Solid-state NMR Studies of Membrane Proteins Using Phospholipid Bicelles

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Membrane proteins in highly oriented lipid bilayer samples are useful for membrane protein structure determination. We used in the past planar lipid bilayers which were aligned and supported on the glass slide. These samples were mechanically aligned in a magnetic field. However, these stacks of glass slides with planar lipid bilayers are not well suited for use with a commercial solid-state NMR probe with a round coil. Therefore, a homebuilt solid-state NMR probe was built and used with a stack of thin glass plates wherein the RF coil was wrapped directly around the flat square sample. Recently, we began to use magnetically aligned bicelles that are suitable for the structure determination of membrane proteins by solid-state NMR spectroscopy without any effort to build a flat square coil probe. These bicelle samples are well suited for use with a commercial solid-state NMR probe with a round coil, are very easy to prepare and are very stable, so that they can be kept for more than a year. In this paper, we present the solid-state NMR spectra of optimized and magnetically oriented bicelle samples of membrane proteins.

Key Words : Solid-state NMR, Membrane proteins, Phospholipid bilayers, Phospholipid bicelles

Introduction

X ray crystallography and conventional solution NMR spectroscopy techniques are difficult to use for the study of membrane protein structures, because the lipids required for the structural integrity and functionality of the membrane proteins prevent the crystallization of the proteins as well as reducing their rate of overall reorientation in solution. Solid-state NMR experiments on lipid bilayer samples are especially valuable for membrane proteins with a predominantly helical secondary structure. Planar lipid bilayers which were aligned and supported on glass slides were often used for this purpose. In this method, the samples are mechanically aligned in the magnetic field. However, commercial solid-state NMR probes with a round coil are difficult to use with a stack of glass slides with planar lipid bilayers Therefore, a homemade solid-state NMR probe was built for use with a stack of thin glass plates, wherein the RF coil is wrapped directly around the flat square sample.[1-6] Recently, we started to use magnetically aligned bicelle samples for the structure determination of membrane proteins by solid-state NMR spectroscopy. These bicelle samples are well suited for use with a commercial solid-state NMR probe with a round coil, the sample preparation of the bicelles is very easy and the bicelle samples are very stable, so that they can be kept for more than a year. These bicelle samples were composed of long-chain phospholipids and short-chain phospholipids, as shown in Figure 1. The long-chain lipids constitute the planar bilayers and the short-chain lipids form the edge of the bicelles. Bicelles are distinguished by their q value, which is the ratio of the long-chain to short chain lipids. The large bicelles which have q value greater than 2.5 are aligned, as shown in Figure 2. Aligned bicelle samples have their orientation of bilayer normal perpendicular to the direction of the applied magnetic field. The bicelle disks could be flipped by ninety degrees by the addition of lanthanide ions. The flipped bicelles have the same orientation as the mechanically oriented phospholipids bilayers situated between the glass plates, but the unflipped bicelles are used because of the instability of the sample due to the presence of lanthanide ions.[7-14]

Experimental Methods

Unflipped and flipped bicelle samples. 14 : 0 Diether PC(1,2-Di-O-Tetradecyl-*sn*-Glycero-3-Phosphocholine) and 06:0 Diether PC: (1,2-Di-O-Hexyl-*sn*-Glycero-3-Phosphocholine) were used to make bicelles having a lyotropic liquid

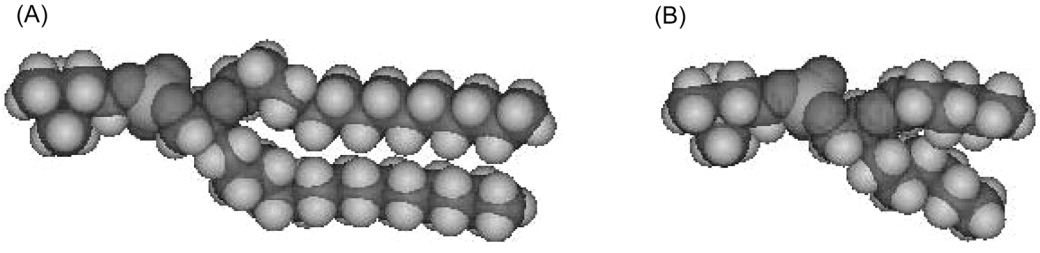

Figure 1. (A) 14 : 0 Diether PC; 1,2-Di-O-Tetradecyl-*sn*-Glycero-3-Phosphocholine. (B) 06 : 0 Diether PC: 1,2-Di-O-Hexyl-*sn*-Glycero-3-Phosphocholine The bicelle was made from two phosphatidyl cholines with different chain lengths. q = 3.2, 20% (w/v)

