Journal of Magnetic Resonance 204 (2010) 303–313

Contents lists available at ScienceDirect

Journal of Magnetic Resonance

journal homepage: www.elsevier.com/locate/jmr

Low-temperature dynamic nuclear polarization at 9.4 T with a 30 mW microwave source

Kent R. Thurber, Wai-Ming Yau, Robert Tycko *

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0520, USA

ARTICLE INFO

Article history:
Received 21 January 2010
Revised 11 March 2010
Available online 23 March 2010

Keywords:
Solid state nuclear magnetic resonance
Sensitivity enhancement
Hyperpolarization
Nitroxide
TEMPO
Spin–lattice relaxation
Paramagnetic relaxation

ABSTRACT

Dynamic nuclear polarization (DNP) can provide large signal enhancements in nuclear magnetic resonance (NMR) by transfer of polarization from electron spins to nuclear spins. We discuss several aspects of DNP experiments at 9.4 T (400 MHz resonant frequency for ^{1}H, 264 GHz for electron spins in organic radicals) in the 7–80 K temperature range, using a 30 mW, frequency-tunable microwave source and a quasi-optical microwave bridge for polarization control and low-loss microwave transmission. In experiments on frozen glycerol/water doped with nitroxide radicals, DNP signal enhancements up to a factor of 80 are observed (relative to ^{1}H NMR signals with thermal equilibrium spin polarization). The largest sensitivity enhancements are observed with a new triradical dopant, DOTOPA-TEMPO. Field modulation with a 10 G root-mean-squared amplitude during DNP increases the nuclear spin polarizations by up to 135%. Dependencies of ^{1}H NMR signal amplitudes, nuclear spin relaxation times, and DNP build-up times on the dopant and its concentration, temperature, microwave power, and modulation frequency are reported and discussed. The benefits of low-temperature DNP can be dramatic: the ^{1}H spin polarization is increased approximately 1000-fold at 7 K with DNP, relative to thermal polarization at 80 K.

Published by Elsevier Inc.

1. Introduction

Dynamic nuclear polarization (DNP) is a powerful method to increase nuclear spin polarizations by transferring polarization from unpaired electron spins. Because the electron spin energy splitting is 660 times greater than that of ^{1}H nuclei, ^{1}H nuclear spin polarizations can be increased by more than a factor of 100. Similar enhancements of nuclear magnetic resonance (NMR) signals are then observed. Since the time required for an NMR experiment limited by random noise depends on the square of the signal-to-noise, a signal increase by a factor of 100 reduces the experiment time by a factor of 10,000, all other things being equal. This time reduction enables experiments that would be impractical otherwise.

DNP was predicted in 1953 by Overhauser [1], and seen experimentally in the same year by Carver and Slichter [2]. Since then, many techniques have been discovered for transferring polarization from electron spins to nuclear spins. For examples, see recent DNP reviews [3,4]. In this article, we focus on the relatively simple technique of DNP driven by continuous microwave irradiation of electron paramagnetic resonance (EPR) lines. For applications in solid state NMR of organic and biochemical systems, related experiments were performed in the 1980s and 1990s by Wind et al. [5], Singel et al. [6], and Afeworki et al. [7] at magnetic fields of 1.4 T (40 GHz microwave frequency). DNP experiments at higher fields have been pioneered by Griffin and coworkers, using gyrotron microwave sources capable of providing many watts of microwave power at frequencies of 140 GHz and above [8–11]. High-frequency diode-multiplier-based sources have also become commercially available, physically smaller and less expensive than a gyrotron, with power outputs in the 10–100 mW range. Such sources have also been used for DNP at 140 GHz [12–16].

