Hyperpolarization by DNP is Applicable to ^{13}C Isotopomer Analysis

M. Merritt[1], D. Blazina[2], C. Malloy[1]
[1]Advanced Imaging Research Center, UTSW Medical Center, Dallas, TX, United States, [2]Oxford Instruments Molecular Biotools, Ltd., Tubney Woods, United Kingdom

Introduction

Magnetic resonance experiments are uniformly plagued by low sensitivity. Despite advances in probe sensitivity and increasing magnetic field strengths, the detection limits of MR remain orders of magnitude less than of more sensitive techniques like mass spectroscopy. Dynamic nuclear polarization (DNP) transfers magnetization from free radical electrons added to a sample resulting in a massive increase in the polarization of the nuclear species. We have studied samples of uniformly ^{13}C labeled acetate and glutamate using DNP to assess the suitability of the technology for isotopomer analysis to measure flux in tissue extracts, tissues, and whole animals.

Methods

Samples of uniformly ^{13}C labeled acetate and glutamate were purchased from Cambridge Isotope Laboratories. A stock solution of 15mM trityl radical in 1:1 DMSO/H2O was used as the solvent for the samples. The DMSO serves as a glassing solvent; crystallization in the sample prevents formation of the fully dipolar coupled electron bath necessary for hyperpolarization by the thermal effect. For the acetate sample, 10ul of a mixture of a 1:1:1 mixture of [1-^{13}C], [2-^{13}C], and [1,2-^{13}C] acetate was used. The final concentration of the acetate was .05mg/ml or 1.16mM. The [U-^{13}C] glutamate sample was run at a final concentration of .4mM using the same DMSO/H_2O mixture. The experiments were carried out at the Oxford Instruments Molecular Biotools site at Tubney Woods using a homebuilt system operating at 3.35Tesla. Following hyperpolarization, the sample was transferred by heated tube to a 9.4T Varian Unity+ high resolution NMR system. A 5mm broadband probe was used for the ^{13}C spectroscopy. The probe temperature was held at 50C to match the temperature of the incoming sample and therefore optimize the ^{13}C lineshape.

Results and Discussion

Figure 1 illustrates the ^{13}C spectrum of a sample of [U-^{13}C] glutamate obtained after a single scan following hyperpolarization. In order to obtain maximum sensitivity, the decoupling was gated on during acquisition only, otherwise the NOE effect will reduce the observed signal. Despite only being able to shim on a standard sample prior to the experiment, the shimming was good enough to monitor the multiplets caused by the uniform ^{13}C labeling. Since the relative populations of spin up and spin down nuclei determine the asymmetry of the j-coupled peaks, it is possible to estimate the relative polarization by determining the areas of each portion of the doublet. In this case the polarization was estimated to be ~12%.

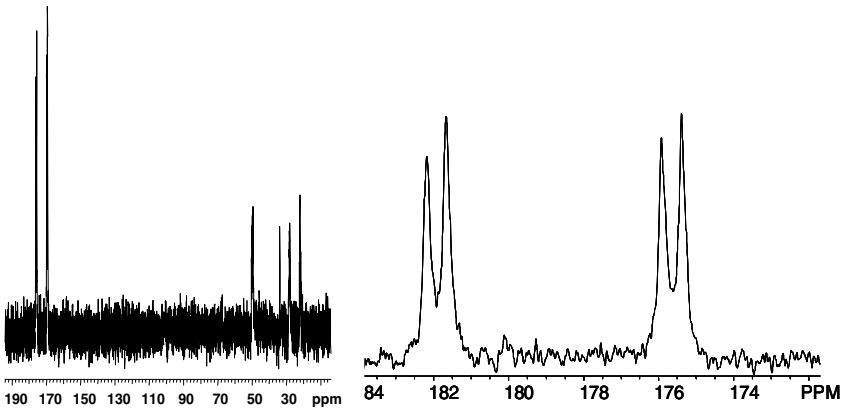

Figure 1. (left) ^{13}C spectrum of uniformly labeled glutamate obtained after hyperpolarization. Note that the shorter T_1's of the aliphatic carbons cause a decreased intensity relative to the carbonyl's in the single pulse spectrum. (Right) Expansion of the carbonyl region, illustrating the asymmetry of the doublets.

Conclusion

DNP has been shown to be applicable for polarization enhancement in uniformly labeled glutamate. To take full advantage of this new methodology, large volume probes that have optimal shimming characteristics would generate the best spectra. Higher field magnets would still provide better resolution, though the polarization would far exceed thermal levels achievable by the magnet. Sensitivity enhancements of this level should allow detection of a variety of Kreb's cycle intermediates previously unattainable by NMR.