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Separation of a Pair of Interleaved Sideband Families in Magic Angle Spinning NMR by a Pair of One-Dimensional Experiments

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Interleaved sideband families that arise from distinct isotropic chemical shifts and the associated chemical shift anisotropy (CSA) tensors in magic angle spinning NMR are conventionally disentangled using two-dimensional schemes. However, for the case of just a pair of distinct chemical sites, a scheme is suggested that involves a pair of one-dimensional experiments whose sum and difference spectra separate the interleaved sideband families. This method was applied to phospholipid bilayers. The ^{31}P NMR sidebands of phosphatidylcholine and dioleoylphosphatidylethanolamine are separated and CSA values for each phospholipid were calculated. © **1997 by John Wiley & Sons, Ltd.**

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INTRODUCTION

It is common practice to resort to magic angle spinning (MAS) in solid-state and wide-line NMR to gain higher sensitivity, higher resolution and chemical shift anisotropy (CSA) information.[1–3] In a simple one-dimensional (1D) experiment, the spinning speed ω_r determines which of the goals is achieved better. At high spinning speeds (ω_r much higher than the CSA interaction) only the isotropic shifts remain and a high-resolution spectrum results. At $\omega_r = 0$, a superposition of broad static spectra, each centered about its own isotropic shift, is obtained (and if the static pattern widths are much larger than the isotropic chemical shift differences, the super-position smears out the information regarding the isotropic chemical shift differences). At intermediate spinning speeds, (ω_r comparable to the CSA interaction), the static spectrum is split into sidebands uniformly spaced at ω_r and centered about the isotropic chemical shift frequency ω_{iso}. This has the advantage of increasing sensitivity and resolution (by being able to identify the different isotropic chemical shifts) while at the same time retaining the anisotropic information.

In certain situations, such as ^{31}P NMR of phospholipid multilayers, a combination of factors such as large anisotropies, broad lines and small isotropic shift separations lead to considerable overlapping of sidebands from different sites. This prevents the resolution of different spinning sideband families. A number of 2D techniques have been developed which address this problem and extract information regarding both the isotropic shifts and the CSA principal values.[4–8]

An important application of ^{31}P NMR spectroscopy has been the study of phospholipids in model membranes (bilayers). The ^{31}P signal arises solely from the phosphate headgroup, and the static spectrum provides information about the structural organization of phospholipids (bilayer *vs* hexagonal phase) and the orientation/motions of the headgroup region.[9] However, the very broad lines in non-spinning ^{31}P spectra of unoriented bilayers (the powder pattern) make the detection of different headgroups difficult when more than one species is present. To extend the usefulness of ^{31}P NMR from simple model membranes comprised of one phospholipid species to mixtures of phospholipids and even biological membranes requires newer approaches of MAS NMR.

Here we outline a method which is applicable to the situation of a pair of distinct sites and which employs a pair of simple 1D experiments to resolve the sideband families in ^{31}P spectra of model membranes comprised of egg phosphatidylcholine (PC) and dioleoylphosphatidylthanolamine (PE). Most biological membranes contain two major phospholipid species, which in most cases are PC and PE.[10]

EXPERIMENTAL

All experiments involved ^{31}P NMR on a sample consisting of multilamellar phospholipids. The bilayers

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were made up of PC and PE in a mass ratio of 2.0 or 3.0. For these two phospholipids, the mass ratio is very similar to the molar ratio. Solutions of lipids in chloroform (20 mg ml^{-1}) from Avanti Polar Lipids (Pelham, AL, USA) were mixed in the desired proportion. The solvent was dried and the sample was placed under vacuum for about 12 h and hydrated with 70% D_2O by mass.

All experiments were conducted at 121.5 MHz on a Bruker AMX300 solids level-1 spectrometer equipped with MAS accessories and a spinning speed controller. The sample temperature was about 25 °C.

For wideline (non-spinning) experiments, the spectral width employed was 50 kHz and the number of data points was 1024. All free induction decays were processed with a line broadening of 25 Hz.

For MAS experiments, the spectral width employed was 20 kHz and the number of data points was 4096, resulting in a digital resolution of better than 5 Hz. All free induction decays were processed with a line broadening of 1 Hz.

