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High-field dissolution dynamic nuclear polarization of [1-^{13}C]pyruvic acid

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[1-^{13}C]pyruvate is the most widely used hyperpolarized metabolic magnetic resonance imaging agent. Using a custom-built 7.0 T polarizer operating at 1.0 K and trityl radical-doped [1-^{13}C]pyruvic acid, unextrapolated solution-state ^{13}C polarization greater than 60% was measured after dissolution and rapid transfer to the spectrometer magnet, demonstrating the signal enhancement attainable with optimized hardware. Slower rates of polarization under these conditions can be largely overcome with higher radical concentrations.

Introduction

Compared to other analytical techniques, nuclear magnetic resonance spectroscopy (NMR) is hampered by low sensitivity, which is due to the low net nuclear spin polarization of several parts per million at equilibrium under typical conditions. In recent years, the advent of dissolution dynamic nuclear polarization (DNP), where a cold, highly polarized solid-state sample is rapidly dissolved in a heated solvent, has offered a way to temporarily overcome to this limitation. Dissolution DNP can enhance the spin polarization of quaternary carbon-13 nuclei in labeled small molecules by greater than 10,000-fold, and this has enabled the rapid imaging of metabolic transformations in live subjects using magnetic resonance methods. The ability to interrogate non-invasively the chemical state of a labeled compound with high sensitivity is perhaps the most compelling application of the technique, and it is in clinical development for early detection of cancer on the basis of its characteristic metabolism.[1] The vast majority of dissolution DNP studies reported to date have used [1-^{13}C]pyruvate, which has the appropriate physical and metabolic characteristics to be very well suited to this application.[2-4]

DNP is effected in a magnetic field with saturating microwave irradiation of a target sample doped with a paramagnetic species, usually a stable free radical, near the electron Larmor frequency in order to transfer the higher spin order of the electrons to surrounding nuclei. For efficient solid-state DNP, the radical must usually be uniformly distributed in the matrix. This is typically achieved by freezing a viscous solution of the radical-containing compound and the nuclear spin-labeled compound to be polarized into a glassy solid. The polarization process proceeds more rapidly with higher nuclear spin concentrations, where their closer proximity facilitates more efficient spin diffusion.[5] Pyruvic acid has a neat concentration of 14.2 M and readily forms a glass when cooled rapidly. A variety of different radicals have been used to polarize [1-^{13}C]pyruvic acid. These include the trityl radicals OX063, AH111501 (an O-methylated analogue of OX063),[1,6] BDPA (pyruvic acid in sulfolane solution),[7] a hydroxy-methylated BDPA analogue soluble in neat pyruvic acid,[8] as well as with free radicals generated *in situ* with UV-irradiation.[9] Frozen [1-^{13}C]pyruvate salt solutions have also been polarized with trityl or nitroxyl radicals in solution[10,11] or nitroxyl radicals immobilized in a solid matrix,[12] although generally not to as high a level as the free acid. An exception is the choline salt, which glasses well in aqueous solution and polarized to 33% in the solid state, outperforming the free acid at 28%.[13] Solid-state polarization levels as high as 70% have been reported in [1-^{13}C]pyruvic acid polarized at 4.6 T and 1.15 K with a trityl and Gd^{3+}-doped sample.[14] In the absence of Gd^{3+} doping, a maximum solid-state polarization of 75% was obtained at 5 T and 0.93 K.[15] However, the solution-state polarization that is routinely obtained using the commercial hyperpolarizer SPINLab^{TM} operating at 5 T and 0.85 K, is about 50%.[16]

Despite the very large gains in sensitivity made possible by dissolution DNP, the relaxation of the polarization to equilibrium generally limits the practical experiment duration

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to about one minute post-dissolution. Thus, often only rapid metabolic transformations can be detected using this technique and less abundant metabolites are signal limited. Pyruvate is normally present in the blood in the range of 0.028 to 0.208 mM,[17] but hyperpolarized pyruvate administration can substantially increase its blood concentration[18] such that its metabolic transformation may potentially differ from normal physiology. Therefore increased polarization levels would allow the use of more physiological dosages as well as the possibility to measure both less abundant metabolites and the products of slower metabolic processes. This provides a strong motivation to achieve the highest possible ^{13}C spin polarization. As polarization increases with higher magnetic field strength and lower temperature, the custom-built dissolution DNP equipment in our laboratory[19] was recently upgraded to operate at 7 T and 1.0 K, and it is capable of polarizing nitroxyl-doped sodium [1-^{13}C]acetate up to 35%, measured after dissolution.[20] DNP of trityl-doped [1-^{13}C]pyruvic acid under these conditions is examined in this study, with an emphasis on the usable ^{13}C polarization obtained after rapid dissolution and transfer to a scanner.

