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Low-ε Static Probe Development for ^{15}N-^{1}H Solid-state NMR Study of Membrane Proteins for an 800 MHz NB Magnet

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A low-ε solid-state NMR(Nuclear Magnetic Resonance) probe was developed for the spectroscopic analysis of two-dimensional ^{15}N-^{1}H heteronuclear dipolar coupling in dilute membrane proteins oriented in hydrated and dielectrically lossy lipid environments. The system employed a 800 MHz narrow-bore magnet. A solenoid coil strip shield was used to reduce deleterious RF sample heating by minimizing the conservative electric fields generated by the double-tuned resonator at high magnetic fields. The probe's design, construction, and performance in solid-state NMR experiments at high magnetic fields are described here. Such high-resolution solid-state NMR spectroscopic analysis of static oriented samples in hydrated phospholipid bilayers or bicelles could aid the structural analysis of dilute biological membrane proteins.

Key Words : Solid-state NMR probe, Low-ε, Probe design, Membrane proteins, Bicelles

Introduction

Membrane proteins are important in various essential systems and regulatory processes, ranging from the complicated network of cellular communications to the metabolism and breaking down of unwanted substances in the human body. Hundreds of diseases involve the mis-folding or mis-assembling of integral membrane proteins and over 60% of all drug molecules target membrane proteins. Despite their importance, these protein's intrinsic structure-function relationships are not understood as their three-dimensional structures have not been fully elucidated. The study of membrane proteins by current methods is hampered by the need of a native bilayer environment, which is difficult using most biophysical techniques. They are notoriously difficult to crystallize for X-ray crystallography and their slow reorientation rates when combined with lipids or detergents hinder the use of conventional solution NMR (Nuclear Magnetic Resonance) techniques.

Solid-state NMR spectroscopy is a relatively new means of investigating the structures of solid biological samples.[1] It can be used to study the structure and dynamics of membrane proteins, whose low solubility, low yield, and low crystallization would prevent conventional structural analysis. Unlike solution-state NMR, whose resolution is heavily dependent on the molecular size of the analyte, the resolution of solid-state NMR under static conditions relies only on the degree of the sample's alignment and the ability to implement various multiple-pulse line-narrowing decoupling techniques. Solid-state NMR analysis of phospholipid bilayers or bicelles is valuable to the study of membrane proteins with predominantly helical secondary structures because analysis of these oriented samples takes advantage of the spectral simplifications that result from their uniaxial orientation parallel to the direction of the applied magnetic field. Orientational restraint of membrane proteins in a lipid environment can be obtained by ^{1}H-^{15}N heteronuclear dipolar coupling solid-state NMR experiments such as polarization inversion spin exchange at the magic angle (PISEMA), high-resolution separated local field spectroscopy based on magic-sandwich pulses (SAMMY), and SAMPI4.[2-4]

Solid-state NMR techniques constitute a promising area of structural biology as membrane proteins can be studied using the higher magnetic fields, more advanced instruments, novel NMR methodologies and advanced model membrane systems now available. However, solid-state NMR spectroscopy in conductive and dielectrically lossy protein samples with a high-frequency field (> 700 MHz) is hindered the significant heating of samples by RF irradiation during cross-polarization (CP) and decoupling pulses.[5] Most biological samples contain much water and salt, giving then high dielectric properties, which reduce the probe's efficiency by severely reducing its Q-factor (quality factor) and significantly shifting down the tuned frequency. Incident decoupling power of ~100 W for tens to hundreds of milliseconds can induce temperature increases of ~50 °C. With increasing magnetic field, this heating increases in magnitude as a sample's dielectric heating is proportional to f^3 (where f is the frequency of the irradiating field).[6,7] Therefore, a specific probe with high efficiency and high capability is required for the solid-state NMR study of these biological samples. Several probe designs have been developed and tested to improve efficiency described below.