THEORY

The pulse sequence pair employed in these experiments is shown in Fig. 1. The time domain signals $s_{a,b}(t)$ are given by[11,12]

$$s_q(t) = f_q \exp[-R(t+\tau)] \sum_{k=-\infty}^{\infty} \exp(\iota k\omega_r t) \times \{a_k^{(1)} \exp[\iota\omega_{iso}^{(1)} t] + g_q a_k^{(2)} \exp[\iota\omega_{iso}^{(2)} t]\}, \quad q = a, b \quad (1)$$

where a_k is the kth sideband amplitude from a given site, R is the exponential decay rate constant (equal to inverse of the transverse relaxation time T_2), $f_a = g_a = 1$, $f_b = \exp[\iota\omega_{iso}^{(1)}\tau]$, $g_b = \exp(\iota 2\pi\Delta_{iso}\tau)$, $2\pi\Delta_{iso} = \omega_{iso}^{(2)} - \omega_{iso}^{(1)}$ and $\omega_{iso}^{(\,)}$ are the isotropic chemical shifts. The common phase factor $\exp[\iota\omega_{iso}^{(1)}\tau]$ can be eliminated by a suitable choice of ω_{ref} or zeroth-order phase correction. By imposing a double restriction on the time interval τ that it equals both $1/2\Delta_{iso}$ and an integral multiple of τ_r, the relative phase shift is rendered equal to -1. In the actual pulse sequence we prefer the integral multiple of τ_r to be an even number, as this makes the task of combining signals from the two pulse sequences a and b easier:

$$\tau = \frac{1}{2\Delta_{iso}} = n2\tau_r \quad (2)$$

where n is a natural number. The sum and difference of these signals, $s_a \pm s_b$, readily separates the contributions from the two different sites. (The above manipulations can be carried out in either the time or frequency domain.)

Attention is drawn to the fact that the sideband amplitudes a_k are in fact ensemble averaged over randomly oriented bilayers, and depend on the CSA principal values and the spinning speed. Precisely this dependence enables us to extract the CSA information from the sideband amplitudes, and the relevant hierarchy of relationships is outlined below:[11,12]

$$a_k = \langle C_k^2(\alpha, \beta)\rangle$$

$$C_k(\alpha, \beta) = \frac{1}{2\pi}\int_0^{2\pi} d\Psi \exp(-\iota k\Psi)\exp[\iota\xi(\alpha, \beta; \Psi)]$$

$$\Psi = \omega_r t - \gamma$$

$$\xi(\alpha, \beta; \Psi) = \sum_{k=\pm 2} \frac{1}{k}\exp(\iota k\Psi) d_{k0}^{(2)}(\tan^{-1}\sqrt{2}) \times \sum_{m=0,\pm 2} \mathscr{A}_m\, d_{mk}^{(2)}(\beta)\exp(-\iota m\alpha)$$

The angle brackets and the integral over Ψ indicate averaging over α, β and γ, the Euler angles specifying a given orientation of the PAS of CSA tensor $\mathscr{A}$ (here in second rank spherical tensor notation); d^2 are the second rank reduced Wigner rotation matrix elements from PAS to rotor frame.

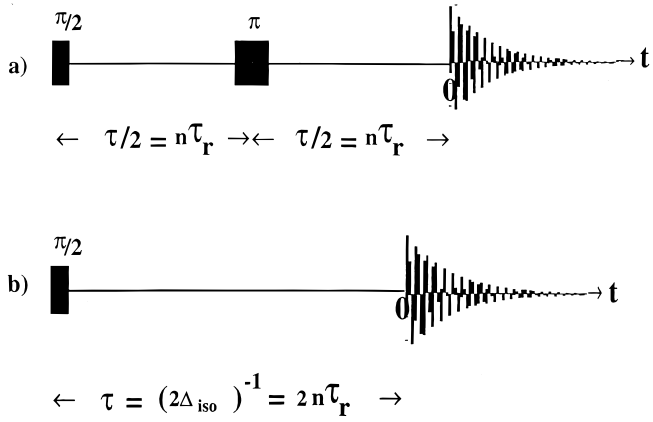

Figure 1. A pair of pulse sequences which can be used to separate interleaved sidebands arising from a pair of distinct chemical sites in MAS NMR. Pulse sequence (a) produces the usual MAS spectrum but with a suitable scaling for comparison with the signal from sequence (b). Sequence (b) inverts one entire sideband family with respect to the other sideband family. In the equations shown, n is a natural number.

