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Solid-State NMR Spectroscopy of Aligned Lipid Bilayers at Low Temperatures

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Determination of structure and dynamics of membrane-associated proteins is one of the most important aspects of structural genomics. In addition to the high-resolution structure and dynamics, recent studies have shown that it is possible to obtain the topology of proteins embedded in cell membranes using solid-state NMR spectroscopy.[1] In these studies, mechanically aligned phospholipid bilayers are used as model membranes in which the uniaxial orientation of molecules provides narrow spectral lines. The ability to alter the composition of bilayers to mimic a variety of cell membranes including bacterial, mammalian, and myelin membranes is highly useful in understanding the role of individual membrane components on the function of a protein.[2] Such model membranes are also used to understand the mechanism of antimicrobial peptides, toxins, fusion peptides, and channel-forming peptides by characterizing peptide−lipid, peptide−peptide, and lipid−lipid interactions.[1,3]

While the combination of mechanically aligned bilayers and solid-state NMR experiments seems to be a powerful approach to investigate biological problems related to cell membranes, there are some intrinsic difficulties that pose a major hurdle to such applications. The degree of alignment and hydration of mechanically aligned bilayers are heat sensitive, and any heat dissipation decreases the stability of the sample inside the NMR probe and also reduces the resolution and sensitivity of the experiment. Since heat dissipation is common in high-power solid-state NMR experiments, usually the samples are stable only for a few days. Therefore, unlike in solution NMR applications, it is not possible to complete all necessary solid-state NMR experiments on a single sample. Sometimes, the sample may not be stable to complete a single solid-state NMR experiment, even if the spectrometer is quite stable, because the sample contains less protein and extensive signal averaging is necessary to obtain a descent signal intensity, which would increase the experimental time. It is also difficult to increase the sample size due to the volume restriction provided by the rf coil (in some cases the amount of peptide/protein should be kept low, for example, <5 mol % of peptide in the case of membrane-disruptive systems) or the low abundance of the desired peptide/protein. For example, a 2D PISEMA experiment could take a week, and a 3D experiment may run more than a week on some systems. Thus, one sample per experiment is needed, which is highly demanding. Therefore, there is a great demand for developing procedures to prepare stable aligned bilayer samples.

This study demonstrates that some of these difficulties can be overcome by performing experiments on aligned bilayer samples at a low temperature. Mechanically aligned 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC) bilayers were prepared using a recently developed naphthalene procedure.[4] ^{31}P chemical shift and ^{14}N quadrupole coupling spectra of POPC and ^{2}H quadrupole coupling spectra of POPC-d_{31} bilayers were acquired at various temperatures with the bilayer normal oriented parallel to the applied magnetic field. Representative ^{31}P (Figure 1A), ^{14}N (Figure 1B), and ^{2}H (Figure 1C) spectra obtained at 37 and −4 °C are given. A single narrow line at the parallel edge of the ^{31}P powder pattern suggests that the sample is well aligned at all temperatures above the gel-to-liquid crystalline phase transition temperature (T_c = −5 °C). However, the frequency of the peak and the span of the ^{31}P chemical shift interaction slightly increased as the temperature is decreased, but the peak position remained at the parallel edge of the powder spectrum. The increase in the span of the ^{31}P chemical shift with the decrease in the temperature could be attributed to the changes in the mobility of the lipid headgroup. Most importantly, the line width decreased and the signal intensity increased as the temperature decreased. For example, the signal-to-noise ratio (S/N) of the ^{31}P chemical shift spectrum at 0 °C is 35% more than that at 37 °C. Since the gel-to-liquid crystalline phase transition temperature of POPC is −5 °C, spectra obtained below this temperature showed line broadening (data not shown). These experiments demonstrate that it is possible to perform solid-state

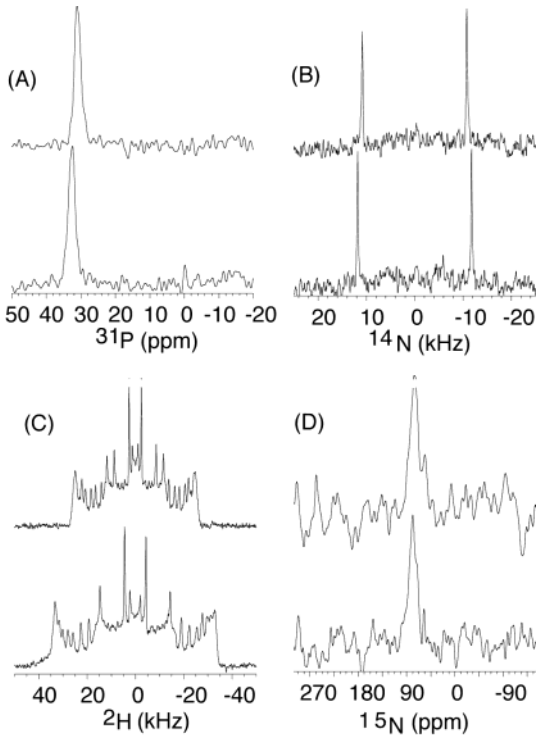

Figure 1. Solid-state NMR spectra of (A) ^{31}P and (B) ^{14}N of POPC, (C) ^{2}H of POPC-d_{31}, and (D) ^{15}N of [^{15}N]Phe$_6$-LL37 embedded in POPC. Five milligrams of POPC was used to obtain (A), (B), and (C), and for (D) 6 mg of LL37 was mixed with POPC to prepare 2 mol % of the peptide concentration. Top and bottom spectra were collected at 37 and −4 °C, respectively.

