Home-built Solid-state NMR Probe for Membrane Protein Studies

Yongae Kim,* Jung-Hyun Hwang, and Jae-Joon Park

Dept. of Chemistry, Hankuk University of Foreign Studies, San 89, Mohyun, Yongin 449-791, Korea
Received May 26, 2003

Proteins in highly oriented lipid bilayer samples are useful to study membrane protein structure determination. Planar lipid bilayers aligned and supported on glass slide were prepared. These stack of glass slide with planar lipid bilayers are not well fit for commercial solid-state NMR probe with round coil. Therefore, homebuilt solid-state NMR probe was built and used for a stack of thin glass plates and RF coil is wrapping directly around the flat square sample. The overall filling factor of the coil is much better and the large surface area enhances the extent to orientation by providing uniform environments for the phospholipids and the high ratio of circumference to area reduces edge effects. ^{1}H and ^{15}N double resonance probe for 400 MHz NMR (9.4T) with a flat coil (coil size: 11 mm × 20 mm × 4 mm) is constructed and tested.

Key Words : Home-built solid-state NMR probe, Membrane proteins

Introduction

Approximately 30% of all expressed polypeptides are membrane-associated but neither X-ray crystallography nor solution nuclear magnetic resonance (NMR) spectroscopy is very effective for these proteins. The lipids required for the structural integrity and functionality of membrane proteins impede crystallization as well as the rate of overall reorientation in solution. Solid-state NMR experiments on lipid bilayer samples are especially valuable for membrane proteins with predominantly helical secondary structure. High resolution solid-state NMR spectroscopy is capable of determining the backbone and side chain structures of membrane proteins. Solid-state NMR spectra of immobile molecules have very broad resonance signals because the anisotropic spin interactions are not averaged out by molecular motions. Irradiation of RF pulse and/or sample manipulations can lead to the selective averaging and separation of the spectral manifestations of the anisotropic spin interactions. The spin interactions can be probed through radio frequency (rf) irradiations and sample manipulations that lead molecular reorientation as a line-narrowing mechanism. Solid-state NMR of oriented samples takes advantages of the spectral simplifications that result from uniaxial orientation parallel to the direction of the applied magnetic field. The spin interactions at ^{15}N-, ^{13}C, and ^{2}H labeled sites yield signals that can be characterized by single resonance frequencies in each of several dimensions.[1-6] The observed frequencies depend on the orientations of the principal axes of the spin-interaction tensors, present at each site, relative to the direction of the applied magnetic field.

A commonly used technique for aligning membrane bilayers involves the deposition of a mixture of proteins and lipids onto glass plates followed by application of pressure, shear, centrifugal forces.[7-9] In this Study, about 30 rectangular glass plates are stacked in parallel so that the bilayer normal is parallel to the field of the magnet. Planar lipid bilayers aligned and supported on glass slide are prepared for uniaxial orientation.

Experimental Methods

Circuit Diagram for a Home-built ^{1}H-^{15}N Double resonance Probe. Figure 1 shows a picture of a flat-coil probe designed for ^{1}H-^{15}N double resonance experiments. Figure 1(a) is a whole probe body and Figure 1(b-d) are different view of the flat square coil (dimension is 11 mm × 20 mm × 4 mm) with a sample in a polyethylene bag to keep the humidity. Figure 2 shows the general circuit diagram for a flat square coil probe. Capacitor C1, C2, and C6 are selected in conjunction with the $\lambda/4$ line to tune and match the high frequency channel. The length of $\lambda/4$ line can be calculated by using an equation (1).

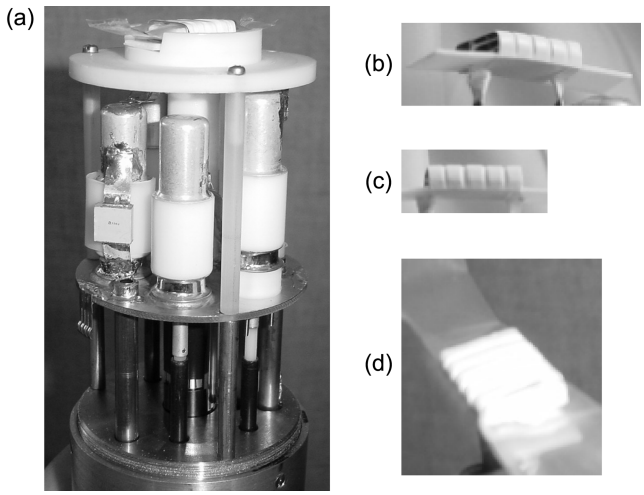

Figure 1. The pictures of the 400 MHz (9.4T) home-built solid-state NMR probe with a flat square coil designed for ^{1}H and ^{15}N double resonance experiments. (a) Whole probe (b-d) different view of the flat square coil (dimension is 11 mm × 20 mm × 4 mm) with a sample in a polyethylene bag to keep the humidity.

