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A High-Resolution Solid-State NMR Approach for the Structural Studies of Bicelles

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In addition to providing the shape and structure of cells, biological membranes play a variety of important roles in the function of membrane-associated peptides and proteins, cell signaling, drug delivery, and other cellular activities. Therefore, there is considerable current interest in obtaining the high-resolution structure of biological membranes to better understand their function.[1] While the complex nature of membranes poses a tremendous challenge to the existing biophysical techniques, the use of model membranes, such as lipid bilayers and bicelles, enabled the applications of NMR spectroscopy. Recent studies have shown that bicelles are attractive model systems that can easily be prepared with a desired degree of order, aligned in the magnetic field to enhance the resolution, and can be combined with sophisticated high-resolution NMR studies to solve the structures of membrane proteins.[2] Particularly, solid-state NMR experiments are well suited to determine the atomistic-level resolution structures of molecules embedded in highly ordered bicelles and also to determine the structural disorders on bicelles due to ligand binding. These existing NMR methods either require isotopic labeling or cannot resolve parameters, such as chemical shift and dipolar couplings, that are essential to determine the conformation. In this communication, we demonstrate the usefulness of a solid-state NMR approach that can be used to measure the heteronuclear dipolar couplings between ^{1}H, ^{13}C, and ^{31}P nuclei without the need for isotopic enrichment.

A 2D proton-evolved separated-local-field experiment[3] is used to measure ^{1}H$-^{13}$C, ^{1}H$-^{31}$P, and ^{13}C$-^{31}$P dipolar couplings by ^{13}C detection using a pulse sequence, as shown in the Supporting Information. In this experiment, the ^{1}H$-^{13}$C dipolar couplings are measured when a π pulse is applied in the middle of the dipolar evolution (t_1) period in both the rf channels, while the ^{1}H$-^{31}$P dipolar couplings are measured along with the ^{1}H chemical shift when the π pulse on the proton channel is removed. In both experiments, ^{13}C$-^{31}$P couplings are obtained from the evolution during the direct detection period. A multiple pulse sequence BLEW-48 is applied during t_1 to suppress ^{1}H$-^{1}$H dipolar interactions. In the present study, this 2D sequence was used to obtain the experimental spectrum of 3.5:1 DMPC:DHPC bicelles that correlates the ^{13}C chemical shift and ^{1}H$-^{13}$C dipolar coupling (Figure 1). It is evident that most of ^{13}C spectral lines are well resolved in the 1D chemical shift spectrum (shown on the left of Figure 1) due to magnetic alignment of bicelles as indicated by the ^{31}P spectrum (data given in the Supporting Information). The high-resolution ^{1}H$-^{13}$C dipolar coupling slices shown in Figure 1 demonstrate the remarkable efficiency of the pulse sequence. While the full width at half-maximum of the dipolar coupling spectral lines depends on the magnitude of the dipolar coupling, the resolution parameter defined as the ratio of the splitting to the line width is about 20 for most of the peaks. It should be noted that to achieve this resolution a high radio frequency power is not essential unlike with the rotating-frame separated-local-field (SLF) experiments, such as PISEMA,[4] SAMMY,[5] and HIMSELF.[6] In addition, multiple dipolar couplings (including weak couplings) can be measured even

