Journal of the Korean Magnetic Resonance Society **2004**, *8*, 55 - 61

^{2}D NMR Probe Development for Investigation of Biosupramolecular Systems

Andre Kim, Shin Won Kang and Jang-Su Park*

Department of Chemistry, Pusan National University, Busan, 609-735, KOREA
Received April 16, 2004

Abstract : Biosupramolecular systems such as biological membranes usually fluid under physiological conditions[1]. Therefore, solid-state NMR has been used to investigate biosupramolecular systems. But solid-state NMR spectra contain a large number of overlapping resonances and are rather difficult to analyze. These problem has to be overcome by selective isotope labeling. We constructed a deuterium NMR probe for AM400 NMR spectrometer, which is mainly used for liquid samples. To overcome the fluidity problem, a saddle type coil was designed. The efficiency was systematically investigated for two kinds of coil geometry, solenoid and saddle types. Our results suggest that solenoids are superior to saddle type coils in the sensitivity. However, the letter fits better to fluid samples such as biosupramolecular systems.
keyword : Biosupramolecular system, solenoid type coil, saddle type coil, solid-state NMR, isotope labeling

INTRODUCTION

Biosupramolecular systems such as biological membranes have complex composites. They are usually fluid under physiological conditions.[1] Therefore, it is difficult to get a high resolution of atomic level in X-ray crystallospectroscopy and high resolution liquid NMR spectroscopy. Recently, solid-state NMR has been used to investigate biosupramolecular systems. This technique has a merit that it can measure the samples in the same circumstances as biological condition. However, in solid compound the molecular motions are suppressed. As a result the solid-state NMR spectra contain a large number of

* To whom correspondence should be addressed. E-mail: jspark@pusan.ac.kr

overlapping resonances and are rather difficult to analyze. This problem has to be overcome by selective isotope labeling. To investigate physicochemical properties of deuterium labeled lipid membrane systems,[2] we constructed a deuterium NMR probe for AM400 NMR spectrometer, which is mainly used for liquid samples. To overcome the fluidity problem, a saddle type coil was designed. The efficiency was systematically investigated for two kinds of coil geometry.

Finally, the usefulness of this home-built probehead was examined by measurement of actual ^{2}H-NMR spectra of lipid bilayer samples.

EXPERIMENTS AND RESULTS

A solid-state ^{2}H-NMR probe was prepared by modification of a probe for liquid NMR measurement. The deuterium nuclear resonance frequency fits to 61.4MHz. To get high resolution spectra, it is important to have a homogenious radio frequency power in the coil. We have examined two types of coil, namely, solenoid and saddle forms. The sample tube is set horizontally in the former and vertically in the latter. These coil geometries were shown in Fig. 1. A solenoid coil has to be mounted after wounding copper wire of 1 mm diameter. The exact number of turns was determined empirically. The 90° pulse width was 12μs for the solenoid with 4mm coil diameter. Then the probe circuit was simplified as shown in Fig. 2, resulting in the shortening of the 90° pulse width to 4.3μs. Using this probe, the ^{2}H-NMR spectrum of phospholipids bilayers[3-7] was obtained by accumulation of about 10,000 times for 40mg of deuterated lipids (60% deuteration). However, since the sample tube is set horizontally in the solenoid coil, the fluid sample moved away from the center position of the probe coil. It resulted in the distortion of the spectral shape and remarkably reduced spectral sensitivity. To solve this problem, the probe coil geometry was replaced with the saddle form. It made the 90° pulse width was 7.8μs. A reasonable ^{2}H-NMR spectrum was obtained by accumulation of about 15,000 times with this probehead for the 60mg of deuterated lipids. The ^{2}H-NMR spectra measured by solenoid and saddle coils are illustrated in Fig. 3A and 3B, respectively. Both spectra reveal reasonable signal to noise ratio. RF power is about 30kHz when 90° pulse width is 7.8μs. Therefore, the effectively excited range is about 60kHz. To examine the coverage of the RF power, spin-lattice relaxation (T_1)

measurement was carried out. The result of T_1 measurement for cardiolipin bilayers[8] is

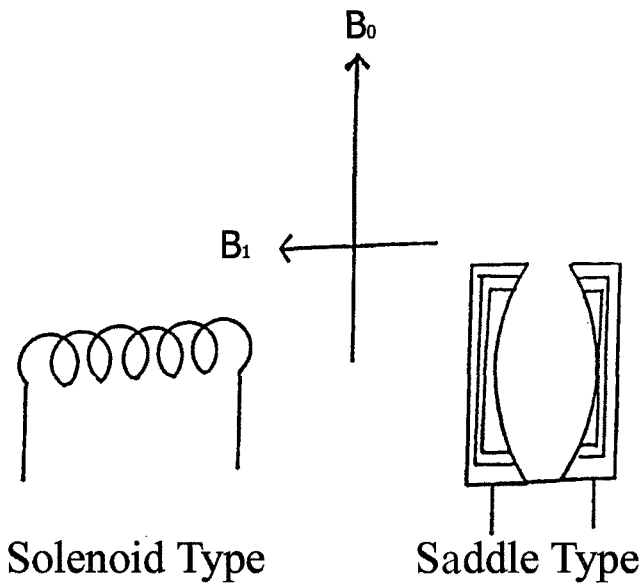

Fig. 1. Types of the NMR probe coils.

