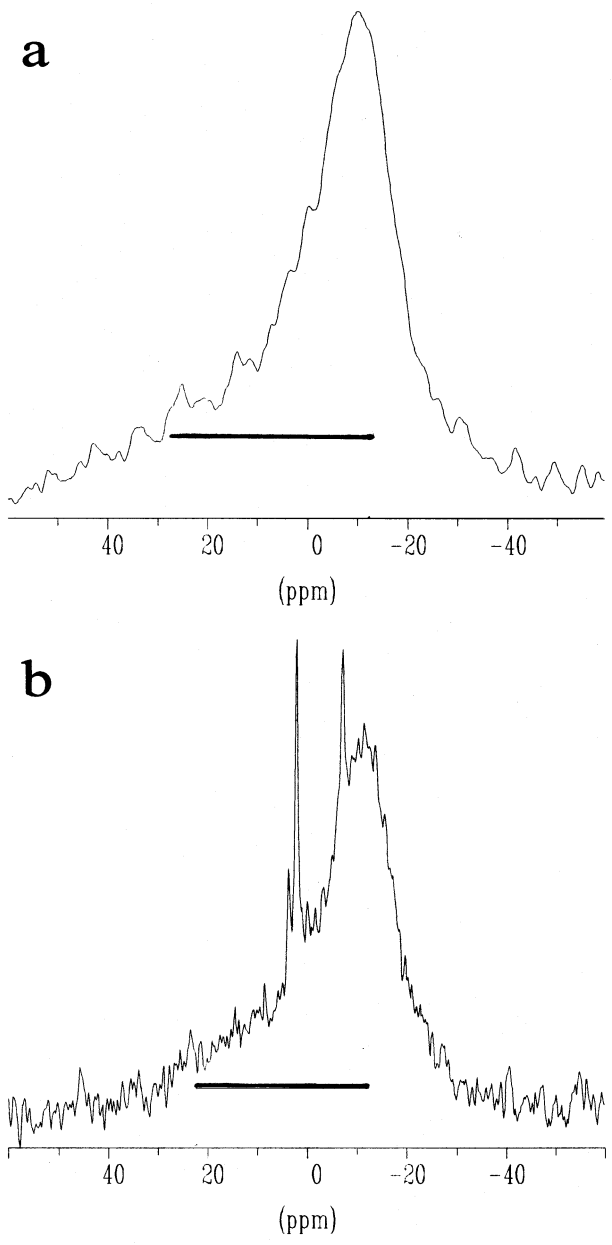

Fig. 2. Static ^{31}P NMR spectra at 121.9 MHz for (a) EM and (b) SMS at room temperature. Acquisition was recorded over 50 kHz spectral width, using 1 s repetition delay and 4000 scans were accumulated. ^{1}H decoupling was not performed because of lack of improvement with decoupling at high field as previously reported [19,31]. The horizontal bar represents the CSA.

sample. This short delay could be insufficient for a total relaxation thus inducing partial signal saturation. The delay was therefore set at 5 s in two cases which showed that for both peaks, the area obtained with the 1 s delay was 40% of the area obtained with a 5 s delay. However, as the relative areas of the two peaks were identical, suggesting that T_1 relaxation times were identical, no saturation factor was applied to spectra acquired with 1 s delay. The areas of the two peaks were significantly different, peak 2 amounting to about one-third of the total for EM, while inversely, peak 2 amounted to about two-thirds of the total for SMS (Table 2).

The two sharp peaks observed at 2.2 and −7.1 ppm in the static spectrum of SMS were also present in the MAS spectrum at the same resonance frequencies. A spectrum obtained from buffer A (not shown) evidenced two peaks at the same chemical shifts as these two peaks: a peak at 2.2 ppm from inorganic phosphate (Pi) and a peak at −7.1 from sodium pyrophosphate. As SMS were studied in buffer B which did not contain phosphorus nuclei, Pi and sodium pyrophosphate were inside the SMS vesicles. By contrast, the yet unidentified peak at 3.6 ppm was no longer visible in the MAS spectrum as its intensity (1%) was too low to be extracted from noise.