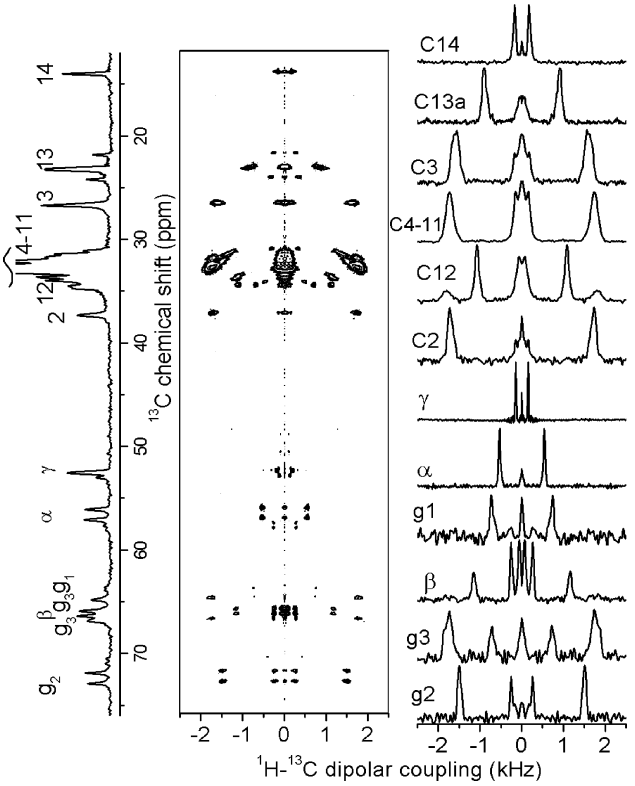

Figure 1. ^{13}C chemical shift spectrum (left), 2D spectrum (middle) that correlates the ^{13}C chemical shift (vertical dimension), and ^{1}H$-^{13}$C dipolar coupling (horizontal dimension) and ^{1}H$-^{13}$C dipolar coupling slices (right) of 3.5:1 DMPC:DHPC bicelles obtained from a Varian/Chemagnetics 400 MHz solid-state NMR spectrometer. The pulse sequence given in the Supporting Information was used; 64 scans were accumulated for each of 200 points in t_1 dimensions with the increment time of 384 μs and recycle delay of 5 s. ^{1}H rf field strength of 31 kHz was used during t_1 evolution. Contact time for CP transfer was set to 3.0 ms.

in the presence of a strong dipolar coupling, which is not possible with the rotating frame SLF methods. For example, three different C$-$H couplings are measurable in the C_β dipolar coupling spectrum (Figure 1). Moreover, the dipolar resolution and the scaling factor are insensitive to the frequency offset unlike in other 2D SLF sequences. Because of these reasons, resonances in Figure 1 are better resolved than in a spectrum that was obtained using SAMMY[7] or other rotating frame sequences. On the other hand, PISEMA and HIMSELF could provide better resolution on more rigid solids, such as proteins embedded in bilayers, than the proposed method. Most importantly, the ability to reveal dipolar couplings with ^{31}P makes this technique unique for studies on bicelles.

The values of ^{1}H$-^{13}$C dipolar couplings were converted into an order parameter (S_{CH}) profile for the lipid molecule, as given in Figure 2 using the relationship $D_{CH} = D_0 S_{CH}(3\cos^2\vartheta_{NB} - 1)/2$, where $D_0 \approx 21.5$ kHz is the dipolar coupling constant for a rigid C$-$H bond and the angle $\vartheta_{NB} = 90°$ defines the orientation of the

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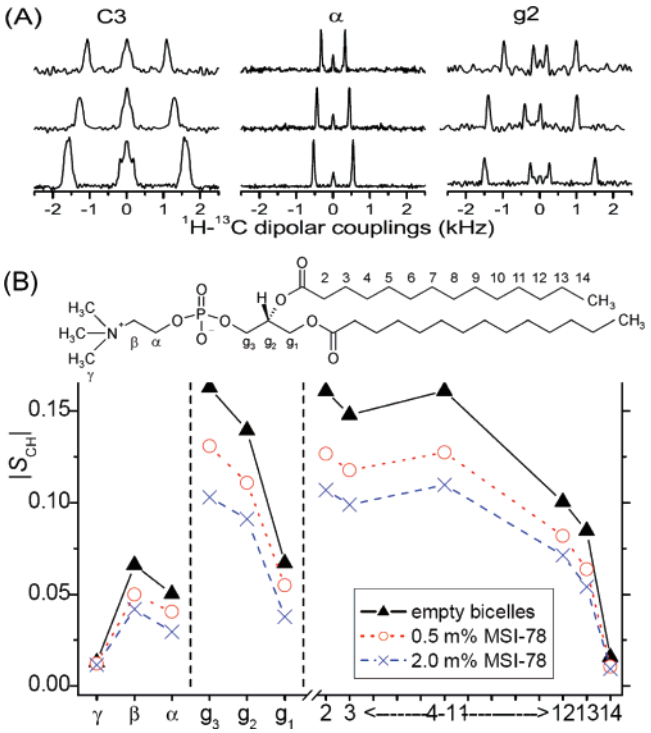

Figure 2. (A) ^{1}H−^{13}C dipolar coupling extracted from 2D spectra from 3.5:1 DMPC:DHPC bicelles with 0 (bottom), 0.5 (middle), and 2 (top) mol % MSI-78. (B) The order parameter profile for DMPC determined from the dipolar coupling values.

bicelle-normal relative to the magnetic field direction. The motionally averaged dipolar coupling constant, D_{CH}, is extracted from the experimental splitting $\Delta\nu = 2k[D_{CH} + J_{CH}/2]$, where J_{CH} is the scalar coupling constant and $k = 0.42$ is the scaling factor of the homonuclear decoupling sequence applied during the t_1 period. The sign and values of J_{CH} are known from the solution spectrum, while the signs of D_{CH} can be derived from a previous study[8] assuming that the DMPC conformation in bicelles is similar to that in multilamellar bilayers.