The development of NMR instruments that can prevent membrane proteins from heating, especially the design of resonator probes, encompasses many areas of new research. Resonator designs developed for lossy biological samples include low-ε coil, scroll coil, modified Alderman-Grant coil (MAGC) and solenoid coil with strip-shield.[8-11] The conventional solenoid coil can perform well at high and low

frequency channels but its use is restricted by the detrimental effects on lossy samples at ^{1}H frequencies above 700 MHz. Modified coils are required for the study of ^{1}H resonances at the currently available high magnetic field strengths that are essential for solid-state NMR experiments. A strip shield can retain the favorable features of solenoid coils while protecting lossy samples. To prevent the coil from arcing, the shield's dielectric component has to be thick enough to cover the copper strips.[11] A coil with a strip shield of such resonators is largely compatible with lossy biological samples, allowing adequate levels of ^{1}H and ^{15}N RF heating, efficiency and homogeneity.[5]

This paper reports the optimized design, construction, and efficiency of a 800 MHz narrow-bore (NB) ^{1}H-^{15}N solid-state NMR probe with a 5 mm solenoidal RF coil for use in high-power, multi-pulse sequence experiments, such as 2D SAMMY or 2D SAMPI4. To reduce the effects of lossy samples on the probe's performance during solid-state NMR at high fields, a liner strip-shield consisting of copper strips and PTFE insulator inserted between the high-inductance solenoid coil and the glass sample tube. The probe could provide short high-power pulses and good RF homogeneity. ^{1}H-^{15}N 2D SAMPI4 spectra from a single crystal of ^{15}N labeled *N*-acetyl Leucine (NAL) were successfully recorded at 800 MHz using this lab-built solid-state NMR probe.

Experimental Methods

Probe Design. An 800 MHz ^{1}H-^{15}N double resonance NB probe was designed to match a bicelle sample for the observation of membrane proteins in membrane-like environment. The probe designed here is an adaptation of a previously reported static probe.[12,13]

Between the outer solenoid coil and the sample was a shield containing an inner copper strip. The strip-shield comprised 15 strips of 5 mm ID rolled copper sheet; each strip was 14/32 in. long and 1/45 in. wide. The strip-shield was inserted between a 5.2 mm ID solenoid coil and bicelle membrane protein sample in 5 mm OD flat-bottom glass tube. The RF receiver coil was constructed to 5 turn round coil made by gold plated flat copper wire. The receiver coil plate was made using POM (polyoxymethylene) that has no perturbation for ^{15}N labeled protein sample.

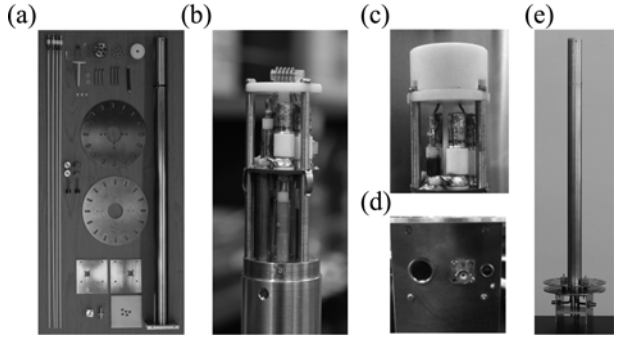

Figure 1. The 800 MHz NB lab-built strip-shield low-ε solid-state NMR probe. (a) Parts of the probe assembly. (b) The completed probe head, the strip shield was used to prevent heating of the bicelle sample and the coil. (c) PTFE coil cap for the sample. (d) Probe heat sensor and heater for temperature control. (e) The whole probe.

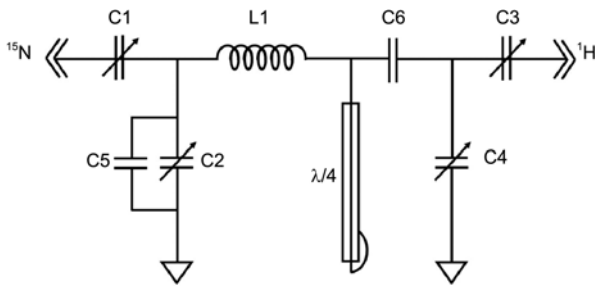

Figure 2. Circuit diagram of the 800 MHz WB ^{1}H and ^{15}N double resonance probe with a solenoidal coil. C3 and C4 are tuning and matching capacitors, respectively, for the decoupled frequency channel (800 MHz). C1 and C2 are tuning and matching capacitors, respectively, for the observed frequency channel (80 MHz). Capacitors C1, C2, C3, and C4 are 1-10 pF variable capacitors. The fixed capacitors are: C5 = 22 pF, C6 = 1 pF. L1 represents the five-turn 5.2 mm round solenoidal coil. λ/4 is a quarter lamda coaxial wave length cable of length 6.3 cm. The resonator is impedance matched to 50 Ω.