4. Discussion

The major findings of our work were (i) that natural plasma membranes showed typical static ^{31}P NMR powder-like spectra without any additional component at the resonance of isotropic phospholipids and (ii) that MAS was able to reduce the static broad spectrum into two resonances with line widths comparable with those obtained with synthetic phospholipid mixtures.

Both membrane preparations showed typical 'powder-like' spectra with CSA ranging over 30–40 ppm, characteristic of phospholipids with a restricted mobility in a bilayer arrangement [4,5], and in the same 35–50 ppm range established for pure PC [27] or phospholipids mixtures [4,5,10]. However, this type of spectrum has only been demonstrated with high concentrations of large pure phospholipid vesicles (multilamellar vesicles, MLV) while small unilamellar vesicles (SUV) show a sharp symmetrical ^{31}P NMR peak (for review, [3]). In MLV, the multi-bilayer arrangement in large vesicles prevents macromolecular phospholipid mobility, while individual phospholipids retain their own mobility inside the bilayer (rotation, lateral diffusion). Thus, it can be deduced from the spectra obtained in EM and SMS first, that phospholipids were arranged in bilayer and secondly, that the preparations were in large vesicles and/or at a concentration which prevents macromolecular mobility. SMS measured 0.2–0.3 μm in diameter which is greater than SUV (<0.05 μm) and less than MLV (>0.5 μm) but the density of the preparation was very high at about 1.5 mg protein/100 μl. EM were not resealed as vesicles but appeared as long fragments with a very high density in the rotor.

The presence of three sharp peaks in the SMS spectrum was striking. Many ^{31}P NMR spectroscopy studies of liposomes have found a sharp peak superimposed on the broad line. This sharp peak was attributed to the contribution of phospholipids in small vesicles or micellar phase (frequency at about 0 ppm) or sometimes to hexagonal phases of phospholipids (frequency at about +2.3 ppm) [10,28]. Such peaks appear at increasing temperature, or in the presence of drugs or proteins [6–10], or in the presence of diacylglycerol and/or bivalent cations [28–30]. The three sharp peaks we observed were in the static SMS spectrum. Two of them can undoubt-

Table 1
Chemical shift anisotropy (CSA), $\sigma_{\parallel}$ and $\sigma_{\perp}$ values in ppm from static ^{31}P NMR spectra of EM and SMS

	$\sigma_{\parallel}$	$\sigma_{\perp}$	CSA
EM	27 ± 1	−12.2 ± 0.2	40 ± 1
SMS	22 ± 3*	−12.0 ± 0.6	34 ± 3*

Mean ± S.D. of four experiments.
*$P<0.015$ from EM.