These experiments were also performed on 3.5:1 DMPC:DHPC bicelles containing a designed antimicrobial peptide, MSI-78 (or commercially known as plexiganan) (2D data not shown). Previous studies have shown that this peptide has a broad spectrum of antimicrobial activity and is more potent than the naturally occurring magainins (magain2 and PGLa), forms an amphipathic α-helical structure in lipid bilayers, and alters the conformation of the lipid headgroup upon binding. Representative dipolar coupling slices are given in Figure 2A to show the peptide-induced changes in the structural order of the lipid. The order parameters determined from the dipolar coupling values are given in Figure 2B. All ^{1}H−^{13}C dipolar couplings are reduced, and therefore, the peptide binding increases the disorder in lipid bilayers even at 0.5 mol % peptide concentration. On the other hand, this disorder is measurable using ^{2}H experiments only for a concentration ≥1% of the peptide, as shown in a recent study.[9]

A 2D experiment as mentioned above to simultaneously measure ^{1}H−^{31}P and ^{13}C−^{31}P dipolar couplings was carried out on bicelles with and without the peptide. A part of the 2D correlation spectrum that contains the resonances from the nuclei in the headgroup and glycerol region of the lipid is given in Figure 3. Dipolar splittings in the ^{13}C resonances of g_2, g_3, α, and β appear as tilted doublets. This tilt arises from the simultaneous splitting due to ^{13}C−^{31}P and ^{1}H−^{31}P dipolar interactions along the vertical axis (direct dimension) and the horizontal axis (indirect dimension), respectively. Upon adding peptide, the measured splittings decrease by a factor

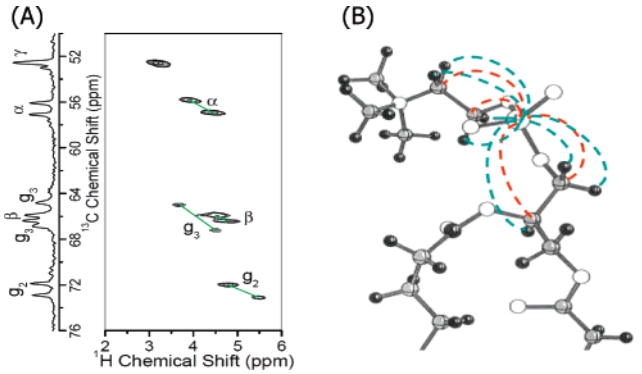

Figure 3. (A) A 2D correlation of ^{13}C chemical shift and ^{13}C−^{31}P dipolar coupling (*y*-axis) with the ^{1}H chemical shift and ^{1}H−^{31}P dipolar coupling (*x*-axis) of 3.5:1 DMPC:DHPC bicelles (left). The ^{1}H−^{31}P dipolar split peaks of α, β, g_2, and g_3 are connected by a green line. (B) The measured ^{1}H−^{31}P (blue) and ^{13}C−^{31}P (red) dipolar couplings in DMPC lipid molecule are highlighted.

comparable to that found for the scaling of ^{13}C−^{1}H dipolar and ^{31}P anisotropic chemical shift interactions. Since ^{13}C−^{31}P and ^{1}H−^{31}P dipolar couplings are not between directly bonded nuclei, they could be very sensitive to the conformational changes in the headgroup region around the phosphorus site. Therefore, the changes in the lipid headgroup conformation can be measured using this experimental approach. Spin pairs in the headgroup for which dipolar couplings were measured are indicated in Figure 3B.

Another significant advantage of this 2D approach is in assigning and separating peaks in the ^{13}C spectrum that otherwise overlap due to ^{13}C−^{31}P dipolar coupling. This difficulty can be seen for g_3 and β carbon sites, while the peaks from g_2 and α are assignable in pure bicelles but may not be possible in bicelles containing additives. We expect this approach to be useful in the structural studies of membrane-associated molecules and also in studies that utilize bicelles as an alignment medium.

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Supporting Information Available: Pulse sequence and ^{31}P NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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