The circuit of probe was shown in Figure 2 for tuning of two frequencies for ^{1}H and ^{15}N. This probe circuit used the Cross-Waugh type circuit.[14] The ^{1}H decoupled channel was tuned and matched by C3, C4 and C6 capacitors and λ/4 coaxial cable. The length of the λ/4 coaxial line for ^{1}H channel was calibrated for ^{1}H resonance frequency (800.130 MHz) in an 18.8 Tesla magnet using the below equation.[12-14]

$$\text{Length of } \lambda/4 \text{ cable} = \frac{c \cdot k}{4 \cdot \nu}$$

$$= \frac{3 \times 10^{10}(\text{cm} \cdot \text{sec}^{-1}) \times 0.667}{4 \times 800.130 \times 10^{6}(\text{sec}^{-1})}$$

$$= 6.25 \fallingdotseq 6.3 \text{ (cm)}$$

Where c is the speed of light, ν is the frequency of the proton channel, and k is shortening factor of the coaxial cable with PTFE insulator. The ^{15}N observe channel was tuned and matched C1, C2 and C5 capacitors. The C5(22 pF) and C6(1 pF) capacitors were fixed capacitor for determination of ^{1}H and ^{15}N frequency (American Technical Ceramics, USA). The C1-C4 capacitors were variable capacitors (1-10 pF) for tuning and matching of sample in each channel (Polyflon, USA). The probe body (O.D. = 39.5 mm) was made by aluminum 6061, non magnetic material. As radio frequency transmitting cable, coaxial cable contained PTFE insulator was used. The whole resonant circuit of probe was grounded to a probe body and cap through copper coated circuit board and gold copper fingers. The whole circuit was well matched at 50 like other nmr console and tuned at the resonance frequencies of each channel using a network analyzer (Hewlett Packard 85046A, USA) outside a magnet. As temperature affects the structures of biological samples, a temperature control unit, comprising a ceramic heater, Cu-Const. sensor, and a dewar, was installed as shown in Figure 1(d). The temperature range of Cu-Const. sensor is −50 °C to 150 °C.

The temperature control unit was was calibrated by observing ^{1}H signal of 100% neat MeOH.[15] (data not shown)

Before mounting the lab-built solid-state NMR probe into the magnet, a network analyzer was used to tune and match the probe circuit and measure its electrical properties. Q-factors of the probe's ^{1}H and ^{15}N channels were measured as follows from a plot of reflected power *versus* frequency:

$$\text{Q-factor} = \frac{\text{Resonant frequency}}{\text{Bandwidth}} = \frac{f_0}{|f_1 - f_2|}$$

where f_0 is the resonant frequency of the respective channel, and f_1 and f_2 are the frequencies at −3 dB from the baseline of total reflection of the respective resonant frequency. After the probe was inserted into the magnet, resonance of its circuit was observed using a function of the Bruker spectrometer software (Topspin 2.1).

Solid-state NMR Experiments. All experiments were conducted using a Bruker Avance III spectrometer with a 800 MHz (18.8 T) narrow-bore (NB) magnet (Bruker BioSpin, Germany). The position of the home-built probe inside a magnet was determined and calibrated with the proton resonance of H_2O with 50 mM $CuSO_4$ that reduced spin-lattice relaxation time. The circuit's efficiency and B_1 RF field homogeneity for the ^{1}H and ^{15}N channels were characterized by nutation experiments with H_2O with 50 mM $CuSO_4$, and ^{15}N labeled glycine powder, respectively.