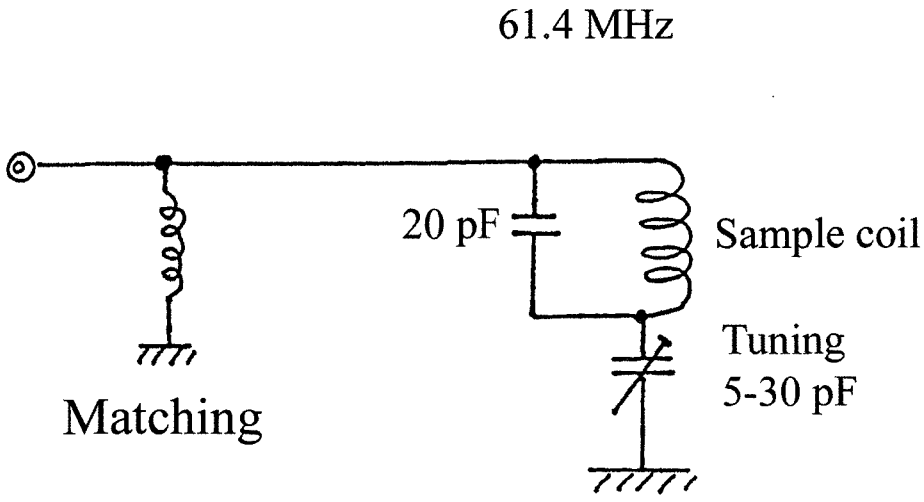

Fig. 2. A circuit used for the ^2H-NMR probe.

