brief communication

Two-dimensional $^1H/^{13}C$ heteronuclear chemical shift correlation spectroscopy of lipid bilayers

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ABSTRACT Using solid-state magic angle spinning nuclear magnetic resonance (NMR) techniques, we have obtained two-dimensional (2D), $^1H/^{13}C$ chemical shift-correlated spectra of liquid crystalline 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC) bilayers in 30 wt% PO_4/D_2O buffer. Linewidths in both the ^{13}C and the ^1H dimensions were <0.3 ppm wide. The 2D spectrum consists of chemical shift correlations between all resolvable, directly bonded ^{13}C–^1H pairs and exhibits considerably greater spectral dispersion than either ID ^1H or ^{13}C MAS spectra. This approach promises to be an important tool in structural studies of biological membranes.

INTRODUCTION

Although nuclear magnetic resonance (NMR) spectroscopy has been used extensively for many years to investigate membrane structure and dynamics, high-resolution spectra of intact bilayers and membranes are a recent observation. Early efforts to record high-resolution NMR spectra were limited to studies of membrane components in organic solvents (Chapman and Morrison, 1966) or sonicated membrane fragments (Chapman et al., 1967; Sears, 1975; London et al., 1975). Whether sonicated bilayers are suitable as a model membrane system has been extensively debated since the observed line narrowing could be artifactual due to the creation of small vesicles with highly curved surfaces (Sheetz and Chan, 1972; Lichtenburg et al., 1975; McKay et al., 1978). Solid-state NMR techniques have been successfully employed to obtain spectra of unsonicated bilayers and intact cell membranes, but most of the studies have concentrated on dynamical studies using static NMR techniques and lineshape analysis (Davis, 1979; Griffin, 1981; Wittebort et al., 1982). Due to problems with spectral overlap of very broad lines, these experiments necessitate isotopic labeling of membrane components which is both time-consuming and expensive.

High-resolution solid-state NMR spectra may be obtained by employing magic angle spinning (MAS) (Andrew et al., 1958; Lowe, 1959; Andrew, 1971), which averages any inhomogeneous interactions such as chemical shift anisotropies (Maricq and Waugh, 1979). The resulting spectrum exhibits a series of centerbands, corresponding to the isotropic chemical shifts and an associated collection of rotational sidebands, whose number and intensity depend on the size of the individual anisotropy relative to the spinning speed. Using this approach, high-resolution ^{13}C-MAS spectra of a variety of model phospholipid bilayers have been reported (Haberkorn et al., 1978; Sefcik et al., 1983; Oldfield et al., 1987).

Very recently, high-resolution ^1H-MAS spectra of phospholipid bilayers were described (Forbes et al., 1988). Even with relatively slow spinning speeds, it was possible to narrow the spectrum into a series of centerbands and rotational sidebands since the dipolar broadening arises from a special "inhomogeneous" form of the dipolar Hamiltonian. In particular, fast lateral and axially diffusive motions in the L_α phase of lipid molecules result in an "inhomogeneous" Hamilitonian. Although the resolution in MAS spectra of lipid bilayers is much improved, particularly for ^1H spectra, the number of problems that can be examined with the current linewidths (~0.3 ppm) is still quite limited. Hence, approaches that further increase spectral resolution will extend the applicability of the technique. The observation of both ^{13}C and ^1H spectra suggest that two-dimensional (2D) NMR techniques can now be readily applied to intact membranes (Ernst et al., 1987). 2D spectroscopy is used extensively in solution state NMR studies of biomolecules for determining intramolculear connectivities, chemical shift correlations, and spatial proximities (Wagner and Würthrich, 1982; Bax and Lerner, 1986). Recently, 2D heteronuclear correlation techniques have been successfully implemented to increase the resolution in $^1H/^{13}C$ correlation spectra of spinning solids (Roberts et al., 1984; Caravatti et al., 1983).