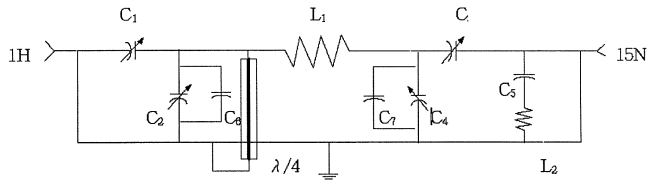

Figure 2. Circuit diagram for the ^{1}H and ^{15}N double resonance probe with a flat square coil. C1 and C2 are tuning and matching capacitor for 1H side that are 1.0-10pF variable capacitance (Polyflon). C3 and C4 are tuning and matching capacitor for ^{15}N side that are 1.0-10pF variable capacitance (Polyflon). C5, C6, and C7 are fixed capacitors (ATC). L1 is a flat square copper coil of 5 turns which dimension is 11 mm × 20 mm × 4 mm. $\lambda/4$ is a quarter lamda line which length is 12.5 cm.

$$\lambda/4 = c/4 \quad (1)$$

$$= \frac{3 \times 10^{10}(\text{cm/sec}) \times 0.667^{*}}{4 \times 400 \times 10^{6}(\text{sec}^{-1})}$$

$$= 12.5 \text{ (cm)}$$

(*: Inside the dielectric of the coaxial cable is 0.667)

Then L1 flat square coil is added to the circuit and tuned. Capacitor C3, C4, and C7 are added to tune and match the low frequency channel. High frequency trap of C5 and L2 are used to tune the decoupled channel and provide the higher isolation between the decoupled frequency channel and observed channel. Tuning and matching of each frequency channel and isolation between two channels were measured by network analyzer (HP 8712ES).

Oriented lipid bilayer samples. To make an oriented lipid bilayer samples, codissolved vpu which are 81 residue accessory protein of human immunodeficiency virus type-1 (HIV-1) protein and phospholipids (DMPC and DMPG) are deposited from organic solvents followed by evaporation and lipid hydration with the glass surface. The stacked glass slide sample is wrapped in a thin layer of parafilm, and then placed in a thin film of polyethylene that is heat sealed at both ends to maintain sample hydration during the experiments. Figure 1D shows an oriented protein in oriented bilayer sample in a sealed polyethylene bag.

Solid-state NMR Experiments. Solid-state NMR spectrum was obtained on a Chemagnetics-Otsuka Electronics (Ft. Collins, CO) spectrometer with a wide bore Oxford 400/89 magnet, using a home-built flat square coil probe double tuned to the resonance frequencies of 1H at 400.3 MHz, and 15N at 40.5 MHz. 1 dimensional 15N chemical shift spectra were obtained with 1 ms contact time, CPMOIST (Cross-Polarization with Mismatch Optimized IS Transfer) cross-polarization to generate 15N magnetization. The 2D PISEMA experiment was using flip-flop, phase- and frequency switched Lee-Goldburg homonuclear decoupling to provide line-narrowing in the 1H-15N dipolar coupling dimension.

Results and Discussion

Specifications of the home-built square flat coil probe are summerized at Table 1. Quality factor of home-built probe and isolation between high frequency channel and low frequency channel is high enough to measure high resolution solid-state NMR spectrum. RF power durability of the home-built solid state NMR probe is high enough for more than 1 kW. Especially for ^{1}H and ^{15}N cross polarization experiments like PISEMA (Polarization Inversion with Spin Exchange at the Magic Angle)[10-16] require more than 1kW for low frequency channel. Figure 3 shows 1D and 2D solid-state NMR spectra of vpu which are 81 residue accessory protein of human immunodeficiency virus type-1 (HIV-1) in oriented phospholipids bilayers obtained with home-built solid-state NMR probe. More than 1kW RF power was required for ^{15}N frequency channel but there is no probe arcing detected. The ^{15}N chemical shift depends on the helix orientation are obtained from 1D spectrum. The ^{15}N chemical shift and ^{1}H-^{15}N dipolar coupling frequencies were

Table 1. Specification of the home-built flat square coil probe of ^{1}H and ^{15}N double resonance for 400 MHz

Coil Dimension	11 × 20 × 4 mm (5 Turns)
Inductance	49 Ω
Quality factor	1H; 220 ; 15N; 105
Isolation	High → Low; 29 dB; Low → High; 29.6 dB
Power Capability	1H Dec; 2.3 μs 90° ; 15N 90°; 4.5 μs