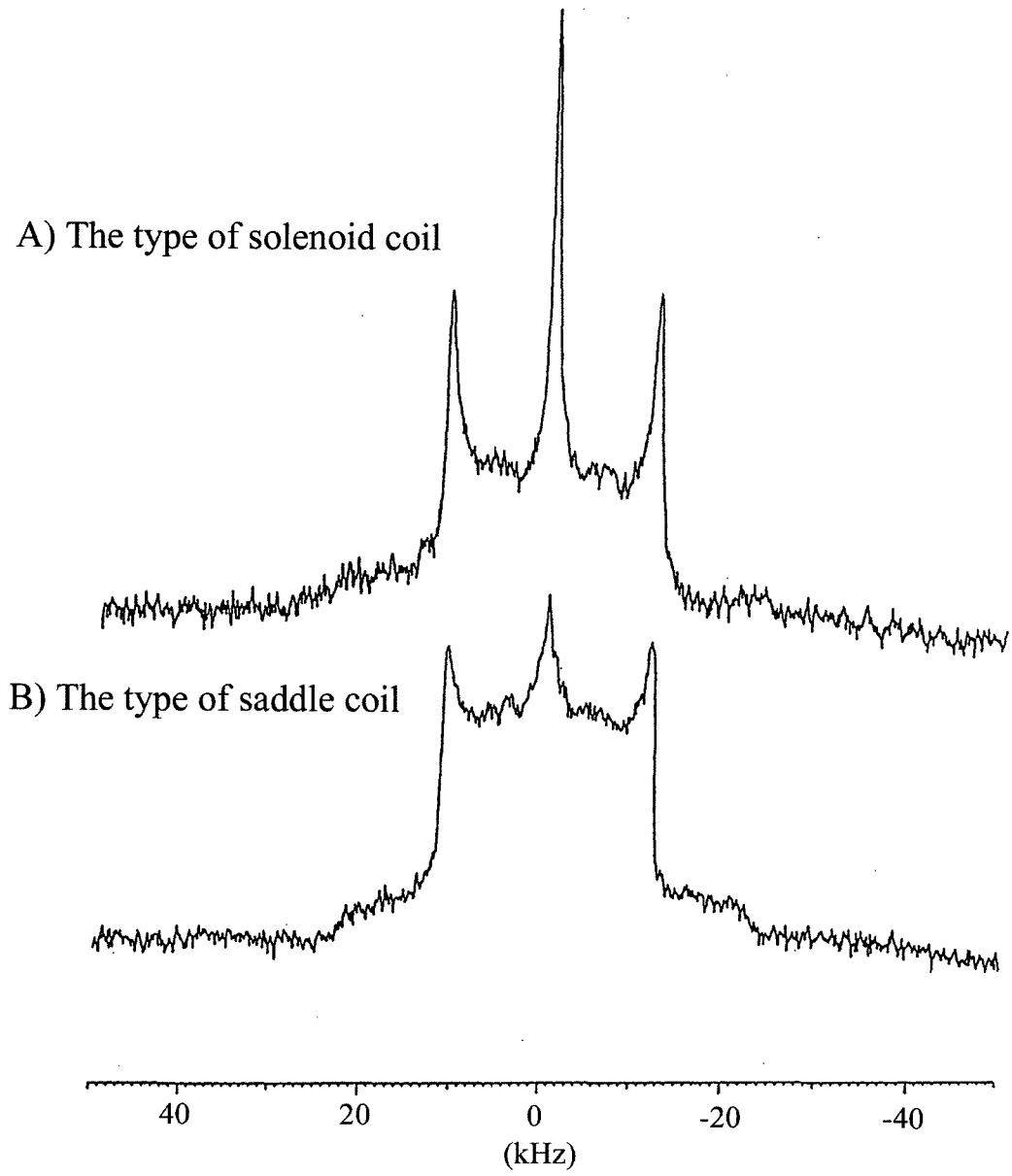

Fig. 3. ^{2}H-NMR spectra of [2-$^{2}H_{1}$]glycerol backbone of phosphatidylethanolamine(PE).