DNP mechanisms generally become weaker at higher fields [3], suggesting that high-field DNP experiments might not be successful. However, Griffin and coworkers have shown that large solid state NMR signal enhancements are readily obtained with the aid of biradical dopants that improve DNP efficiencies by providing electron spins in coupled pairs [17–19]. Coupled electron spins are crucial for several DNP mechanisms. For the cross effect, an inhomogeneously broadened EPR line is required, with two electrons whose resonance frequencies are separated by the NMR frequency [18,20,21]. This allows an energy-conserving three-spin process in which the two electrons exchange spin states, with the energy difference between them provided by a simultaneous nuclear spin flip (which tends to polarize nuclear spins when the two electrons have different initial polarizations). For a homogeneously broadened EPR line, with a linewidth greater than the NMR frequency, the DNP process is described as thermal mixing

* Corresponding author. Address: National Institutes of Health, Building 5, Room 112, Bethesda, MD 20892-0520, USA. Fax: +1 301 496 0825.
E-mail address: robertty@mail.nih.gov (R. Tycko).

1090-7807/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.jmr.2010.03.016

[5]. Off-center irradiation of the EPR line creates a polarization of the electron dipolar system, which can then be transferred to the nuclei. Recent developments in microwave sources and biradical dopants has led to renewed interest in high-field DNP for NMR spectroscopy [22–30].

In this paper, we report the results of DNP experiments at 9.4 T using a compact and relatively inexpensive (but relatively low-power) solid state microwave source, in which we observe ^{1}H NMR signals from frozen glycerol/water solutions that are doped with several nitroxide radical compounds. Glycerol/water solutions are of particular interest because of their relevance to our studies of proteins and protein complexes in frozen solutions [31–37]. We present data regarding the dependencies of ^{1}H NMR signals, nuclear spin relaxation times, and DNP build-up times on dopant, dopant concentration, temperature (7–80 K range), and microwave power. We introduce a new triradical dopant, DOTOPA-TEMPO, that produces the largest sensitivity enhancements in our experiments. We also show that DNP enhancements can be increased substantially by application of magnetic field modulation during microwave irradiation, and we discuss possible mechanisms for this effect. The primary conclusion of this work is that quite large enhancements of NMR sensitivity can be achieved in high fields with relatively low microwave powers, provided that low temperatures are also employed. Relative to signals at 80 K with thermal equilibrium nuclear spin polarization and taking into account the temperature dependence of the DNP build-up time, the sensitivity of ^{1}H NMR measurements is increased by factors greater than 10, 50, 180, and 400 at 80 K, 35 K, 16 K, and 7 K, respectively, in our experiments with 30 mM DOTOPA-TEMPO as the dopant.

2. Materials and methods

2.1. Nitroxide dopants

4-Amino-TEMPO and 4-hydroxy-TEMPO were used as purchased from Sigma–Aldrich. The biradical TOTAPOL and the triradical DOTOPA-TEMPO (4-[N,N-di-(2-hydroxy-3-(TEMPO-4′-oxy)-propyl)]-amino-TEMPO) were synthesised based on the procedure given in Song et al. for TOTAPOL [17]. Chemical structures are shown in Fig. 1. The synthesis was modified by reacting 4-amino-TEMPO and 4-(2,3-epoxypropoxy)-TEMPO in 1:1.7 ratio, rather than a 1:1 ratio. Both the biradical and triradical were purified from the same synthesis by chromatography. ESI TOF mass spectrometry indicated a mass of 628.49 Da for the triradical, in good agreement with the proposed structure ($C_{33}H_{63}N_4O_7$, theoretical average mass = 627.87 Da). For TOTAPOL ($C_{21}H_{41}N_3O_4$, theoretical average mass = 399.57 Da), the measured mass was 400.32 Da.

