J|A|C|S COMMUNICATIONS

Published on Web 08/24/2010

Triton X-100 as the "Short-Chain Lipid" Improves the Magnetic Alignment and Stability of Membrane Proteins in Phosphatidylcholine Bilayers for Oriented-Sample Solid-State NMR Spectroscopy

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Abstract: A mixture of phospholipids and Triton X-100 in a molar ratio of 5:1 forms well-aligned and stable bilayers that give superior solid-state NMR spectra of proteins. In a comparison, the oriented-sample (OS) solid-state NMR spectrum of Pf1 coat protein in aligned phospholipid bilayers displayed better resolution than the equivalent solution NMR spectrum of the same protein in micelles. Both samples and experimental parameters were fully optimized.

Molecular alignment is an integral part of solution NMR spectroscopy of soluble proteins and solid-state NMR spectroscopy of membrane proteins in phospholipid bilayers. Starting with Pake's demonstration that the splittings between doublets due to the dipole−dipole interaction depend on the angle with respect to the field,[1] there is a long history of devising ways to measure these splittings, the most powerful of which is separated local field (SLF) spectroscopy, in part because it is equally applicable to single crystals[2] and uniaxially aligned[3] samples. Once it had been shown that the natural tendency of proteins in solution to align in a strong magnetic field could be boosted by the use of alignment media such as bicelles,[4] filamentous bacteriophages,[5] or stressed polyacrylamide gels,[6] it became possible to routinely measure residual dipolar couplings (RDCs) for structure determination and refinement, including those of small membrane proteins in micelles or isotropic bicelles. Similarly, once it had been shown that membrane proteins in phospholipid bilayers could be aligned magnetically in mixtures of long-chain and short-chain lipids to an extent superior to that obtainable by mechanical alignment between glass plates,[7] it became possible to routinely obtain solid-state NMR spectra with single-site resolution and directly measure individual dipolar couplings as input for structure calculations.[8] Because of the importance and widespread implementation of dipolar coupling measurements, there has been continuous development of the alignment media for use with both soluble and membrane proteins.

Here we report an advance in sample preparation that increases the resolution of the spectra and the stability of the samples, resulting in a marked improvement in the applicability of oriented-sample (OS) solid-state NMR spectroscopy to structure determination of membrane proteins in phospholipid bilayers. Mixtures of "long-chain lipids" (i.e., phosphatidylcholine lipids whose hydrophobic chains vary in length and unsaturation) with the "short-chain lipid" Triton X-100 in a molar ratio of 5:1 ($q = 5$) form planar magnetically alignable bilayers[9] that are stable over a wide temperature range for long periods of time (months) and yield protein spectra whose resolution and sensitivity are superior to those we have obtained in any other combination of lipids.

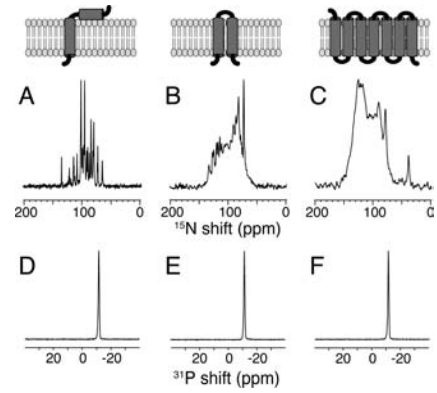

Figure 1. (top) Schematic drawings of the three membrane proteins aligned in planar "long-chain lipid":Triton X-100 ($q = 5$) bilayers. (A−C) One-dimensional solid-state ^{15}N NMR spectra of uniformly ^{15}N-labeled proteins. (D−F) One-dimensional solid-state ^{31}P NMR spectra of the phospholipids. (A, D) The 46-residue Pf1 coat protein. (B, E) The 78-residue mercury transport protein MerE. (C, F) The 350-residue G-protein-coupled receptor CXCR1. The ^{15}N NMR spectra were obtained at 35 °C on a Bruker 700 MHz spectrometer using a home-built ^{1}H/^{15}N double-resonance probe with a MAGC coil for the ^{1}H channel and a solenoid coil for the ^{15}N channel.[12] Pf1 coat protein and MerE were aligned in DMPC:Triton X-100 bilayers, and CXCR1 was aligned in DMPC/POPC (8:2 w/w):Triton X-100 bilayers.

This is illustrated in Figure 1 with one-dimensional ^{15}N solid-state NMR spectra of uniformly ^{15}N-labeled membrane proteins with one, two, and seven transmembrane helices and the corresponding ^{31}P NMR spectra of the phospholipids in the same samples. As is the case for conventional DMPC:DHPC bicelles ($q > 2.5$), the bilayer normal is perpendicular to the direction of the applied magnetic field, and narrow single-line resonances are observed because both the lipids and the proteins undergo fast rotational diffusion about the bilayer normal. It is also possible to "flip" the direction of alignment to make it parallel to the field through the addition of lanthanide ions (Figure S1 in the Supporting Information).[10]

All of the spectra in Figure 1 are distinguished by the narrow line widths of their resonances. There is only a single narrow (<1 ppm) ^{31}P resonance from the phospholipids; the resonance frequency demonstrates the perpendicular alignment and planarity of the DMPC bilayers. The narrow (∼0.5 ppm) line widths of the ^{15}N resonances from the amide backbone sites in the proteins are most readily appreciated in Figure 1A, where essentially every signal is resolved. Although there is significant overlap in the spectra in Figure 1B,C from the larger proteins, the sharp features of the overlapped resonances indicate that the line widths of individual resonances are similar to those in Figure 1A.

