Sensitivity-Enhanced NMR of Biological Solids: Dynamic Nuclear Polarization of Y21M fd Bacteriophage and Purple Membrane

Melanie Rosay,[‡] Ana-Carolina Zeri,[†] Nathan S. Astrof,[‡] Stanley J. Opella,[†] Judith Herzfeld,[§] and Robert G. Griffin*[,‡]

Department of Chemistry and Francis Bitter Magnet Laboratory Massachusetts Institute of Technology Cambridge, Massachusetts 02139
Department of Chemistry, University of Pennsylvania Philadelphia, Pennsylvania 19104
Department of Chemistry and Keck Institute for Cellular Visualization, Brandeis University Waltham, Massachusetts 02454

Received October 2, 2000
Revised Manuscript Received December 7, 2000

Solid-state NMR (SSNMR) is established as a powerful tool for extracting structural parameters from biological and chemical systems.[1−3] However, the inherent low sensitivity of NMR often limits the scope of structural studies. With dynamic nuclear polarization (DNP) it is possible to address this issue since signal intensities can be enhanced by 2 to 3 orders of magnitude. This gain in sensitivity is accomplished by transferring polarization from electrons in exogenous paramagnetic molecules to nuclear spins via microwave irradiation at or near the EPR Larmor frequency.[4] The size of the DNP signal enhancement is dependent on a number of factors including the nuclear spin lattice relaxation time, T_1, which is maximized by performing experiments at low temperatures. Systems with an abundance of methyl groups, such as virus particles and membrane proteins, often have short T_1's even at low temperatures and could be particularly challenging candidates for DNP experiments. However, these are also some of the most interesting cases for SSNMR structural studies since they often cannot be examined with either solution NMR or X-ray diffraction. In this communication we demonstrate that it is possible to efficiently polarize archetypal examples of each of the systems mentioned above-namely the viral particle fd and the purple membrane containing bacteriorhodopsin (bR) and its accompanying lipids. Furthermore, by comparing the DNP signal enhancements in the ^{15}N and ^{31}P spectra of fd bacteriophage, we show that ^{1}H spin diffusion evenly distributes the enhanced polarization throughout a large macromolecular assembly. These results suggest that DNP may be a generally applicable approach for sensitivity enhancement in SSNMR experiments.

We have been developing DNP techniques for studies at high magnetic fields [initially at 5 T and more recently at 9 T (140 and 250 GHz EPR and 211 and 380 MHz ^{1}H NMR frequencies)]. To date, our most successful polarization transfer experiments are based on thermal mixing[5,6] with the nitroxide radical 4-amino-TEMPO doped into water/glycerol. The experiment is implemented as illustrated in Figure 1 and consists of microwave irradiation and cross polarization from ^{1}H to a low-γ nucleus. We have achieved ^{13}C signal enhancements of ∼400 in H_2O/glycerol mixtures at 13 K using a microwave cavity[7] and ∼50 in the ^{15}N magic angle spinning (MAS) spectrum of T4 lysozyme at 50 K,[8] both at 5 T.

Filamentous fd bacteriophages are long rod-shaped viruses comprising a 6408 nucleotide single-stranded DNA genome surrounded by 2700 copies of the α-helical pVIII (major coat) protein. The dimensions of a single virion are 8000 × 65 Å.[9] Due to the shape anisotropy and the alignment of the coat proteins approximately parallel to the virion long axis, the phage particles orient in a magnetic field, enabling high-resolution static SSNMR experiments.[10,11] The Y21M mutation of the coat protein decreases the conformational heterogeneity of the coat protein assembly and increases the NMR spectral resolution for oriented samples.[12] Since the fd virus is ∼90 wt % protein and 10 wt % DNA, the ^{15}N NMR spectrum is dominated by signals from the coat protein backbone. In contrast, the ^{31}P spectrum is due exclusively to the encapsulated DNA.[10]

