In vivo Hyperpolarized ^{13}C MRS using DPPH as polarizing agent

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Target audience: Scientists who have an interest in *in vivo* hyperpolarized MR applications

Introduction: The preparation of hyperpolarized solutions using dissolution DNP necessitates the use of stable free radicals as polarizing agents. It has been recently demonstrated in an *in vitro* study that 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is an efficient alternative radical for DNP applications[1]. The hydrophobic nature of this compound results in its precipitation upon dissolution in aqueous solutions and it can be readily filtered to prepare radical-free hyperpolarized solutions[1]. Hyperpolarized pyruvate and acetate have been shown to be particularly interesting metabolic precursors for *in vivo* DNP applications[2-5]. To obtain large ^{13}C polarization in concentrated solutions, it is often required to use of highly concentrated acidic preparations[6]. It is thus important to incorporate a radical that is relatively stable in acidic media. Although DPPH undergoes quenching in acidic environment, the relatively slow reactions can be visualized by a color change from purple to yellow and thus monitored during sample preparation. The aim of the present study was to formulate a protocol based on rapid sample freezing in order to hyperpolarize pyruvic acid (PA) and acetic acid (AcOH) using DPPH and to demonstrate that it can be used to perform *in vivo* hyperpolarized experiments in conjunction with an automated filtration and injection procedure.

Method: 100 µL solution containing 50mM DPPH in sulfolane was rapidly mixed with 100 µL of 3.6 M [1-^{13}C]PA or 4.4 M [1-^{13}C]AcOH solutions in deuterated glycerol. The mixture was immediately poured into a sample cup and readily freezed in liquid nitrogen to minimize quenching reactions between the acidic compound and the radical. A bead, 16 uL, of 10 M NaOH was added. The sample was polarized in a 7 T custom-designed DNP polarizer[7] (196.75 GHz /1.00 ± 0.05 K). Once reaching the maximal polarization, the sample was rapidly dissolved with deuterated phosphate buffer (~pH 7.5) and transferred into a separator/infusion pump placed inside a 9.4 T/31 cm actively shielded MR scanner (Varian/Magnex)[8]. A mechanical filter was mounted at the outlet of the infusion pump to strain the radical from the infusate solution (Fig. 1). Polarization levels were determined after sample filtration by comparing the hyperpolarized NMR signal of the solution to its thermal counterpart. *In vivo* ^{13}C MRS measurements were acquired in in the brain of C57BL/6j mice (<23> g) following a series of 30° BIR4 pulses every 2 s using a home-built ^{1}H quadrature ^{13}C single loop coil that was placed on top of the mouse head.

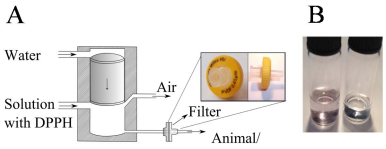

Figure 1: (A) Sketch of the separator/infusion pump used in this study. The inlet of the pump is connected to the dissolution transfer line and outlet is connected through a filter to femoral vein catheter. (B) Unfiltered (left) and filtered (right) dissolution liquids of acetic acid samples doped with DPPH.

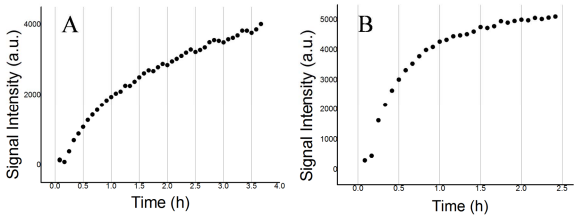

Figure 2: ^{13}C build-up curves measured when polarizing PA (A) and AcOH (B) with DPPH as a polrizing agent at 7T.

Results: ^{13}C DNP polarization build up curves of [1-^{13}C]PA (A) and [1-^{13}C]AcOH samples doped with 25 mM DPPH are shown in Figure 2. Maximal PA polarization was achieved after 4 hr of microwave irradiation and after 2 hr in the case of AcOH (7%). The sum of dynamic spectra acquired in the mouse brain following the infusion of 400 uL hyperpolarized pyruvate solution prepared with DPPH radical is shown in Figure 3A. The hyperpolarized metabolites [1-^{13}C]lactate and ^{13}C-bicarbonate were readily detected and we deduced pyruvate-to-lactate and bicarbonate-to-lactate ratios of 1.72±0.055 and 0.1±0.020 respectively. These ratios characterizing PDH and LDH enzymatic activities were calculated from the signal integrals deduced from fits obtained with the AMARES protocol in JMRUI.

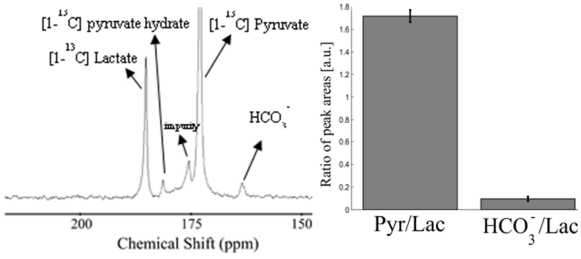

Figure 3: Hyperpolarized [1-^{13}C]Pyr and its metabolites measured in mouse brain (A) metabolite ratio compared to lactate (B).

Discussion: The results show that it is feasible to use DPPH as polarizing agent to enhance the ^{13}C polarization of acidic preparations. It requires the use of a protocol including rapid freezing of the preparations. A completely automated protocol consisting in sample dissolution, pH balancing, filtration and *in vivo* injection of radical-free solutions was designed and the delay between dissolution and injection was fixed to 4 s. Although the *in vivo* signal-to-noise ratio was sufficient to observe metabolic products of [1-^{13}C]pyruvate, the optimal radical concentration for polarization at 7 T remains to be determined to improve the ^{13}C polarization level and hence the sensitivity of the experiments.

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