The ^{15}N-^{1}H 1D CPMOIST and ^{15}N-^{1}H 2D SAMPI4 pulse sequences are established methods for determining the structures of membrane proteins in solid-state NMR experiments.[16] The CPMOIST and SAMPI4 pulse sequences were optimized using NAL crystals. The ^{15}N chemical shift was calibrated by ^{15}N labeled ammonium sulfate (Cambridge Isotope Laboratories, USA; δ = 26.8 ppm) in a 5.0 mm OD glass tube. One-dimensional ^{15}N NMR spectra of a single crystal of NAL and bicelle sample were obtained using 1.0 ms CP-MOIST (cross-polarization with mismatch-optimized IS transfer) with SPINAL-16 ^{1}H decoupling for RF mismatch compensation.[16-18] Two-dimensional ^{1}H-^{15}N heteronuclear dipolar coupling solid-state NMR spectra of NAL were obtained using the SAMPI4 pulse sequence. All 1D data were processed by TOPSPIN 2.1 (Bruker BioSpin, germany), 2D SAMPI4 spectra were processed by NMR PIPE/NMR DRAW software.[19]

Results and Discussion

Both channels of the lab-built probe showed high Q-factors of over ~250. Isolation between the ^{1}H and ^{15}N channels was measured 35 dB from high to low frequency and 55 dB from low to high frequency. The high isolation factors and the high Q-factor were sufficient to measure high-resolution solid-state NMR spectra. After mounting the lab-built probe into the magnet, final tuning and matching were achieved using the Topspin software.

RF performance of the 800 MHz ^{1}H-^{15}N round coil probe was tested by delivering various pulse widths at different input powers in H_2O with 50 mM $CuSO_4$ and ^{15}N labeled glycine powder, respectively. B_1 homogeneity was measured with respect to pulse length (Figure 3). Homogeneity is reported as the comparison of the signal intensities obtained from the 0° and 810° pulses. Ratios of 55% in the ^{1}H and 80% in the ^{15}N channels were observed. Figure 3(a) and (b) show B_1 nutation profiles obtained at both frequencies. RF field strengths ($\omega_1/2\pi$) were measured to be 45.5 kHz at 60 W input power for ^{1}H (800 MHz) and 45.5 kHz at 450 W input power for ^{15}N (80 MHz) without arcing. The electrical properties of the 800 MHz ^{1}H-^{15}N strip-shield low-ε probe are listed in Table 1.

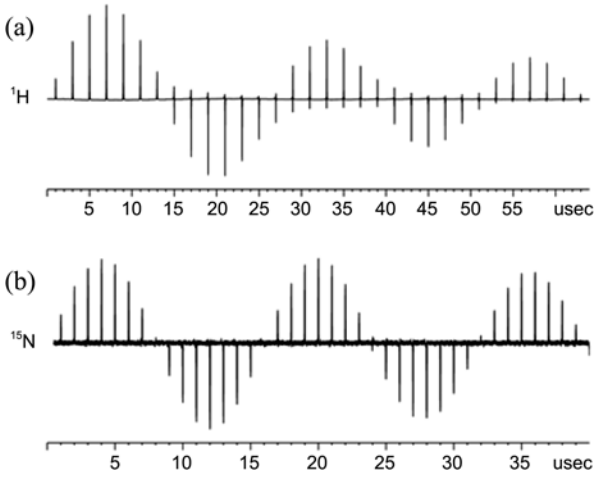

Figure 3. ^{1}H and ^{15}N B_1 nutation profiles for 50 mM aqueous $CuSO_4$ and ^{15}N labeled glycine powder, respectively. (a) RF homogeneity for ^{1}H channel: starting at 1 μs with intervals of 2 μs, a 90° pulse length of 6.5 μs at 60 W is achieved. $A_{810°}/A_{90°}$ = 55%, (b) RF homogeneity for ^{15}N channel: starting at 1 μs with intervals of 1 μs, a 90° pulse length of 4.0 μs at 450 W is achieved. $A_{810°}/A_{90°}$ = 80%.