Differences in the electron–electron interactions for the biradical and the triradical are clearly seen in the low-field (10 GHz) EPR spectra for 4-hydroxy-TEMPO, TOTAPOL, and DOTOPA-TEMPO shown in Fig. 1. The low-field EPR spectra in solution are dominated by hyperfine interactions with ^{14}N nuclei. 4-hydroxy-TEMPO shows the three lines corresponding to the three spin states of a single ^{14}N nucleus. If electrons exchange among the nitroxide sites within a single molecule, the EPR lines of the biradical and triradical reflect an average over the multiple ^{14}N sites. In the fast-exchange limit, this results in five EPR lines for the biradical (total z-component of ^{14}N spin from −2 to +2) and seven EPR lines for the triradical (total z-component of ^{14}N spin from −3 to +3) [38,39]. Although the EPR spectrum of the triradical is not fully resolved, seven peaks in the derivative spectrum are seen (Fig. 1c). Strong features in the derivative spectrum at the edges and in the center are characteristic of exchange-broadened spectra [18,38,40], as EPR lines that correspond to ^{14}N spin states that are the same on all nitroxide moieties (*i.e.*, the $|+1,+1,+1\rangle$, $|0,0,0\rangle$, and $|-1,-1,-1\rangle$ nuclear spin states) remain sharp in the presence of exchange. These lines occur at the same positions as the hyperfine-split lines of 4-hydroxy-TEMPO (Fig. 1a). Similarly, the EPR spectrum of TOTAPOL shows three sharp lines of equal intensity and broad, unresolved features between these lines (Fig. 1b). This is consistent with narrow EPR lines for the $|+1,+1\rangle$, $|0,0\rangle$, and $|-1,-1\rangle$ ^{14}N spin states and broad lines for other ^{14}N spin states [18].

Nitroxide concentrations were verified by titration with ascorbic acid [41], monitored by the UV–visible absorption spectrum. The UV–visible spectrum has a strong absorbance below 300 nm and a weak absorbance at longer wavelength (peaked at 435 nm for DOTOPA-TEMPO) that produces a yellow-orange color, both in the solid form and in solution. Reduction with ascorbic acid attenuates the 435 nm absorption (see Fig. S1 of Supplementary Material). Based on titration of the 435 nm absorption, our DOTOPA-TEMPO had 109 ± 15% of the expected radical concentration (measured at 20 mM in 25/75 mol% glycerol/water). The titration procedure was validated on commercial 4-hydroxy-TEMPO (75 mM in glycerol/water). For TOTAPOL, we measured 85 ± 15%

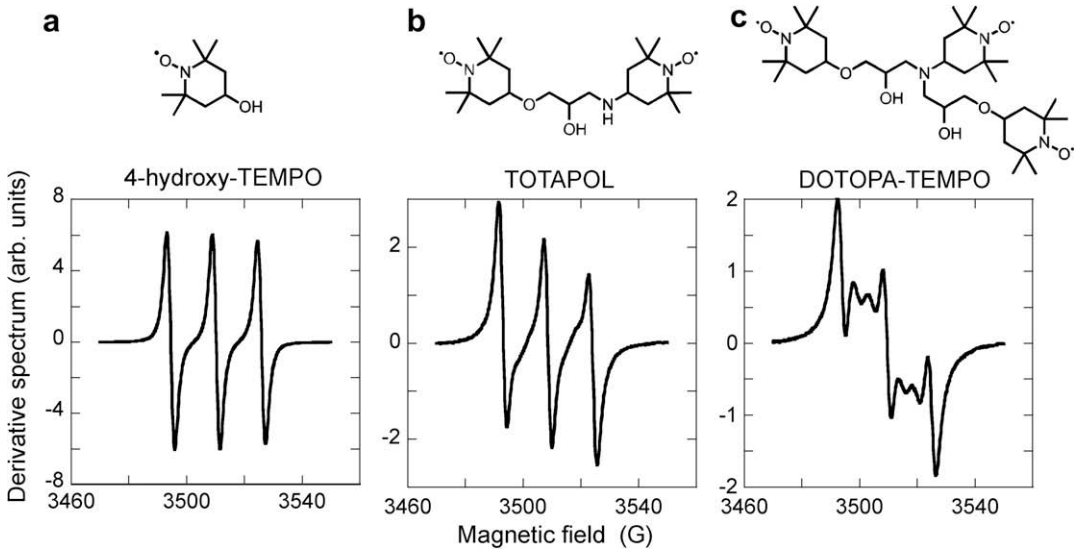

Fig. 1. X-band EPR spectra and chemical structures of 4-hydroxy-TEMPO (a), TOTAPOL (b), and DOTOPA-TEMPO (c). All samples are 0.5 mM solutions in 95/5 vol.% ethanol/water at room temperature.