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NMR experiments on pure lipids at low temperatures as low as the gel-to-liquid crystalline phase transition temperature.

Because of the weak $^{1}H-^{31}P$ dipolar couplings in lamellar phase bilayer samples, cross-polarization from ^{1}H to ^{31}P is not commonly used to obtain the ^{31}P chemical shift spectrum. Instead, a chemical shift echo sequence, $90°-\tau-180°-\tau-$acquire, with ^{1}H decoupling during $\tau-180°-\tau-$acquire, is used. In this study, experiments were performed to examine the efficiency of CP, ramp-CP, and the $90°-\tau-180°-\tau$ sequence. In all experiments, the signal intensity increased as the temperature of the sample decreased. In CP and ramp-CP experiments, the signal intensity increases as a function of the contact time and reaches the steady state at 3.5 ms. Our results suggest that ramp-CP provides a better signal intensity than other sequences at all temperatures and it provides 2 times more signal intensity than the $90°-\tau-180°-\tau$ sequence at all temperatures for a contact time from 2 to 5 ms.

Quadrupole couplings of ^{14}N and ^{2}H nuclei provide valuable information for understanding the properties of lipid bilayers and lipid−peptide interactions. Unlike the large quadrupole coupling (>MHz) associated with the amide-^{14}N in the peptide backbone, the quadrupole coupling of the ^{14}N nucleus in the choline site of POPC headgroup is significantly reduced due to the symmetry, which makes ^{14}N a valuable probe to investigate drug−membrane interactions, as demonstrated in our recent study.[5] The narrow lines in the ^{14}N quadrupole spectra of POPC bilayers (Figure 1B) suggest that the lipids are well aligned above T_c, while line broadening was observed below the transition T_c. The quadrupole splitting increases as the temperature decreases due to the reduction in the mobility of the lipid headgroup, which is in agreement with the ^{31}P results discussed above.

^{2}H spectra of aligned POPC-d_{31} shown in Figure 1C suggest that the bilayers are well aligned even at −4 °C. Unlike the small increase in the ^{31}P CSA span and ^{14}N quadrupole splitting, the ^{2}H quadrupole splitting increases significantly with decreasing temperature. These results suggest that the (motion and its dependence on the temperature) of the hydrophobic core and the headgroup (or the bilayer surface) are significantly different, which is in complete agreement with previous studies in the literature.[6] Therefore, the mechanically aligned bilayers could be used to measure the effects of phase transition and the temperature on the physical properties of bilayers.

We also performed experiments on POPC bilayer samples containing peptides in order to demonstrate that it is possible to obtain aligned spectra for the structural studies on membrane-bound peptides and proteins. As an example, experimental results obtained from bilayers containing LL-37 peptide are discussed below. LL-37 is a 37-residue human cathelicidin antimicrobial peptide. Solid-state NMR experiments have shown that the peptide is oriented on the surface of the lipid bilayer.[3] In addition, the previous study has shown that the secondary structure and orientation in bilayers is independent of the membrane composition and temperature (from 25 to 55 °C). In this study, ^{15}N chemical shift spectra of 2 mol % ^{15}N-Phe_6-LL-37 embedded in POPC bilayers oriented with the bilayer normal parallel to the magnetic field were obtained at various temperatures; representative spectra are shown in Figure 1D. A single peak with the same chemical shift frequency suggests that the peptide is well aligned and the orientation of the peptide does not change with the temperature. Importantly, the line width decreased and signal intensity increased as the temperature decreased. The decrease in the line width as the temperature decreases could be due to the decreased mobility of the peptide, as discussed in our recent publication.[3] The reduction in the mobility of the peptide at low temperatures could increase the cross polarization from proton to ^{15}N, which could have resulted in the increased signal intensity. ^{31}P chemical shift spectra of this sample recorded as a function of temperature (data not shown) were similar to the spectra of pure lipids as shown in Figure 1A.

These fully hydrated aligned bilayer samples were prepared with excess water in order to produce biologically relevant L_α phase.[6] On the other hand, experiments on samples that were not fully hydrated (or prepared by exposing them to water vapor at 100% humidity without the addition of excess water) did not provide aligned spectra at low temperatures. Our experiments suggest that mechanically aligned bilayer samples used in solid-state NMR experiments performed at a low temperature (around 0 °C) are quite stable for a very long time as compared to those used in room-temperature experiments. For example, POPC containing LL-37 was stable even after months of high-power solid-state NMR experiments, while pure POPC samples were stable for more than one year; these samples were kept at 4 °C in a refrigerator when not being used in experiments. However, the stability of the bilayer sample could depend on the property of the peptide or protein embedded in them. In conclusion, the present study demonstrates that it is possible to perform solid-state NMR experiments on mechanically aligned pure lipids and lipids with peptide incorporated at low temperatures (as low as the gel-to-liquid crystalline phase transition temperature). Further, the enhanced S/N of ^{31}P, ^{14}N, ^{2}H, and ^{15}N spectra of aligned bilayers at low temperatures could pave avenues for solid-state NMR applications to study membranes.

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Supporting Information Available: ^{14}N and ^{2}H spectra of aligned POPC bilayers at various temperatures, and a plot of signal intensity versus the CP contact time. This material is available free of charge via the Internet at http://pubs.acs.org.

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