Figure 4(a) shows the one-dimensional ^{15}N chemical shift NMR spectrum of a ^{15}N labeled NAL single crystal recorded using the NMR probe. The NAL signal crystal has four amide nitrogens in a unit cell of crystal.[20] The chemical shifts of each nitrogen signals were dependent with angles for magnetic field. In other words, these chemical shifts were determined by orientations of nitrogens in NAL crystal. These results were similar to orientated membrane in lipid bicelle for magnetic field, therefore the NAL single crystal was frequently used as a standard sample of aligned static probe for membrane protein. The acquisition time was 25.3 ms with 128 accumulations. Although each molecule contained only one ^{1}H-^{15}N bond, the spectrum shows four re-

Table 1. Electrical characterization of the lab-built 800 MHz ^{1}H-^{15}N strip-shield low-ε probe

Channel	^{1}H	^{15}N
Q-factor	> 250	> 250
Isolation	$^{1}H \rightarrow {}^{15}N$; 35 dB	$^{15}N \rightarrow {}^{1}H$; 55 dB
Nutation frequency, B_1	40 kHz	63 kHz
Power	100 Watts	500 Watts

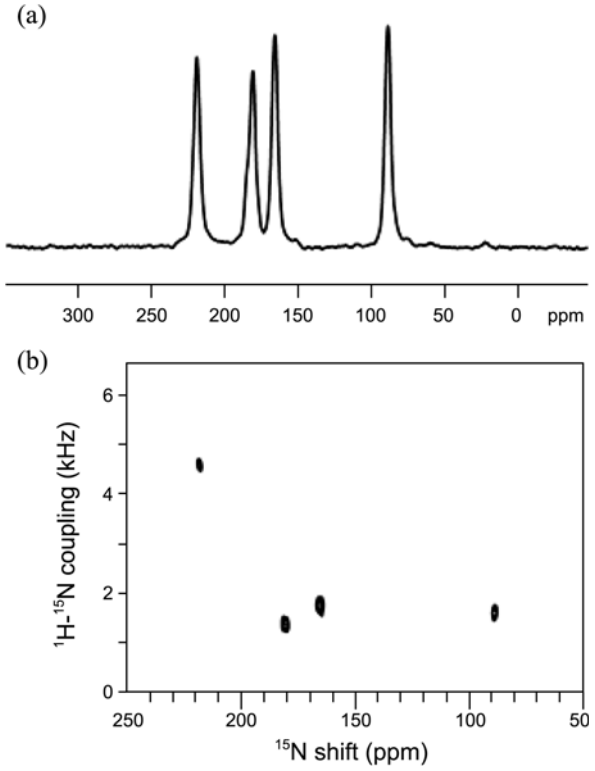

Figure 4. Experimental one-dimensional ^{15}N chemical shift NMR spectrum (a) and two-dimensional ^{1}H-^{15}N heteronuclear dipolar coupling/^{15}N chemical shift SAMPI4 spectrum (b) of a single crystal of NAL. Spectra were obtained at 298 K using a 18.8 T magnet with 800 MHz ^{1}H resonance frequency and the strip-shield low-ε probe. Experimental parameters: (a) 1024 transients with 5 s recycle delay, 45.5 kHz B_1 field strength (5.5 μs, 90 pulse), (b) 184 t1 increments with 256 transients each, 5 s recycle delay, 45.5 kHz B_1 field strength (5.5 μs, 90 pulse).

sonances because there are four different conformational sites per unit cell of the crystal.

The two-dimensional heteronuclear dipolar coupling spectrum (Figure 4(b)) was obtained using the SAMPI4 pulse sequence at a ^{1}H resonance frequency of 800 MHz. 128 t_1 increments, 1024 t_2 complex points, 256 transients, and 5 s recycle delay were used. The π/2 pulse was calibrated at 5.5 μs and 45.5 kHz B_1 field strength was used for the Hartmann-Hahn match during the CP contact period. The dipolar axis is adjusted to account for the scaling factor of 0.81. The spectrum shows an average < 200 Hz linewidth in the dipolar dimension.