Powder patterns for uniformly ^{15}N-labeled Y21M fd bacteriophage in TEMPO/water/glycerol are shown in Figure 2 b and c at 13 K and 5 T with and without DNP, respectively. Since the fd bacteriophage orients in a magnetic field, the sample was frozen prior to insertion into the precooled probe to obtain a powder pattern. The resulting signal is fit with $\Omega = 168$ ppm and $\kappa = -0.75$, representative of an amide backbone (Figure 2 a). The narrow peak on the upfield edge of the experimental powder pattern arises from the amino groups of the five lysine residues and the N terminus. The signal enhancement due to DNP is 26, representing a factor of approximately 675 reduction in signal averaging time. An additional factor of 20 in signal intensity is gained from the increased Bolztmann polarization by performing NMR experiments at 15 K versus room temperature. The ^{1}H $T_1 = 15$ s at 13 K. The high number of methylated amino side chains on the coat protein contribute to this short T_1; specifically, the 50-residue major coat protein of Y21M fd contains 10 Ala, 4 Ile, 2 Leu, 2 Met, 3 Thr, and 4 Val residues.[9] Despite the short ^{1}H T_1 at low temperatures, a substantial signal enhancement is observed with DNP.

To use DNP as a sensitivity enhancement tool for structural studies it is desirable to have uniform signal enhancements throughout the sample. In a typical DNP experiment, the bulk protons are polarized followed by cross-polarization (CP) from ^{1}H's to a low-γ nucleus prior to detection (Figure 1). Since ^{1}H's are highly abundant, we have argued that rapid spin-diffusion distributes the enhanced polarization throughout the solvent and solute.[8] Thus, with the fd bacteriophage ^{1}H spin diffusion should mediate the diffusion of polarization from the solvent to the encapsulated DNA, through the 20 Å layer of coat proteins. Figure 2 e and f show ^{31}P powder patterns with and without DNP acquired immediately after the ^{15}N experiment (with no change in temperature) and a similar signal enhancement is observed, ∼26. Detection of equal DNP enhancements on the coat protein and DNA core confirms that ^{1}H spin diffusion is an efficient mechanism for uniformly distributing enhanced polarization

* Address correspondence to this author.
‡ Department of Chemistry, MIT.
† Current address: Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093.
§ Department of Chemistry, Brandeis University.

(1) Opella, S. J. *Nat. Struct. Biol.* **1997**, *4*, 845−848.
(2) Griffin, R. G. *Nat. Struct. Biol.* **1998**, *5*, 508−512.
(3) van Beek, J. D.; Beaulieu, L.; Schafer, H.; Demura, M.; Asakura, T.; Meier, B. H. *Nature* **2000**, *405*, 1077−1079.
(4) Abragam, A. *The Principles of Nuclear Magnetism*; Clarendon: Oxford, England, 1961.
(5) Wenckebach, W. T.; Swanenburg, T. J. B.; Poulis, N. *J. Phys. Rep.* **1974**, *14*, 181−255.
(6) Duijvestijn, M. J.; Wind, R. A.; Smidt, *J. Physica B* **1986**, *138*, 147−170.
(7) Weis, V.; Bennati, M.; Rosay, M.; Bryant, J. A.; Griffin, R. G. *J. Magn. Reson.* **1999**, *140*, 293−299.
(8) Hall, D. A.; Maus, D. C.; Gerfen, G. J.; Inati, S. J.; Becerra, L. R.; Dahlquist, F. W.; Griffin, R. G. *Science* **1997**, *276*, 930−2.
(9) Marvin, D. A.; Hale, R. D.; Nave, C. *J. Mol. Biol.* **1994**, *235*, 260−286.
(10) Cross, T. A.; Tsang, P.; Opella, S. J. *Biochemistry* **1983**, *22*, 721−726.
(11) Cross, T. A.; Frey, M. H.; Opella, S. J. *J. Am. Chem. Soc.* **1983**, *105*, 7471−7473.
(12) Tan, W. M.; Jelinek, R.; Opella, S. J.; Malik, P.; Terry, T. D.; Perham, R. N. *J. Mol. Biol.* **1999**, *286*, 787−796.