In this paper, we have used MAS techniques to obtain high-resolution, 2D heteronuclear-correlation spectra of 1,2-dimyristoyl-*sn*-3-phosphatidylcholine (DMPC) dispersed in 30 wt% phosphate/D_2O buffer. The structure of such a dispersion is well characterized as a bilayer, and thus serves as a model for biological membranes (Janiak et al., 1979). As will be seen below, resolution in the 2D spectra is considerably improved and should facilitate examination of species exhibiting complex spectra.

MATERIALS AND METHODS

DMPC (Avanti Polar Lipids, Inc., Birmingham, AL) was dispersed in phosphate/D_2O buffer (pD 7). The sample was placed in a Macor rotor with an endcap sealed by Viton O-rings (Doty Scientific, Columbia, SC) to prevent evaporation and leakage of the contents. The rotor was placed in a home-built variable temperature MAS NMR probe equipped with an air-driven stator (Doty Scientific).

Typical sample spinning speeds was 2.5 kHz and were regulated to ± 1 Hz by adjusting the air-flow through a mass-flow controller (de Groot et al., 1988). All NMR spectra were acquired on a home-built spectrometer at a field of 9.4 Tesla, with an operating frequency of 100.0 MHz for ^{13}C and 397.7 MHz for ^1H. Typical 90° pulse lengths were 6.5 μs for ^{13}C and 3.5 μs for ^1H and spectra were acquired by pure phase techniques (States et al., 1982). All spectra are referenced to external tetramethylsilane (TMS). Since long decoupling bursts were used in the acquisition of the spectra, all samples were checked for decomposition by thin-layer chromatography after NMR acquisition.

RESULTS AND DISCUSSION

The pulse sequence used for solid-state heteronuclear correlation spectroscopy is shown in Fig. 1 (Roberts et al., 1984). After a presaturated period on the ^{13}C spins, transverse ^1H magnetization was created in the x-y plane by a 90° pulse. During the evolution period, ^{13}C was decoupled from the protons by a WALTZ-4 sequence (Shaka et al., 1983). Coherence was then transferred from ^1H to ^{13}C by cross-polarization, and CW ^1H decoupling was used during ^{13}C acquisition. Synchronous sampling was employed in the t_1 period to fold all sideband intensity into the centerband region, thus giving an isotropic spectrum in the ^1H dimension.

In Fig. 2 is shown a 2D $^1H/^{13}C$ heteronuclear correlation spectrum of DMPC (Fig. 2) in the L_α phase together with projections of the ^{13}C and ^1H dimensions. The associated chemical shifts for all the different species are

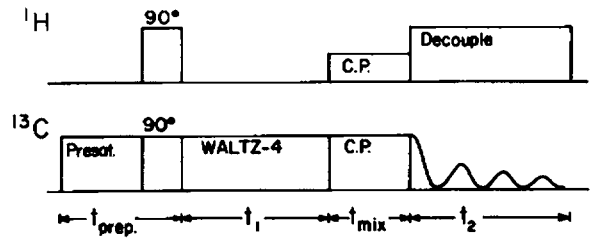

FIGURE 1. Pulse sequence for 2D CP/MAS heteronuclear chemical shift correlation experiment. The preparation period includes presaturation of the carbon spin reservoir followed by a 90_x pulse on the protons. The t_1 evolution period contains a WALTZ-4 sequence to decouple the carbon spins. Cross-polarization is employed in the mixing period followed by ^{13}C detection during t_2.

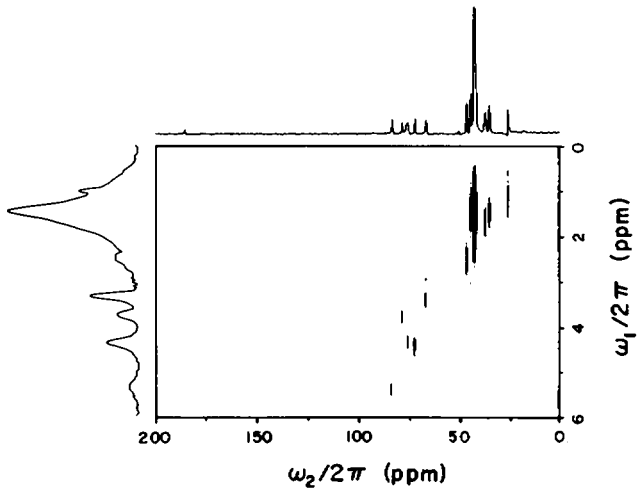

FIGURE 2 2D ^{13}C–^1H heteronuclear chemical shift correlation spectrum for DMPC in 30 wt% phosphate/D_2O buffer. Projections for ^{13}C and ^1H dimensions are also shown.