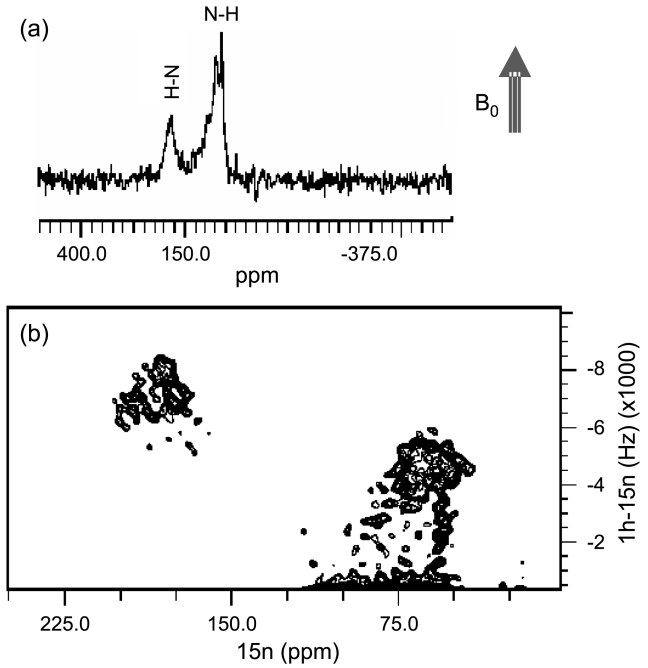

Figure 3. 1D and 2D solid-state NMR spectra of uniformly ^{15}N labeled vpu in oriented bilayers. (a) ^{15}N chemical shift solid-state NMR spectrum of vpu in lipid bilayers oriented on glass slides. Residues in the transmembrane helix have N-H bonds oriented approximately parallel to the field and residues in the protein amphipathic in –plane helix have their N-H bonds perpendicular to the field. (b) The ^{15}N chemical shift and ^{1}H-^{15}N dipolar coupling frequencies measured from the two-dimensional PISEMA spectrum. This 2D solid state NMR spectrum provided the orientational constraints for structure determination of 15N labeled vpu in lipid bilayer membranes.

measured from the two-dimensional PISEMA spectrum. This 2D solid state NMR spectrum provided the orientational constraints for structure determination of ^{15}N labeled vpu in lipid bilayer membranes. Detailed structural infomation will be discussed in other paper.

Conclusions

Home-built solid state NMR probe of ^{1}H and ^{15}N double resonance with square flat coil for 400 MHz was successfully made and tested. All specification including probe quality factor, isolation between high frequency channel and low frequency channel, and RF power durability over 1 kW are satisfactory as Table 1. 1D and 2D solid-state NMR spectra of vpu in oriented bilayers with home-built solid state nmr probe were obtained without any arcing problems.

Acknowledgements. This work was supported by grant No. (R01-2001-00049) from the Korea Science & Engineering Foundation, and HanKuk University of Foreign Studies Research Fund of 2003. This research utilized the resource for Solid-state NMR of Proteins supported by grant P41RR09731 from the Biomedical Research Technology Program, National Center for Research Resources, National Institutes of Health.

References

1. Shon, K.; Kim, Y.; Colango, L. A.; Opella, S. J. *Science* **1991**, *252*, 1303.
2. Shon, K.; Schrader, P.; Kim, Y.; Bechinger, B.; Zasloff, M.; Opella, S. J. *Biotechnology: Bridging Research and Applications* **1991**, 109.
3. McDonnel, P. A.; Shon, K.; Kim, Y.; Opella, S. J. *J. Mol. Biol.* **1993**, *233*, 4474.
4. Opella, S. J.; Kim, Y.; McDonnell, P. *Methods in Enzymology* **1994**, *239*, 536.
5. Kim, Y. *Biochemistry News* **2001**, *21*, 253.
6. Jost, P. C.; Griffith, O. H. *Arch. Biochem. Biophys.* **1973**, *159*, 70.
7. Tanaka, H.; Freed, J. H. *J. Phys. Chem.* **1984**, *88*, 6633.
8. Zhang, Q. W. *et al. J. Magn. Res.* **1998**, *132*, 167.
9. Ramamoorthy, A.; Wu, C. H.; Opella, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 10479.
10. Kim, Y.; Valentine, K.; Opella, S. J.; Schendel, S. L.; Cramer, W. A. *Protein Sci.* **1998**, *7*, 343.
11. Opella, S. J.; Kim, Y.; Valentine, K. G.; Marassi, F. M.; Zasloff, M.; Montal, M.; Cramer W. A. *J. Kor. Magn. Res. Soc.* **1998**, *2*, 120.
12. Marassi, F. M.; Gesell, J. J.; Valente, A. P.; Kim, Y.; Montal, M.; Opella, S. J. *J. Bio. NMR* **1999**, *14*, 141.
13. Opella, S. J.; Marassi, F. M.; Gesell, J. J.; Valente, A. P.; Kim, Y.; Montal, M. *Nature Structural Biology* **1999**, *6*, 374.
14. Ramamoorthy, A.; Wu, C. H.; Opella, S. J. *J. Magn. Reson.* **1999**, *140*, 131.
15. Marassi, F. M.; Opella, S. J. *J. Magn. Reson.* **2000**, *144*, 150.