Secondary substrate assisted dynamic nuclear polarization

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Introduction: Development of techniques to retain highly polarized spins in solution via DNP has enabled the use of ^{13}C labeled metabolic intermediates such as pyruvate to investigate enzymatic exchange processes *in vivo* with high temporal/spatial resolution (1-2). To achieve high polarization enhancement in reasonable amount of time (~1hr), the technique thus far has been limited to substrates with low molecular weight and high solubility in organic solvent/water. In conditions where DNP is dominated by solid state and thermal mixing effects, the electron and nuclear interaction is anisotropic and there is a distribution in the DNP enhancement factor across the sample. These local spin temperature differences are then equilibrated via nuclear dipolar interaction (spin diffusion) (3). Thus it may be possible to enhance the polarization of a target substrate that has poor DNP properties (low solubility, poor enhancement) with a secondary substrate that demonstrates high DNP enhancement and can be added in high concentration at the given condition, allowing the more concentrated and highly polarized nuclei to assist the polarization of the target nuclei via this mechanism. The goal of this study was to develop and test such a method.

Methods and materials:

Sample preparation: [1-^{13}C] N-acetyl-methionine (target substrate) (Isotec, Miamisburg, OH) and ^{13}C urea (secondary substrate) were prepared in a water/dimethylacetymide mixture (50/50 by vol.) for a final solution of ~1.4M N-acetyl-methionine and ~5.5M urea. Samples with identical formulation but with either non-labeled target substrate or non-labeled secondary substrate were also prepared. All samples contain 15mM OX 63 trityl radical (Oxford Instruments, Abingdon, UK). All DNP experiments were performed with 50ul of sample in solid state and 5ml of 100mM phosphate buffer (7.2pH) for dissolution. All samples were polarized and consequently dissolved with a Hypersense DNP polarizer (Oxford Instruments) at ~1.4K, with 25mW microwave irradiation at the optimal MW freq. for a given sample. The solid state polarization buildup were fitted to the equation: $P(t) = P_{eq}(1-\exp^{(-t/Tbuildup)})$+baseline, where P_{eq} is the equilibrium polarization achieved for the sample and $T_{buildup}$ is the polarization buildup time constant.

MR measurements in solution: All solution state measurements were performed with a GE Signa 3T scanner (GE healthcare, Waukesha, WI) equipped with multinuclear package. A dual-tuned ^{13}C/^{1}H micro-strip rat coil was used (Magvale, Palo Alto CA). Polarization enhancement at 3T was measured by quickly transferring ~3ml of the polarized solution from DNP polarizer into the RF coil in a syringe. NMR signals of the solution at hyperpolarized state (5 degree tip angle, 3s TR, 64 transients to allow estimation of T1) as well as at thermal equilibrium polarized state (solution doped with Magnevist™ (Bayer Healthcare) (8µl/ml), 90 degree tip angle, 10s TR, 128 transients) were measured to calculate the polarization enhancement in solution.

Results: Representative solid-state polarization buildup curves of the three different samples are plotted in Figure 1. $T_{buildup}$ was ~700s for ^{13}C N-acetyl-methionine + ^{13}C urea (red curve); ~900s for non-labeled N-acetyl-methionine + ^{13}C urea (green curve) and ~1800s for ^{13}C N-acetyl-methionine + non-labeled urea (blue curve). Spectra of [1-^{13}C] N-acetyl-methionine + ^{13}C urea sample in solution are shown in Figure 2. [1-^{13}C] N-acetyl-methionine polarization enhancement (as compare to thermal-equilibrium polarization at 3T, room temperature) achieved in solution was 31000 (std. 4800) and 26000 (std 4100) for samples containing ^{13}C urea (n=4) and samples containing non-labeled urea (n=3) respectively. T1s of [1-^{13}C] N-acetyl-methionine in solution were the same for samples containing either ^{13}C urea or non-labeled urea (~28s). ^{13}C urea polarization enhancement achieved in solution was 1200 (std. 400) and 1400 for samples containing [1-^{13}C] N-acetyl-methionine (n=4) and non-labeled N-acetyl-methionine (n=1), respectively.

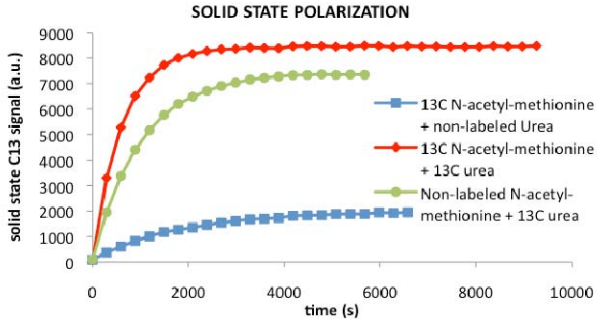

Figure 1. Representative solid-state DNP buildup curves of the three different samples prepared. Plots were normalized to 55mg of sample in the polarizer.

Discussion: Addition of ^{13}C urea allowed much faster DNP buildup for the [1-^{13}C] N-acetyl-methionine sample, while achieving slightly higher enhancement in solution as compared to the sample with non-labeled urea. The decrease in polarization time achieved by addition of ^{13}C urea may be utilized to increase experimental throughput. Also, if the concentration of the radical is reduced in the sample with ^{13}C labeled on both substrates, the polarization time constant may be lengthened, but we may see a bigger increase in polarization for the target substrate (4). The relatively large polarization observed in solid state for ^{13}C urea was not reflected in the low enhancement measured in solution. The low solution polarization for urea is likely not related to T1 decay in solution, as ^{13}C urea T1 was longer than that of ^{13}C N-acetyl-methionine (Figure 2, left).

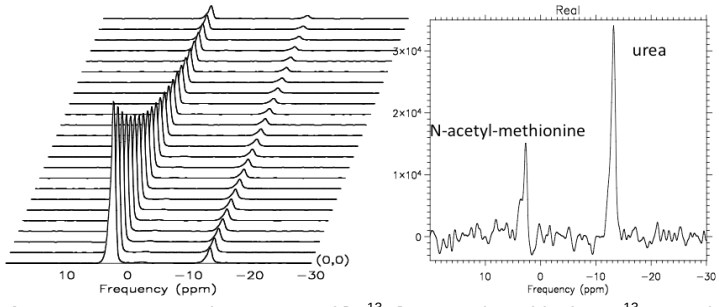

Figure 2. Representative spectra of [1-^{13}C] N-acetyl-methionine + ^{13}C urea in solution while hyperpolarized (left) and at thermal polarization (right). T1s of hyperpolarized [1-^{13}C] N-acetyl-methionine and ^{13}C urea in solution were ~30s and 45s in solution, respectively.

References:

1. Ardenkjaer-Larsen, J.H. et al. PNAS, 2003;100:10158-10163.
2. Golman, K. et al. Proc Natl Acad Sci U S A, 2006; 103:11270-11275.
3. Wind, R.A. et al. Progress in NMR Spectroscopy, 1985; 17: 33-67.
4. Wolber, J. et al. Nuclear Instruments and Methods in Physics Research A, 2004; 526 173–181.