RESULTS AND DISCUSSION

The non-spinning spectra of PC and PE in mass ratios 2.0 and 3.0 [Fig. 2(a) and (b), respectively] exhibit broad overlapping powder patterns, from which it is difficult, if not impossible, to extract chemical shift information (both isotropic and anisotropic). We have also observed in more heterogeneous mixtures with even small amounts of other phospholipids that the powder patterns from individual headgroups are not recognizable.[14]

The spectrum obtained of PC and PE multilayers (mass ratio 2.0) from the pulse sequence in Fig. 1(a) is shown in Fig. 3(a) spinning speed 1512 Hz). Baseline resolution of the center bands was obtained, surpassing the resolution in previously published spectra.[15] This corresponds to the usual MAS spectrum, but with scaling suitable for comparison with pulse sequence (b). A spinning speed of 1512 Hz was employed to satisfy the double constraint [Eqn (2)] as the isotropic chemical shift difference Δ_{iso} between PC and PE is 75.6 Hz.

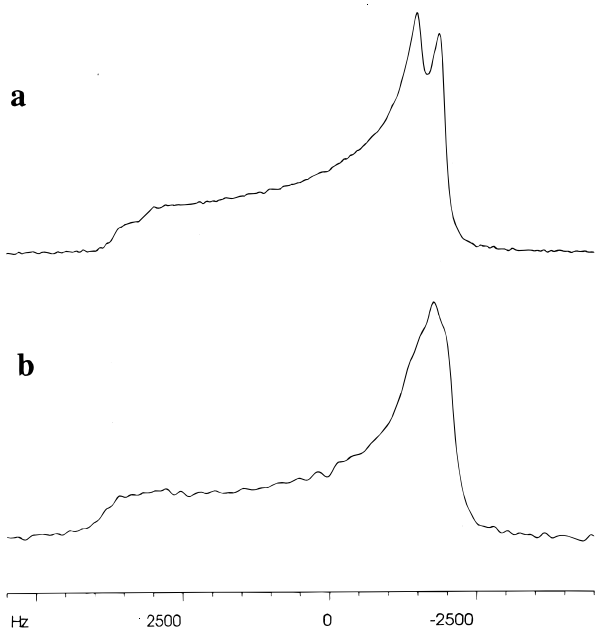

Figure 2. Non-spinning ^{31}P NMR spectra at 121.5 MHz of multilamellar PC–PE phospholipid mixtures in the mass ratios (a) 2 and (b) 3.

Thus τ accommodates ten rotor periods at this spinning speed.

Figure 3(b) shows the spectrum resulting from pulse sequence (b), which phase shifts the signals and imparts opposite signs to the two sideband families. All the signals from PC are positive, whereas all those for PE are negative.

The sum and difference of spectra in Fig 3(a) and (b) (spinning speed 1512 Hz) are displayed in Fig. 3(c) and (d), revealing a separation of sidebands due to PC and PE, respectively.

The relative intensities of the centerband and the accompanying sidebands are preserved by these manipulations. The separation of the two components aids in the calculation of the CSA from the spinning sidebands.[16] Such an analysis for the case of egg PC yielded an 'axially symmetric' CSA tensor with an anisotropy of 46.29 ppm (in good agreement with the literature value for egg PC[17]). Similarly for PE, an axially symmetric CSA anisotropy of 37.03 ppm was obtained.

To summarize, we have demonstrated experimentally that for the case of a pair of distinct chemical sites, we can employ a pair of 1D experiments (Fig. 1) to separate the interleaved sideband families. Although presented for a case of closely spaced but resolved signals, the method will allow the calculation of more accurate CSA values for poorly resolved signals than are achievable with the usual 1D spectra. This scheme, when applicable, may be found appealing because of the ease of implementation and excution and offers the advantage of economy of time and disk space. The strategy will enhance studies of the effects of other membrane components, such as cholestrol and proteins, on individual types of phospholipids in model membranes, and have applications to biological membranes composed of phospholipids with different headgroups, and where the resolution of individual species is expected to be lower than with the model system studied here.