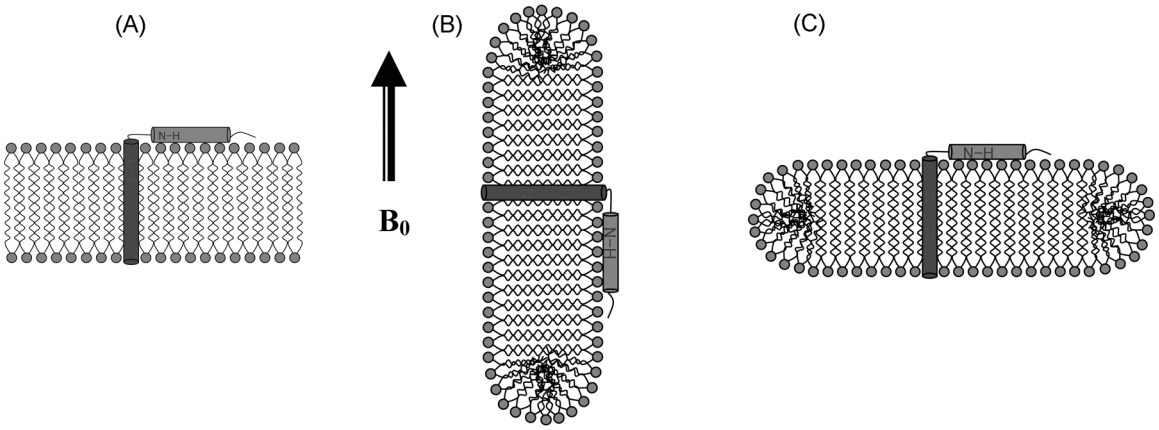

Figure 2. Bilayers and Bicelles. (A) Bilayers, (B) Unflipped bicelles, (C) Flipped bicelles. The helices (Blue: Transmembrane helix, Red: In plane helix) in the oriented bilayers (A) and in the oriented unflipped bicelles (B) were oriented perpendicular to each other. The unflipped bicelles (B) could be flipped by 90° by the addition of lanthanide ions. The flipped bicelles (C) have the same orientation as the mechanically oriented bilayers between the glass plates. Herein, we used unflipped bicelles because of the instability of the sample containing the flipped bicelles due to the presence of lanthanide ions.

crystalline phase. These non-hydrolyzable ether-linked lipids contribute to the stability of the sample. The bicelles were made of two phosphatidyl cholines having different chain lengths with q = 3.2, 20% (w/v). The resulting bicelles have near-perfect uniaxial alignment, as shown in Figure 2-B, and are referred to as unflipped bicelles. The total volume of the samples was 160 μL and they contained the protein and 28% (w/v) lipids. The protein concentration and salt contents were optimized. Unflipped bicelle samples could be flipped by 90 degrees by the addition of lanthanide ions (~2.5 mM).

Solid-state NMR experiments. The solid-state NMR spectra were obtained on a Bruker Avance console, using a 16.4 T Magnex magnet with a ^{1}H resonance frequency of 700 MHz, and a home-built probe with a 5 mm ID double-resonance solenoid coil at 315 K. The probe was doubly tuned to the resonance frequencies of ^{1}H at 700.3 MHz and ^{15}N at 70.8 MHz. A commercial probe with a solenoidal round coil could be used for this purpose. One dimensional ^{15}N chemical shift spectra were obtained with a contact time of 160 μs and CPMOIST (Cross-Polarization with Mismatch Optimized IS Transfer) cross-polarization to generate ^{15}N magnetization using SPINAL-16 for heteronuclear decoupling.

Results and Discussion

The ^{15}N Solid-state NMR spectra of a 47-residue polypeptide with a single trans-membrane helix in two different types of aligned bilayer samples are compared in Figure 3, A and B. The bilayers are aligned between the glass plates in Figure 3A. The unflipped bicelles in Figure 3B yield narrow single-resonances. There is no residual powder pattern lineshape. The resonance line widths of 1-2 ppm for the magnetically aligned bicelle samples are narrower than those typically observed for mechanically aligned bilayers or peptide single crystals. The differences in the chemical shift frequencies between the unflipped bicelles (Figure 3B) and