10.1021/ja005659j CCC: $20.00
Published on Web 01/13/2001

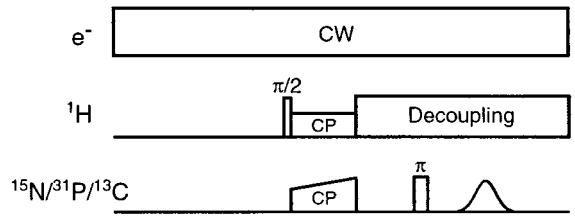

Figure 1. DNP CP pulse sequence. Continuous-wave microwave irradiation (15 mW output power from Gunn Diode, 140 GHz) polarizes ^{1}H's and the enhanced polarization is transferred to ^{15}N, ^{31}P, or ^{13}C (CP) followed by a refocusing pulse to eliminate spectral distortions due to probe ring-down. The spectra with no DNP were obtained with the microwave source off. The DNP enhancement is given as, $\epsilon = [$(signal with DNP)/(signal without DNP) $- 1]$. Typical acquisition parameters are 75 kHz field-strength for the ^{1}H (211 MHz) $\pi/2$ pulse and decoupling, and 40 kHz for CP (3 ms) and the refocusing pulse. Delays of 250 and 60 μs were used before the refocusing pulse for ^{15}N and ^{31}P, respectively. The spectrometer and probe consist of custom-built SSNMR equipment modified to accommodate microwave irradiation and low temperatures, with a minimum attainable temperature of $\sim$10$-$15 K.

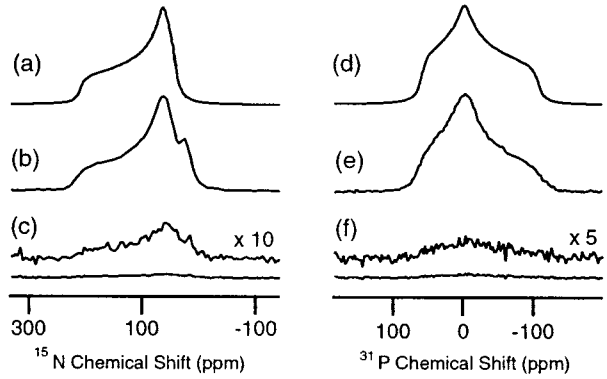

Figure 2. DNP experiments on U-^{15}N-Y21M bacteriophage at 13 K. (a) Simulation of amide powder pattern with $\Omega = 168$ ppm and $\kappa = -0.75$ ($\delta = 105$ ppm, $\eta = 0.2$) and 300 Hz line-broadening. (b$-$c) ^{15}N CP with (b) and without (c) DNP, both with 8 scans and 20 s recycle delay (^{1}H $T_1 = 15$ s). (d) Simulation of phosphodiester powder pattern with $\Omega = 166$ ppm and $\kappa = 0.26$ ($\delta = -90$ ppm, $\eta = 0.68$) and 1 kHz line-broadening. (e$-$f) ^{31}P CP with (e) and without (f) DNP, both with 192 scans. The signal enhancement is $\sim$26 for both experiments. The sample consisted of 60 μL of H_2O/glycerol (50/50) containing Y21M fd bacteriophage (40 mg/ml) and 30 mM 4-amino TEMPO. The Y21M fd phage was prepared following standard procedures.[12]

throughout a large macromolecular assembly. The fd bacteriophage, with a cross-sectional diameter of 65 Å, is a representative system as many proteins have similar or smaller diameter dimensions (for example, the 35 kDa bovine carboxypeptidase A is 50 × 42 × 38 Å[13]).

Bacteriorhodopsin (bR), a light-driven transmembrane proton pump, has been extensively studied by SSNMR spectroscopy.[14−16] Traditional SSNMR studies on purple membrane (75 wt % bR, 25 wt % lipid) require extensive signal averaging. This low sensitivity arises from the large effective size of bR with its accompanying lipids (MW $\approx$ 35 kDa) and the experimental detection of low-γ nuclei (^{13}C, ^{15}N). Such low sensitivity is

