Filterable free radical polarizing agents for dissolution DNP-NMR spectroscopy

Lloyd Lumata[1], Matthew Merritt[1], Craig Malloy[1], A. Dean Sherry[1,2], Johan van Tol[3], Likai Song[3], and Zoltan Kovacs[1]

[1]*Advanced Imaging Research Center, UT Southwestern Medical Center, Dallas, TX, United States,* [2]*Department of Chemistry, University of Texas at Dallas, Richardson, TX, United States,* [3]*National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL, United States*

Introduction

Dynamic nuclear polarization (DNP) amplifies the NMR signals by transferring the high electron spin polarization to the nuclear spins via microwave irradiation at low temperature and high magnetic field. The invention of the dissolution method[1] in DNP in 2003 has allowed production of hyperpolarized liquids with several thousand-fold enhancements of NMR signals, thus extending the application of this technique to chemistry and biomedical research. The ESR properties of the free radical polarizing agents, namely the ESR linewidth *D* and the electron spin-lattice relaxation time T_{1e}, are important factors in attaining maximum nuclear polarization in DNP. The most commonly used free radical polarizing agents are the carbon-centered trityl OX063 and the nitroxide-based TEMPO. In this work, we present three other free radicals that were proven to be efficient polarizing agents: BDPA, galvinoxyl, and DPPH (Figure 1). We have achieved several thousand-fold ^{13}C and ^{15}N NMR signal enhancements with these radicals, and furthermore, we also demonstrated that these hydrophobic free radicals could be easily removed from the dissolution liquid by a simple filtration process.

Experimental Methods

The free radicals BDPA, DPPH, and galvinoxyl were obtained from commercial sources (Sigma-Aldrich, St. Louis, MO). The DNP experiments were performed in the HyperSense commercial polarizer at 3.35 T and 1.4 K as described.[1,2] The concentrations of these free radicals were optimized for ^{13}C DNP in appropriate glassing matrices (40 mM BDPA in sulfolane-based glassing matrix, 40 mM galvinoxyl in ethyl acetate:DMSO, 20 mM-40 mM DPPH in sulfolane).

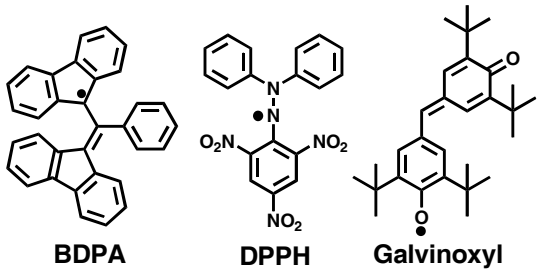

Figure 1 Structures of the free radical polarizing agents discussed in this work.

Results and Discussion

W-band ESR measurements revealed that the base-to-base ESR linewidth *D* of BDPA was 62 MHz, slightly narrower than that of trityl OX063 (*D*=115 MHz). On the other hand, the ESR *D* of galvinoxyl and DPPH are approximately 250 MHz and 290 MHz, respectively, which are narrower than 4-oxo-TEMPO linewidth (*D*=465 MHz). Fig. 2 shows the ^{13}C microwave DNP spectra for samples doped with the radical (BDPA, DPPH, and galvinoxyl). The availability of a wide variety of free radicals for dissolution DNP is important for two main reasons. First, narrow ESR *D* free radicals such as those seen for BDPA[2] and trityl OX063 are better polarizing agents for DNP via thermal mixing of low-γ nuclei such as ^{13}C and ^{15}N because the electron dipolar and nuclear Zeeman energies are comparable. On the other hand, high-γ nuclei such as protons are polarized more efficiently by free radicals with larger linewidths such as TEMPO and are therefore useful in ^{1}H-^{13}C cross polarization DNP experiments.[3] Second, some free radicals work better with certain substrates. For example, trityl OX063 does not work for the DNP of silver complexes whereas BDPA produced excellent $^{107, 109}Ag$ NMR signal enhancements.[4] With regards to the liquid-state NMR signal enhancements ε after dissolution, BDPA was able to enhance [1-^{13}C]pyruvic acid signal by 12,000-fold at room temperature in a 9.4 T magnet, comparable to the results obtained with trityl OX063.[2] DPPH and galvinoxyl produced 5,000-7,000-fold NMR signal enhancement of [^{15}N]choline. Other substrates that could be polarized by these free radicals include [^{13}C]urea, [^{13}C]maleic acid, [^{15}N]pyridine, ^{89}Y- and $^{107,109}Ag$ complexes, among others. After dissolution with water, these hydrophobic free radicals precipitate from an aqueous dissolution liquid making them easy to be removed by simple filtration using a 0.2-micron syringe filter. UV-Vis measurements confirmed the absence of these free radicals in filtered aqueous dissolution liquids.

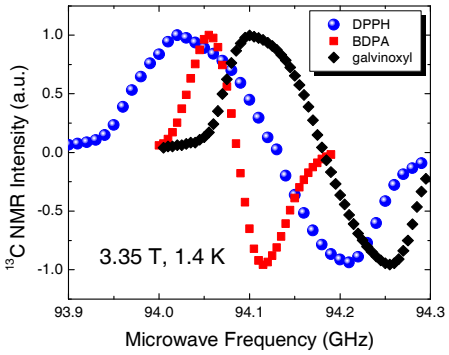

Figure 2 ^{13}C microwave DNP spectra of ^{13}C-ethyl acetate (●), ^{13}C-pyruvic acid (■), and ^{13}C-ethyl acetate (◆) samples doped with DPPH, BDPA, and galvinoxyl, respectively.

Conclusions

We have shown that the stable free radicals BDPA, DPPH, and galvinoxyl can be added to the list of polarizing agents for dissolution DNP. Large liquid-state NMR signal enhancements were achieved for biologically-important labeled substrates such as [1-^{13}C]pyruvic acid and [^{15}N] choline after dissolution DNP. Furthermore, these free radicals can be extracted from aqueous hyperpolarized dissolution liquids, an important consideration for *in vivo* NMR/MRI experiments, using a syringe filter.

References

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