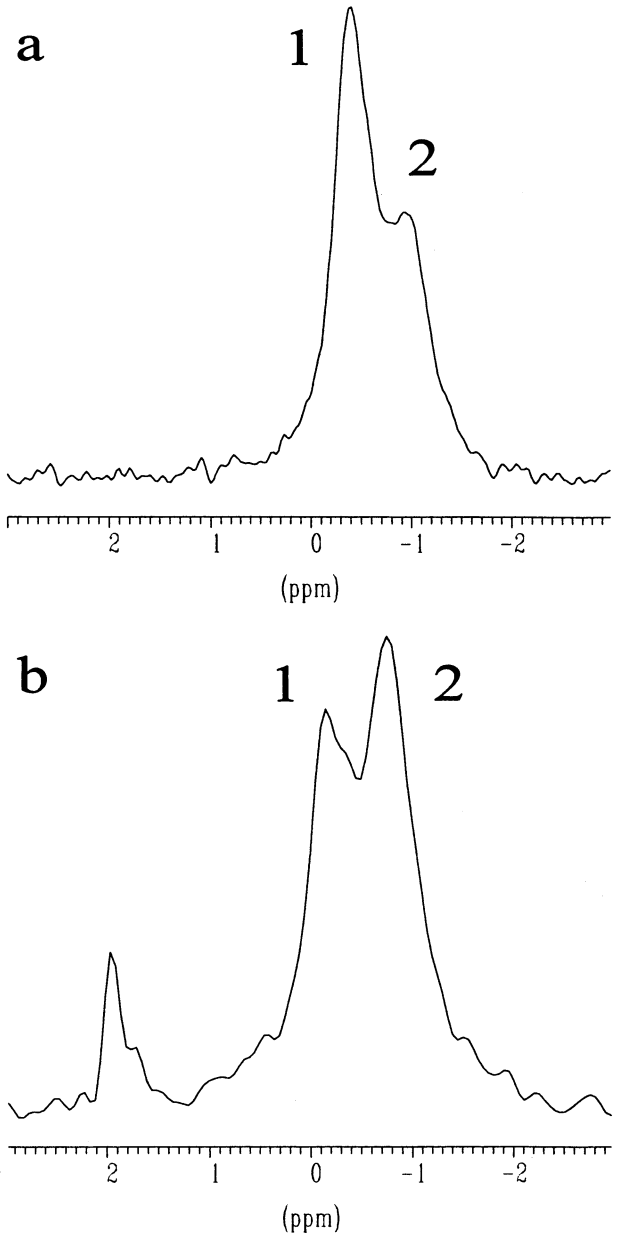

Fig. 3. MAS ^{31}P NMR spectra at 121.9 MHz and 5 kHz spinning rate for (a) EM and (b) SMS. Acquisition was recorded over 50 kHz spectral width, with 1 s repetition delay and 1000 scans were accumulated. As in Fig. 2, no ^{1}H decoupling was performed. The numbers 1 and 2 corresponded to peak 1 and peak 2 in the text respectively.

edly be attributed to phosphorus nuclei present in the buffer used for homogenisation. One remaining peak at 3.6 ppm has not yet been identified. Its intensity (about 1% of the whole spectrum) was too low to be detectable in the MAS spectrum as the signal could not be extracted from noise. Nevertheless, this very narrow line could not correspond to very small quantities of small vesicles or micelles which do not appear at 3.6 ppm but at 0 ppm. This is in contrast with numerous studies on synthetic phospholipid mixtures and the few reports on natural membranes from organelles showing the appearance of small vesicles or micellar structures upon the addition of proteins or drugs. Our results evidenced that even after a long isolation procedure, phospholipids in natural membranes are maintained in a bilayer arrangement, likely due to the presence of cholesterol and protein in plasma membranes.

CSA was larger in EM than in SMS. As shown by the electron microscopy, EM were constituted of membrane fragments which were non-resealed as vesicles, thus reducing the water content of the preparation and increasing the relative concentration of phospholipids in the sample. In comparison, SMS were constituted of resealed vesicles holding water and solutes. This morphological difference could partially account for the CSA difference. However, the essentially identical isotropic chemical shift of the MAS spectrum, which can be calculated from the formula $\sigma_{iso} = (2/3\sigma_{\perp} + 1/3\sigma_{\parallel})$, shows that the poor resolution of the EM powder spectra would most likely explain the CSA difference.

MAS has been used previously on model membranes in order to reduce anisotropic features [17], but, to our knowledge, the MAS ^{31}P NMR spectra presented here are the first to be obtained from two natural membrane systems. MAS reduced the large and asymmetric static spectra to essentially two narrow peaks centred at 0 and −0.50/−0.60 ppm. In human erythrocyte plasma membrane, 30% of total phospholipids are PC [20] and ^{31}P NMR studies of phospholipids in chloroform [31] or in aqueous phase [11,19] show that PC resonance is in the −0.60 to −0.90 ppm range. Consequently, the −0.51 ppm EM peak (peak 2), accounting for 35% of total phospholipids, can be attributed to PC resonance. In addition, the PC resonance at −0.51 ppm is 0.50 ppm away from all the other phospholipids in chloroform solution and, in the MAS spectrum of an aqueous mixture, PC is 0.60 ppm away from PE [11,19]. The resonances of these phospholipids are comprised between −0.1 and 0.1 ppm. As a consequence, peak 1 can be assumed to be constituted of all the other phospholipid resonances.