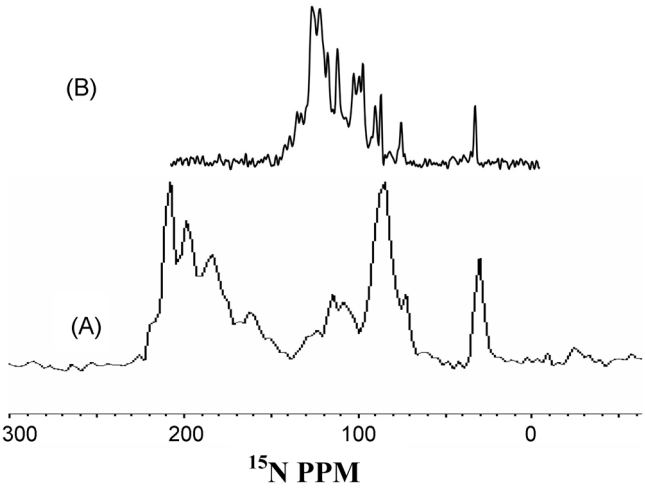

Figure 3. ^{15}N 1D solid-state NMR spectra of uniformly ^{15}N labeled pf1 coat protein in (A) mechanically oriented bilayers and (B) magnetically oriented unflipped bicelles. The resonance line widths for the magnetically aligned bicelle samples are narrower than those typically observed for mechanically aligned bilayers or peptide single crystals. The differences in the chemical shift frequencies between the unflipped bicelles (Figure 3B) and the bilayers between the glass plates (Figure 3A) show that the orientation of the individual peptide planes relative to the magnetic field is reflected in the spectra.

the bilayers situated between the glass plates (Figure 3A) show that the orientation of the individual peptide planes relative to the magnetic field is reflected in the spectra. The helices in the oriented bilayers and in the oriented unflipped bicelles are oriented perpendicular to each other.

The protein concentration and salt contents were optimized in order to obtain bicelles with the best possible behavior, as shown in Figure 4A-C. All three samples in Figure 4 have the same amount of lipids (q = 3.2, 25% (w/v)) in a total of 160 μL of the bicelles. The bicelle sample shown in Figure 4A contains 8 mg of pf1 coat protein as well as residual amounts of Tris buffer and KCl salts. The bicelle sample

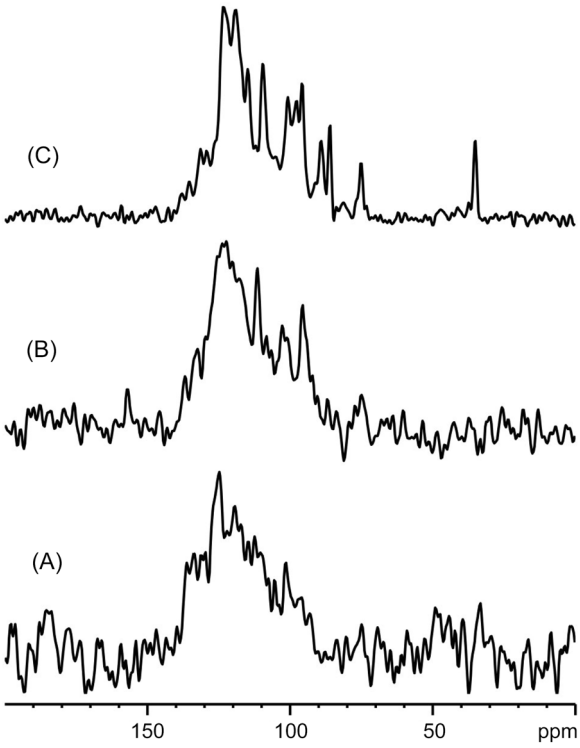

Figure 4. Solid-state NMR spectra of pf1 coat protein in oriented bicelles with different concentrations of protein and salt contents. All three samples have the same amount of lipids (q = 3.2, 25% (w/v)) in a total of 160 μL of the bicelles. (A) contains 8 mg of pf1 coat protein and residual Tris buffer and KCl salts. The number of scans is 2048. (B) contains 4 mg of pf1 coat protein and residual Tris buffer and KCl salts. The number of scans is 6144. (C) contains 4 mg of pf1 coat protein only. The number of scans is 2048. Only the spectrum of Figure 4C shows a distinguishable amino peak near 30 ppm. The ^{15}N 1D solid-state NMR spectra of the bicelles of the pf1 coat protein with residual buffer only and no salts show better resolution and a higher signal to noise ratio.

shown in Figure 4B contains 4 mg of pf1 coat protein as well as residual amounts of Tris buffer and KCl salts. The bicelle sample shown in Figure 4C contains 4 mg of pf1 coat protein only. The ^{15}N 1D solid-state NMR spectra of the bicelles of the pf1 coat protein with only the residual buffer and no salts show better resolution and a higher signal to noise ratio. The spectrum in Figure 4C only shows a distinguishable amino peak near 30 ppm.

Conclusions

Bicelles can be used as samples for the structural determination of membrane proteins. The advantages of using unflipped bicelles are their ease of preparation, the use of a sealed sample tube that ensures the stability of the sample for more than one year, and the fact that the sample can be place inside a solenoid coil for optimal probe performance. These bicelle samples are well suited for use with a commercial solid-state NMR probe with a round coil. In this study, we started to use magnetically aligned bicelles that can be used for the structural determination of membrane proteins by solid-state NMR spectroscopy.

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