Using pyruvic acid as a solvent for dynamic nuclear polarization sample preparation

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Introduction: Dynamic nuclear polarization (DNP) and rapid dissolution methods have been utilized to investigate enzyme mediated reactions *in vivo* (1-2). One of the key requirements for obtaining high degree of polarization for organic substrates is that the DNP samples become amorphous solids at low temperature, which enables the nuclear spins of the substrate and the electronic spins of the free radical to achieve uniform distribution. Some molecules, like neat pyruvic acid, are amorphous solids without the addition of solvents, while others bio-molecules of interest such as amino acids require solvents to act as glassing agents. Organic solvents such as glycerol, DMSO and DMA have been used for preparation of DNP samples that were injected into animals. However, the safety profiles of these solvents at concentrations utilized (>0.1 mmol/kg) may not be adequate for human studies and would require careful evaluations. Recently, hyperpolarized ^{13}C labeled pyruvate has been used in a prove-of-concept clinical trial in prostate cancer patients (3). Although neat pyruvic acid is typically used as the sole substrate in the DNP sample matrix, it may be feasible to use pyruvic acid as the solvent and dissolve other substrates of interest in it. For an appropriate substrate, not only would this solvent-substrate mixture be a glass at solid state, but the safety profile of the solvent has already been determined for potential administration in humans. In this work, N-acetyl-[1-^{13}C]methionine (4), a substrate that was previously utilized via DNP-dissolution method (with DMA as the solvent) was used here to demonstrate the feasibility of using pyruvic acid as the solvent for DNP sample preparation.

Methods: Sample preparation: N-acetyl-[1-^{13}C]methionine (Isotec, 99% enriched) was dissolved in either non-labeled pyruvic acid or [1-^{13}C]pryuvic acid (Isotec, 99% enriched) as a 50/50 mixture (w/w) and doped with 15mM OX63 (Oxford Instruments) and 1mM Prohance (Bracco). For each experiment, approximately 50mg of the mixture was polarized then dissolved using a Hypersense DNP polarizer. 5ml of deionized water was used as the dissolution media, and ~3.5ml of NaOH/Tris (80mM/40mM) solution was used in the receiving vessel to neutralize the pyruvic acid. Experiments using only [1-^{13}C]pyruvic acid as the sample (~25 mg) and the same dissolution protocol were also performed. NMR measurements: All experiments were performed using a GE MR750 3T scanner (GE Healthcare) with a dual-tuned ^{1}H/^{13}C T/R rat coil. For each experiment, spectra from ~3ml of the hyperpolarized solution were acquired using a pulse-acquire pulse sequence (5° tip angle, TR=3s, 96 scans). Data were also acquired from each sample at thermal equilibrium using the same sequence (24µl of Omniscan was added to the sample, 90° tip angle, TR=5s, 384 scans) to estimate the polarization that was achieved by DNP.

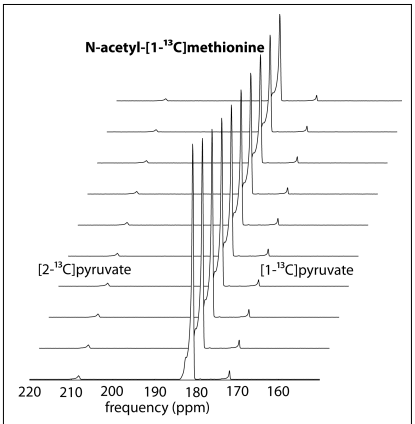

Figure 1. Representative spectra of hyperpolarized N-acetyl-[1-^{13}C]methionine prepared using pyruvic acid as a solvent.

Results: Representative ^{13}C spectra from hyperpolarized N-acetyl-[1-^{13}C]methionine in solution are shown in Fig.1. Resonances from natural abundance [1-^{13}C] and [2-^{13}C]pyruvate from the pyruvic acid solvent were also observed in the spectra. Polarizations achieved for N-acetyl-[1-^{13}C]methionine and [1-^{13}C]pyruvate (from samples using enriched pyruvic acid) are summarized in Fig. 2. More than 2-fold higher polarization level was obtained for N-acetyl-[1-^{13}C]methionine with pyruvic acid as the solvent compared to the preparation in the previous study using DMA as the solvent at similar substrate concentration (>10% vs 4%) (4). The Gd doping used in this study may account for some of the gain in polarization. Similar, yet slightly lower polarization was observed when N-acetyl-[1-^{13}C]methionine was prepared in enriched pyruvic acid as compared to non-labeled pyruvic acid but the enriched mixture shortened the solid-state buildup time constant (2100s vs 950s) (5), and would provide the potential for probing *Acy-1* expression and cellular metabolism *in vivo* in the same study. Lower [1-^{13}C]pyruvate polarization was observed from samples of [1-^{13}C]pyruvic acid and N-acetyl-[1-^{13}C]methionine mixture as compared to [1-^{13}C]pyruvic acid alone.

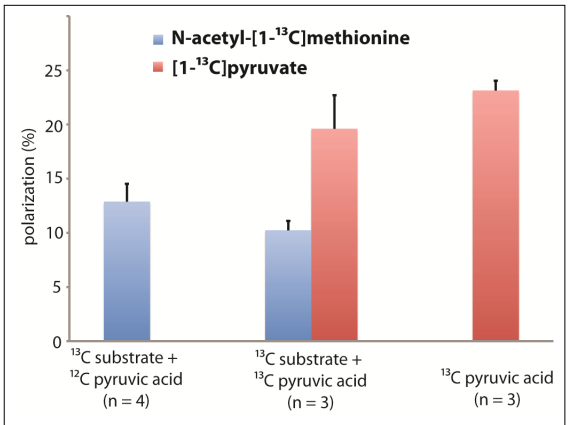

Figure 2. Liquid state polarization of N-acetyl-[1-^{13}C]methionine and [1-^{13}C]pyruvate.

Conclusions: The feasibility of using pyruvic acid as a glassing solvent to prepare samples for DNP studies was demonstrated with N-acetyl-[1-^{13}C]methionine as the substrate, and resulted in considerably higher polarization compared with using DMA as the solvent. Using pyruvic acid as a solvent may provide a favorable safety profile for the hyperpolarized substrate in solution for human studies and an opportunity for probing pyruvate metabolism and other enzyme mediated reactions in the same experiment.

References:

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