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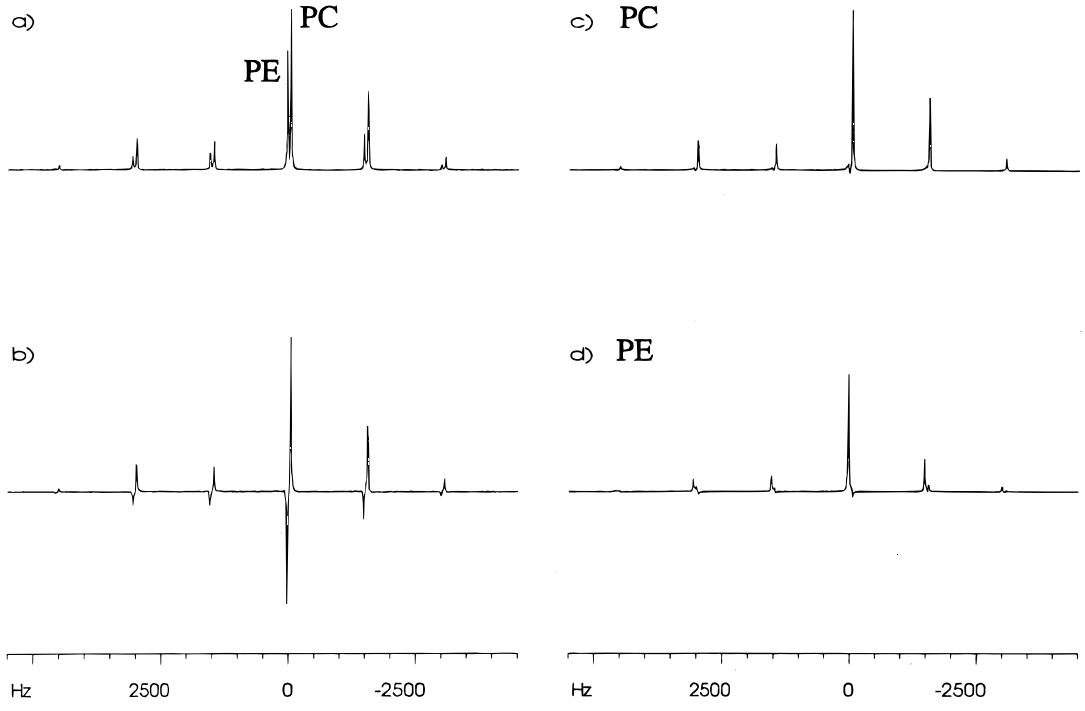

Figure 3. ^{31}P NMR spectra of multilamellar phospholipids made up of PC and PE in the mass ratio 2.0, obtained at 121.5 MHz under a magic angle spinning speed of 1512 Hz and τ adjusted to equal $1/\Delta_{iso}$; isotropic chemical shift difference Δ_{iso} = 75.6 Hz. Number of free induction decays accumulated = 2048. (b) spectrum from sequence (b) in Fig. 1; (c) (a) + (b); (d) (a) − (b).

REFERENCES

1. E. R. Andrew, G. E. Bradbury and R. G. Eades, *Nature* (*London*) **183**, 1802 (1959).
2. I. J. Lowe, *Phys. Rev. Lett.* **2**, 285 (1959).
3. M. M. Mariq and J. S. Waugh, *J. Chem. Phys.* **70**, 3300 (1979).
4. R. R. Ernst, G. Bodenhausen and A. Wokaun, *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*. Clarendon Press, Oxford (1987).
5. R. Tycko, G. Dabbagh and P. A. Mirau, *J. Magn. Reson.* **85**, 265 (1989).
6. A. C. Kolbert and R. G. Griffin, *Chem. Phy. Lett.* **166**, 87 (1990).
7. J. Z. Hu, D. W. Alderman, C. Ye, R. G. Pugmire and D. M. Grant, *J. Magn. Reson. A* **105**, 82 (1993).
8. O. N. Antzutkin, S. C. Shekar and M. H. Levitt, *J. Magn. Reson. A* **115**, 7 (1995).
9. I. C. Smith and I. H. Ekiel, in *Phosphorus-31 NMR, Principles and Applications*, edited by D. G. Gorenstein, Chapt. 15, pp. 447–475. Academic Press, Orlando, FL (1984).
10. M. K. Jain, *Introduction to Biological Membranes*, Chapt. 2, pp. 10–50. Wiley, New York (1988).
11. S. C. Shekar, Z. Song, A. Rupprecht and M. H. Levitt, *Proc. Indian Acad. Sci.* (*Chem. Sci.*) **106**, 1565 (1994).
12. O. N . Antzutkin, Z. Song, X. Feng and M. H. Levitt, *J. Chem. Phys.* **100**, 130 (1994).
13. M. H. Levitt, *J. Magn. Reson.* **82**, 427 (1989).
14. A. Salmon and J. A. Hamilton, unpublished results.
15. T. J. T. Pinheiro and A. Watts, *Biochemistry* **33**, 2459 (1994).
16. J. Herzfeld and A. E. Berger, *J. Chem. Phys.* **73**, 6021 (1980).
17. J. A. Hamilton, D. T. Fujito and C. F. Hammer, *Biochemistry* **30**, 2894 (1991).