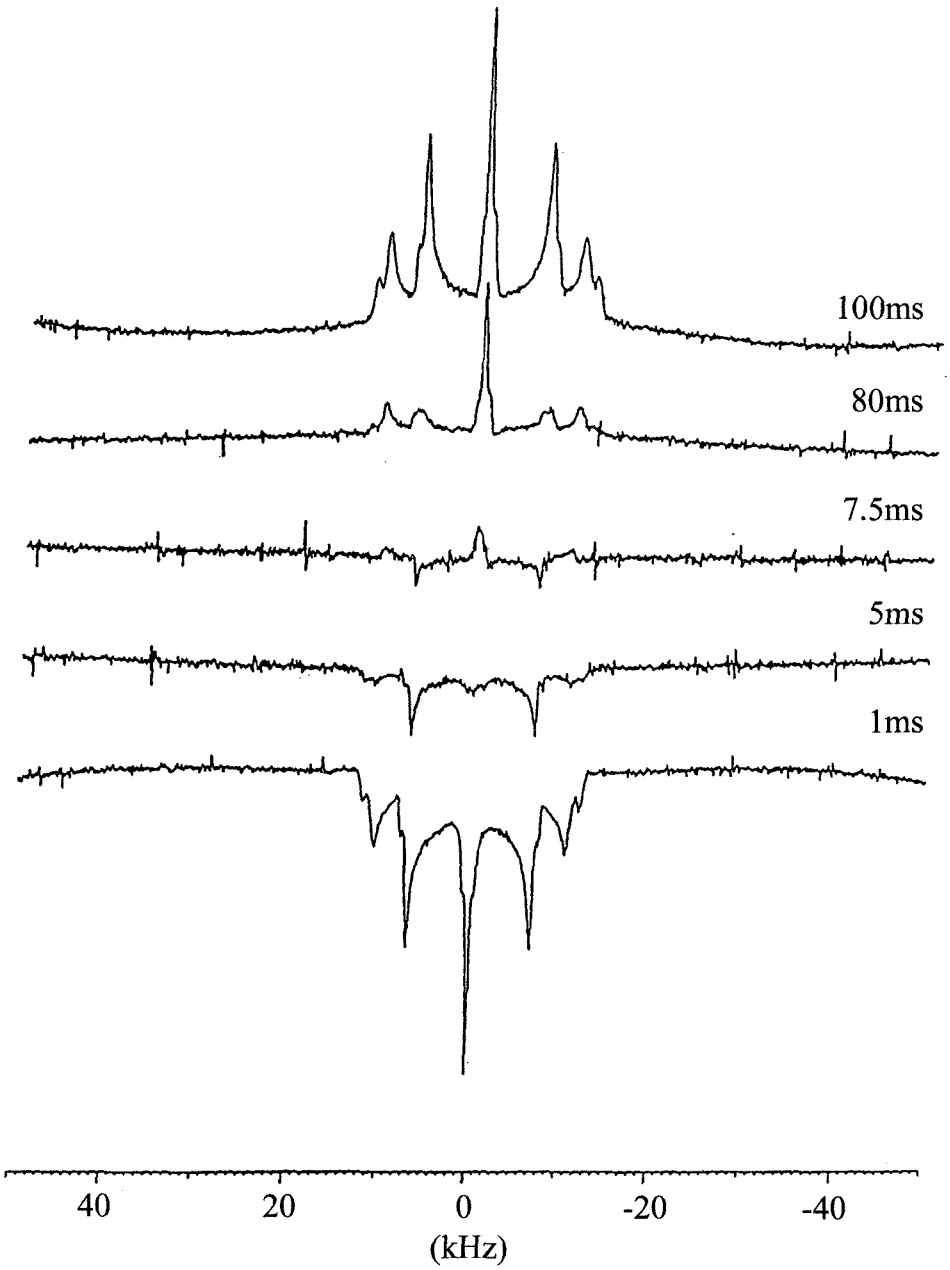

Fig. 4. Spectra of T_1 measurement for cardiolipin(CL) glycerol backbone.

shown in Fig. 4. As can dearly be seen, whole spectrum is completely inverted. Therefore it can be concluded that the home-built probe with a saddle coil is sufficiently useful in a lipid bilayer study.

DISCUSSION

In this study, we constructed a solid-state ^{2}H-NMR probe for measuring biosupramolecular systems. The deuterium nmr frequency was 61.4MHz on BURKER AM400 spectrometer. Microphonics or other spurious ringing might exist in the coil. During the pulse, a high electric current flows in the coil. Strong magnetic field induced in the coil causes mechanical oscillation of the coil. Then, additional ringing may follow a pulse. Epoxy resin has been used to avoid this problem. In addition, teflon tape and silicon rubber has been used to avoid arcing in a circuit of the probe.

For preparation of probe for practical use, probehead coil geometry was replaced from solenoid to saddle form. The coil diameters of solenoid and saddle probeheads are 4mm and 5mm, and numbers of their turns are 14 (1.7cm) and 8 (1.7cm), respectively. Therefore sample volume is 0.69cm^2 for the solenoid coil and 0.94cm^2 for the saddle coil. An other merit of the saddle type is that an ordinary 5 mm NMR tube can be used for the measurement. It simplifies the procedure of the sample preparation. The 90^{o} pulse width was 4.3μs for solenoid type and 7.8μs for saddle type. Namely, the radio frequency powers are 58kHz and 32kHz, respectively. Thus, to achieve the same signal to noise ratio, more sample was required for the saddle type coil. These results suggest that solenoids are superior to saddle type coils in the sensitivity. However, the letter fits better to liquid samples.

Acknowledgements

This work was supported mainly by a Grant from the Pusan National University. The authors are grateful to Prof. H. Akutsu, Institute for Protein Research, Osaka University, for his assistance.

REFERENCES

1. Odahara T, Nishimoto S, Katsutani N, Kyogoku Y, Morimoto Y, Matsushiro A, Akutsu H. J., Dynamic properties of nucleic acids in biosupramolecular systems, as studied by ^{31}P NMR, *Biochem (Tokyo).* 115(2), 270-278. (1994).
2. Itzhaki H, Davis JH, Borochov A, Mayak S, Pauls KP., Deuterium Magnetic Resonance Studies of Senescence-Related Changes in the Physical Properties of Rose Petal Membrane Lipids. *Plant Physiol.*, 108(3), 1029-1033. (1995).
3. Margaret A. Parker, Valencia King, Kathleen P. Howard, Nuclear magnetic resonance study of doxorubicin binding to cardiolipin containing magnetically oriented phospholipid bilayers, *Biochimica et Biophysica Acta*, 1514, 206-216. (2001).
4. Pinheiro TJ, Duralski AA, Watts A., Phospholipid headgroup-headgroup electrostatic interactions in mixed bilayers of cardiolipin with phosphatidylcholines studied by ^{2}H NMR, *Biochemistry*, 33(16), 4896-902. (1994).
5. Schanck A, Deleers M., ^{31}P NMR study of the parameters influencing the formation of non-bilayer phases in model membrane, *Biochem Biophys Res Commun.* 195(2), 654-658 (1993).
6. Shin K, Nagamori T, Kimura Y, Tomoi M, Fujiwara T, Akutsu H., Microdomain formation in phosphatidylethanolamine bilayers detected by ^{2}H-NMR, *Chem Phys Lipids*, 76(1), 55-62. (1995)
7. Weisz K, Grobner G, Mayer C, Stohrer J, Kothe G., Deuteron nuclear magnetic resonance study of the dynamic organization of phospholipid/cholesterol bilayer membranes: molecular properties and viscoelastic behavior, Biochemistry. 31(4), 1100-1112. (1992).
8. Spooner P.J., Duralski A.A., Rankin S.E., Pinheiro T.J., Watts A., Dynamics in a protein-lipid complex: nuclear magnetic resonance measurements on the headgroup of cardiolipin when bound to cytochrome *c.*, *Biophys J.*, 65(1), 106-112. (1993).