One report on rabbit skeletal muscle [22] showed that sarcolemma phospholipid composition is quite different from erythrocyte membrane phospholipid composition where PC content is higher (62% of the total). This is in perfect agreement with the 62% of peak 2 area observed in the SMS preparation and agrees with the attribution of peak 2 to the PC resonance.

Thus, the MAS spectrum provided quantitative data on two classes of phospholipids, namely PC and the others. For both EM and SMS, the PC lines were found exactly at the expected resonance position, in very good agreement with those found in model membrane [4,5]. Moreover, the line widths of the two components in the MAS spectra were as narrow (45–60 Hz) as in model membranes [11,19]. These results clearly show that the natural environment of phospholipids does not modify ^{31}P chemical shift and line widths of the phospholipid head polar groups. This is in agreement with the studies showing that interactions between proteins and liposomes modify dynamic properties of phospholipids but not their structural properties or composition [12].

Nevertheless, the chemical shift scale of mobile phospholipids is about 1 ppm [31] and at 121.50 MHz for ^{31}P as used in our study, we were unable to obtain more information about individual phospholipids. In the study of Pinheiro and Watts [11], MAS was able to provide quantitative data on PC, PE and CL, a phospholipid specific to mitochondrial membrane. However, these authors used a highly ideal mixture since the

Table 2
Chemical shift (CS in ppm), area (in % of total area) and width at half height (ppm) of peak 1 and peak 2 observed by MAS ^{31}P NMR spectroscopy of EM and SMS

	Peak 1			Peak 2		
	CS	Area (%)	Width at half height	CS	Area (%)	Width at half height
EM	0.025 ± 0.12	65 ± 8	0.45 ± 0.06	−0.51 ± 0.10	35 ± 8	0.50 ± 0.08
SMS	−0.072 ± 0.11	38 ± 4*	0.50 ± 0.06#	−0.63 ± 0.11	62 ± 4*	0.61 ± 0.06*

Mean ± S.D. from four experiments.
*$P < 0.05$ from EM.
#$P < 0.05$ from peak 2 of SMS.

low field CL chemical shift was 0.4 ppm from PE. The use of a higher locked static magnetic field combined with high resolution MAS probe heads would certainly provide more insight for further studies.

The line width of MAS lines was very slightly larger in SMS than in EM, possibly suggesting a small distribution of the isotropic chemical shifts of phosphorus nuclei of polar heads or a residual stronger MAS line width in SMS. There is at present no explanation for these differences. Several hypotheses could be put forward: different protein composition and/or interactions between polar head groups and different buffers used for EM and SMS. In addition, one should keep in mind that EM come from circulating highly deformable cells while SMS come from a highly organised tissue with a longitudinal structure whose function is to provide muscular force by cyclic stretching. The membrane properties of EM and SMS would probably be different, molecular arrangements being of importance to fulfil different functions.

In conclusion, this first report of ^{31}P NMR of two natural membranes, erythrocyte and skeletal muscle membranes, exhibited several interesting features. First static spectra unambiguously evidenced a unique, typical bilayer arrangement, no hexagonal phase being observed. MAS NMR line widths of natural membrane components were quite comparable to those of model membranes and distinguished one part of the individual phospholipid (PC) components, estimating its relative percentage with good precision. These results show that NMR data accumulated for over two decades using model membranes can now be transposed to studies of phospholipids in their natural environment.