listed in Table 1. All resolvable, directly bonded ^{13}C–^1H pairs appear in this spectrum, with 6 out of the 14 hydrocarbon chain positions distinguishable from the main methylene peak. In the absence of the dispersion of methylene chemical shifts in the ^{13}C dimensions, spectral overlap in the ^1H dimension would render the different proton methylene shifts indistinguishable. In addition, all headgroup resonances are resolved and correlated to their respective ^1H resonances. Although a weak resonance from the carbonyl is visible in the ^{13}C projection, it is not correlated to any ^1H resonance, which is to be expected from a non-protonated ^{13}C.

TABLE 1 Chemical shifts of dimyristoyl-*sn*-phosphatidylcholine in 30 wt% PO_4/D_2O buffer

Assignment*	Chemical Shift in ppm‡ (^{13}C)	Chemical Shift in ppm§ (^1H)
R—CH_3	14.84	0.90
C_{13}-methylene	24.11	1.35
C_3-methylene	26.07	1.53
—$(CH_2)_n$—	31.44	1.34
C_{12}-methylene	33.39	1.34
C_2-methylene	35.34	2.39
R—$N(CH_3)_3$	55.36	3.27
α-methylene	60.73	4.32
C_1/C_3-glycerol	64.15	4.24
β-methylene	67.08	3.69
C_2-glycerol	71.86	5.35
C═O (carbonyl)	174.5	—

*Nomenclature as in Brown and Seelig, 1978.
‡Error is ±0.05 ppm referenced to external TMS.
§Error is ±0.1 ppm referenced to external TMS.

The linewidths in the ^{13}C dimension are typically 0.2–0.3 ppm (20–30 Hz), whereas the linewidths in the ^{1}H dimension range from 0.2 to 0.6 ppm. The ^{13}C projection compares favorably with a 1D MAS spectrum, but some of the linewidths in the ^{1}H dimension are larger than for a ^{1}H-MAS spectrum. This is especially true for the main methylene peak. At the same time, the linewidths for the $N(CH_3)_3$ peak are comparable in 2D and 1D spectra, suggesting that our decoupling scheme has not been optimized. Nonetheless, the quality of the spectrum is sufficient to show all the ^{13}C–^{1}H correlations.

The use of a mass-flow controller to regulate the spinning speed of the rotor to ±1 Hz greatly enhanced the quality of the 2D spectrum. Since synchronous sampling was used to fold all the sidebands into the centerband region, any short- and long-term fluctuations of the spinning speed would broaden the individual correlated peaks, as errors in spinning speed are multiplied as $\pm n$ ($\Delta\nu_R$) for the nth order sideband. Without controlled spinning speeds, the weaker headgroup resonances would have been lost in the noise.

CONCLUSIONS

The feasibility of performing heteronuclear shift correlation spectroscopy of DMPC bilayers opens new avenues for NMR studies of membranes. MAS combined with 2D NMR techniques can be a powerful tool for structure assignment. Other types of 2D NMR experiments may also be possible and remain to be explored. Due to the motional processes which lead to line narrowing in the bilayer, these experiments are presently restricted to membranes in the liquid crystalline phase only. For gel phase bilayers, and in cases of strong dipolar coupling, one may have to resort to multiple-pulse narrowing techniques together with MAS in order to obtain a high-resolution spectrum. A future extension of this work is to examine mixed lipid membranes as well as incorporation of various small molecules such as drugs, anesthetics, and small peptides into the bilayer structure. Utilization of perdeuterated lipids may aid in the suppression of unwanted lipid resonances in these experiments.

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