Conclusions

An 800 MHz NB ^{1}H-^{15}N strip-shield low-ε solid-state NMR probe with 5.2 mm solenoidal RF coil was designed and constructed for structural studies of oriented biological samples, and preliminary NMR data were acquired to demonstrate its efficiency. The isolation between high and low-frequency channels and the probe's Q factor were optimized. The strip-shield efficiently reduced the RF electric fields E1 in the lossy sample. Optimal resolution and sensitivity in solid-state NMR observation of membrane proteins were obtained at high magnetic fields. High-resolution 1D and 2D NMR spectra of NAL were successfully recorded. These results demonstrate the high performance and efficiency of the lab-built solid-state NMR probe with a narrow-bore magnet for 800 MHz solution NMR.

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References

1. Martin, R. W.; Paulson, E. K.; Zilm, K. W. *Rev. Sci. Instrum.* **2003**, *74*, 3045.
2. Wu, C. H.; Ramamoorthy, A.; Opella, S. J. *J. Magn. Reson. A* **1994**, *109*, 270.
3. Nevzorov, A. A.; Opella, S. J. *J. Magn. Reson.* **2003**, *164*, 182.
4. Nevzorov, A. A.; Opella, S. J. *J. Magn. Reson.* **2007**, *185*, 59.
5. Wu, C. H.; Grant, C. V.; Cook, C. V.; Park, S. H.; Opella, S. J. *J. Magn. Reson.* **2009**, *200*, 74.
6. Dvinskikh, S. V.; Castro, V.; Sandström, D. *Magn. Reson. Chem.* **2004**, *42*, 875.
7. Li, C.; Mo, Y.; Hu, J.; Chekmenev, E. Y.; Tian, C.; Gao, F. P.; Fu, R.; Gor'kov, P. L.; Brey, W. W.; Cross, T. A. *J. Magn. Reson.* **2006**, *180*, 51.
8. Gor'kov, P. L.; Chekmenev, E. Y.; Li, G. C.; Cotton, M.; Buffy, J. J.; Traaseth, N. J.; Veglia, G.; Brey, W. W. *J. Magn. Reson.* **2007**, *185*, 77.
9. Grant, C. V.; Yang, Y.; Glibowicka, M.; Wu, C. H.; Park, S. H.; Deber, C. M.; Opella, S. J. *J. Magn. Reson.* **2009**, *201*, 87.
10. Stringer, J. A.; Bronnimann, C. E.; Mullen, G. C.; Zhou, D. H.; Stellfox, S. A.; Li, Y.; Williams, E. H.; Rienstra, C. M. *J. Magn. Reson.* **2005**, *173*, 40.
11. Wu, C. H.; Grant, C. V.; Cook, G. A.; Park, S. H.; Opella, S. J. *J. Magn. Reson.* **2009**, *200*, 74.
12. Park, T. J.; Kim, J. S.; Um, S. H.; Kim, Y. *Bull. Korean Chem. Soc.* **2010**, *31*, 1187.
13. Choi, S. S.; Jung, J. H.; Park, Y. G.; Park, T. J.; Park, G. H. J.; Kim, Y. *Bull. Korean Chem. Soc.* **2012**, *33*, 1577.
14. Cross, V. R.; Hester, R. K.; Waugh, J. S. *Rev. Sci. Instrum.* **1976**, *47*, 1486.
15. Ammann, C.; Meier, P.; Merbach, A. *J. Magn. Reson.* **1982**, *46*, 319.
16. Pines, A.; Gibby, M. G.; Waugh, J. S. *J. Chem. Phys.* **1973**, *59*, 569.
17. Levitt, M. H. *J. Chem. Phys.* **1991**, *94*, 30.
18. Fung, B. M.; Khitrin, A. K.; Ermolaev, K. *J. Magn. Reson.* **2000**, *142*, 97.
19. Delaglio, F.; Grzesiek, S.; Vuister, G. W.; Zhu, G.; Pfeifer, J.; Bax, A. *J. Biomol. NMR* **1995**, *6*, 277.
20. Sinha, N.; Grant, C. V.; Rotondi, K. S.; Feduik-Rotondi, L.; Gierasch, L. M.; Opella, S. J. *J. Peptide Res.* **2005**, *65*, 605.