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References

[1] Cullis, P.R. and De Kruijff, B. (1979) Biochim. Biophys. Acta 559, 399–420.
[2] Cullis, P.R. and De Kruijff, B. (1978) Biochim. Biophys. Acta 507, 207–218.
[3] Fenske, D.B. (1993) Chem. Phys. Lipids 64, 143–162.
[4] Seelig, J. (1978) Biochim. Biophys. Acta 515, 105–140.
[5] Yeagle, P.L. (1990) Biol. Magn. Reson. 9, 1–54.
[6] Cullis, P.R., Verkleij, A.J. and Ververgaert, P.H.J.Th. (1978) Biochim. Biophys. Acta 513, 11–20.
[7] Auger, M. (1997) Biophys. Chem. 68, 233–241.
[8] Van Bambeke, F., Mingeot-Leclercq, M.P., Brasseur, R., Tulkens, P.M. and Schanck, A. (1996) Chem. Phys. Lipids 79, 123–135.
[9] De Kruijff, B. and Cullis, P.R. (1980) Biochim. Biophys. Acta 602, 477–490.
[10] Yeagle, P.L. (1982) Biophys. J. 37, 227–239.
[11] Pinheiro, T.J. and Watts, A. (1994) Biochemistry 33, 2459–2467.
[12] De Kruijff, B. (1998) in: Lipid-protein interactions, Special issue, Biochim. Biophys. Acta 1376.
[13] McLaughlin, A.C., Cullis, P.R., Hemminga, M.A., Hoult, D.I., Radda, G.K., Ritchie, G.A., Seeley, P.J. and Richards, R.E. (1975) FEBS Lett. 57, 213–218.
[14] Cullis, P.R., De Kruijff, B., Hope, M.J., Nayar, R., Rietveld, A. and Verkleij, A.J. (1980) Biochim. Biophys. Acta 600, 625–635.
[15] Tellier, C., Vallet-Strouve, C., Akoka, S. and Poignant, S. (1987) Eur. Biophys. J. 15, 177–184.
[16] Buton, X., Morrot, G., Fellmann, P. and Seigneuret, M. (1996) J. Biol. Chem. 271, 6651–6657.
[17] Forbes, J., Bowers, J., Shan, X., Moran, L. and Oldfield, E. (1988) J. Chem. Soc. Faraday Trans. 84, 3821–3849.
[18] Lippmaa, E., Mägi, M., Samoson, A., Engelhardt, G. and Grimmer, A.R. (1980) J. Am. Chem. Soc. 102, 4889–4893.
[19] Traikia, M., Langlais, D.B., Cannarozzi, G.M. and Devaux, P.F. (1997) J. Magn. Reson. 125, 140–144.
[20] Szymanska, G., Marx, A., Melzner, I., Sarzala, M.G. and Rÿdel, R. (1986) Gen. Physiol. Biophys. 5, 179–186.
[21] Haest, C.W.M. (1982) Biochim. Biophys. Acta 694, 331–352.
[22] Rosemblatt, M., Hidalgo, C., Vergara, C. and Ikemoto, N. (1981) J. Biol. Chem. 256, 8140–8148.
[23] De Krester, T.A. and Livett, B.G. (1977) Biochem. J. 168, 229–237.
[24] Michalak, M. and Opas, M. (1997) Curr. Opin. Neurol. 10, 436–442.
[25] Ohlendieck, K., Ervasti, J.M., Snook, J.B. and Campbell, K.P. (1991) J. Cell Biol. 112, 135–148.
[26] Steck, T.L. and Kant, J.A. (1974) Methods Enzymol. 31, 172–180.
[27] De Kruijff, B. and Cullis, P.R. (1975) Biochim. Biophys. Acta 406, 6–20.
[28] Micol, V., Sanchez-Pinera, P., Vilalain, J., de Godos, A. and Gomez-Fernandez, J.C. (1999) Biophys. J. 76, 916–927.
[29] De Kruijff, B., Verkley, A.J., Van Echteld, C.J.A., Gerritsen, W.J., Mombers, C., Noordam, P.C. and De Gier, J. (1979) Biochim. Biophys. Acta 555, 200–209.
[30] Yeagle, P.L. (1985) Biochim. Biophys. Acta 822, 267–287.
[31] Merchant, T.E. and Glonek, T. (1992) Lipids 27, 551–559.