10.1021/ja1055565

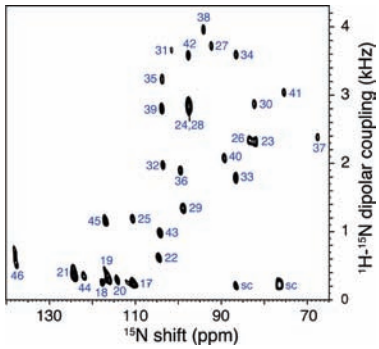

Figure 2. Two-dimensional SLF spectrum of uniformly ^{15}N-labeled Pf1 coat protein in DMPC:Triton X-100 bilayers obtained at 700 MHz with the SAMPI4 pulse sequence.[13] It resulted from signal averaging of 64 transients for each of 128 t1 points, and 1024 points were acquired in the direct dimension. The final data matrix had 1024 by 1024 points.

For simplicity, we show half of the symmetric SLF spectra in Figure 2, so each dipolar coupling is actually twice the value observed for each of the backbone amide ^{1}H$-^{15}$N dipolar couplings. Except for the coincident resonances of residues 24 and 28, this two-dimensional spectrum is fully resolved. It is qualitatively superior to earlier versions[11] (Figure S2), and this improvement can be ascribed solely to the superior alignment and stability of the DMPC:Triton X-100 bilayers. The protein preparation, spectrometer, probe, and pulse sequence are essentially identical to those used previously.

Phospholipid bilayers align in the magnetic field when a short-chain phospholipid (e.g., DHPC) or a detergent (e.g., CHAPSO or Triton X-100) is present. These alignment reagents dissolve in the long-chain phospholipid bilayers and typically reduce the order parameter of both the phospholipids and the proteins to 0.8−0.9, relative to the value of 1.0 observed in DMPC bilayers. Above a certain concentration, they also cause defects in the bilayer. Since it is possible under some circumstances to form aligned bilayers with q as high as 10, where no separate ^{31}P signal can be observed for the short-chain lipids that "cap" the defects or rims of the bilayer discs, it may be the case that the dissolution of DHPC or the detergent into the long-chain bilayers is sufficient to induce magnetic alignment. The ^{31}P NMR signal from the long-chain phospholipids is routinely used as measure of the alignment, since its frequency and width vary significantly with added protein and variation of the experimental parameters, especially temperature. As shown in Figure 1, for membrane proteins with between 46 and 350 residues, the ^{31}P NMR signal from the phospholipids is extremely narrow, indicative of very well aligned planar bilayers. In favorable cases, it is possible to obtain similar line widths from DMPC:DHPC samples, although the quality of the ^{15}N NMR spectra of the proteins in these samples do not approach those shown in Figure 1. Thus, we believe that there is a second factor that contributes to the success of DMPC:Triton X-100 bilayers, namely, the absence of a strong temperature dependence of the alignment. Even when "low E" coils are used to minimize sample heating, it is still difficult to avoid temperature increases of a few degrees in the course of signal averaging under conditions of high-power radio frequency irradiation at high frequencies. With other bilayer preparations, this relatively small temperature variation is sufficient to shift and broaden the resonances of both the ^{31}P and ^{15}N NMR signals. Indeed, part of the setup of the experiments using DMPC:DHPC mixtures involves empirically varying the sample temperature by a few degrees to obtain the narrowest line widths. As shown in Figure S3, DMPC:Triton X-100 bilayers remain well-aligned over a broad range of temperatures. This explains why neither the ^{31}P nor ^{15}N NMR signals are broadened by the small temperature increase due to sample heating inherent in these experiments. This effect may be just as important as the fundamentally high degree and uniformity of the magnetic alignment of the samples.

The combination of narrow line widths in both the dipolar coupling and chemical shift dimensions in two-dimensional spectra and the long-term stability of the samples improves the prospects for resolving signals from individual sites in membrane proteins with multiple transmembrane helices. Although the one-dimensional spectra in Figure 1B,C display limited resolution, this is due to the overlap of many narrow lines, which can be resolved in multidimensional experiments, rather than fundamentally broad line widths. This furthers the application of OS solid-state NMR spectroscopy to membrane proteins, since there is no fundamental reason for the line widths in a large membrane protein to be broader than those in a small membrane protein in phospholipid bilayers

Acknowledgment. We thank Woo Sung Son for the sample of MerE and his advice and Fabio Casagrande, Mignon Chu, Klaus Maier, and Hans Kiefer for their assistance with the preparation of the CXCR1 sample. This research was supported by grants from the National Institutes of Health and utilized the Biomedical Technology Resource for NMR Molecular Imaging of Proteins at UCSD.

Supporting Information Available: Experimental details and ^{15}N and ^{31}P NMR spectra of a DMPC:Triton X-100 sample at various temperatures. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA1055565