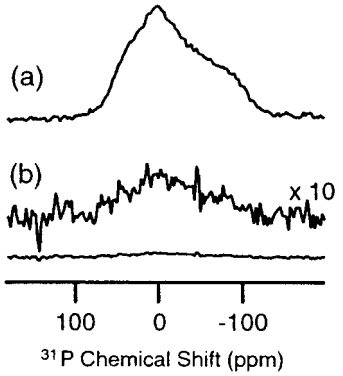

Figure 3. DNP experiment on purple membrane (PM). (a) DNP-enhanced ^{31}P spectrum of the PM phospholipids with 32 scans and a recycle delay of 20 s (^{1}H $T_1 = 15$ s) at 14 K. (b) ^{31}P CP experiment with no DNP and 144 scans. The DNP enhancement is 25. PM in 0.1 M NaCl/H_2O, pH 10, was centrifuged at 95800*g* for 45 min and the pellet mixed in a 40:60 ratio with glycerol doped with 80 mM 4-amino TEMPO. PM was isolated following standard procedures.[17]

common for large membrane proteins that require a hydrated lipid environment for structural integrity.

Figure 3 shows a DNP signal enhancement of $\sim$25 at 14 K from purple membrane (PM) mixed with glycerol/TEMPO. Similarly to the fd bacteriophage, a ^{1}H T_1 of 15 s is observed due to numerous methyl groups in the protein (29 Ala, 15 Ile, 36 Leu, 9 Met, 18 Thr, and 21 Val residues among the 248 amino acids[18]) and in the PM lipids which contain diphytyl chains. In this experiment, the ^{31}P signal from the phospholipid headgroups is detected, however the enhanced polarization should equally propagate to the protein through ^{1}H spin diffusion.

The thermal mixing polarization rate is typically longer than the nuclear relaxation rate. Therefore, the experimentally observed polarization time constant is determined by T_1 and maximal DNP signal enhancements are expected for samples with long T_1's ($>$50$-$100 s). We have shown that significant DNP enhancements can still be obtained for two important classes of biological systems with relatively short relaxation times. The fd bacteriophage and purple membrane both have ^{1}H T_1's in TEMPO/water/glycerol of 15 s. Nevertheless, signal enhancements of about 25 have been measured with only a few milliwatts of microwave power applied to the sample. Higher enhancements are expected with our custom-designed gyrotron (5$-$20 W output power) or samples with deuterated methyl groups. The sensitivity gain derived from DNP will drastically shorten the experimental time for MAS experiments on randomly oriented samples and static experiments on oriented systems. Finally, with studies of the ^{15}N and ^{31}P spectra of the fd bacteriophage, we have experimentally confirmed that polarization diffuses to the inside of the capsid, which is not in direct contact with the bulk solvent. This demonstrates that ^{1}H spin diffusion provides an efficient mechanism for dispersing enhanced polarization throughout a large macromolecule.

Acknowledgment. This research was supported by the National Institutes of Health under Grants GM-38352, GM-36810, GM-24266, RR-00995, and RR-09731. N.S.A is supported by a National Institutes of Health postdoctoral fellowship Grant 1 F32 NS10964-01. We thank R. Perham for the Y21M mutant bacteriophage.

JA005659J

(13) Creighton, T. E. *Protein Structures and Molecular Properties*; W. H. Freeman and Company: New York, 1984.
(14) Hu, J. G.; Sun, B. Q.; Bizounok, M.; Hatcher, M. E.; Lansing, J. C.; Raap, J.; Verdegem, P. J. E.; Lugtenburg, J.; Griffin, R. G.; Herzfeld, J. *Biochemistry* **1998**, *37*, 8088−8096.
(15) Petkova, A. T.; Hu, J. G. G.; Bizounok, M.; Simpson, M.; Griffin, R. G.; Herzfeld, J. *Biochemistry* **1999**, *38*, 1562−1572.
(16) Luecke, H.; Schobert, B.; Richter, H. T.; Cartailler, J. P.; Lanyi, J. K. *Science* **1999**, *286*, 255−260.
(17) Oesterhelt, D.; Stoechenius, W. *Methods Enzymol.* **1974**, *31*, 667−678.
(18) Khorana, H. G.; Gerber, G. E.; Herlihy, W. C.; Gray, C. P.; Anderegg, R. J.; Nibei, K.; Biemann, K. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 5046−5050.