Results and Discussion

Microwave sweep

A microwave sweep to find the optimal frequency for ^{13}C polarization was performed at 3.6 K and the highest solid-state polarization was obtained at the negative maximum at 196.81 GHz (Figure 1), with a zero crossing corresponding to the ESR absorption at approximately 196.774 GHz. The magnetic field was optimized for DNP with nitroxyl radicals; consequently, the limited tuning range of the microwave source only covers part of the positive polarization regime, down to 196.75 GHz, and the positive peak, expected around 196.737 GHz, could not be directly measured.

Polarization buildup rates

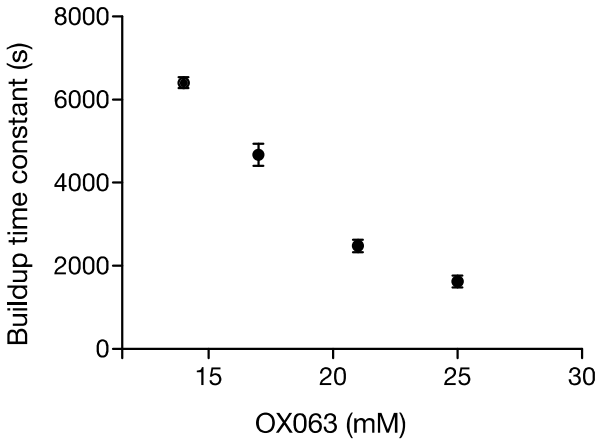

Figure 2. Solid-state ^{13}C polarization buildup rate of [1-^{13}C]pyruvic acid doped with OX063 trityl radical at 7 T and 1.0 K as a function of radical concentration. Error bars indicate standard deviation of three independent experiments (two experiments for 14 mM OX063).

The polarization buildup is markedly slower at 7 T and 1.0 K compared to a HyperSense polarizer operating at 3.35 T and 1.4 K. Here, with 17 mM OX063, the monoexponential buildup time constant was 4670 ± 270 s, as opposed to 838 s for 15 mM in the HyperSense.[21] The slow buildup rate follows the trends described in prior studies[14,15] where increased magnetic field strength or decreased temperature allow a higher maximum polarization but with a longer time required to reach maximum. The buildup rate is inversely related to the radical concentration (Figure 2), ranging from 1620 ± 140 s with 25 mM OX063 to 6400 ± 130 s with 14 mM, following the expected trend seen at lower fields and temperatures with [1-^{13}C]pyruvic acid[14,15] and [^{13}C]urea,[22,23] but with slower rates overall. For applications of dissolution DNP, a long polarization time limits the number of experiments that can be performed, but at high field and low temperature the buildup rates with higher trityl concentrations in the 20- to 25 mM range are practical for routine use.

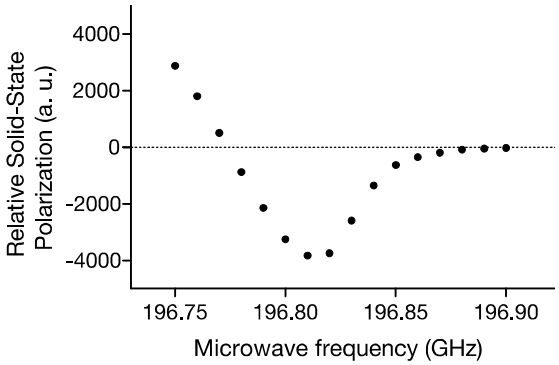

Figure 1. ^{13}C polarization of [1-^{13}C]pyruvic acid doped with 25 mM OX063 trityl radical at 7 T and 1.0 K as a function of microwave frequency. With the magnetic field optimized for DNP with nitroxyl radicals, the positive peak is out of range of the microwave source.

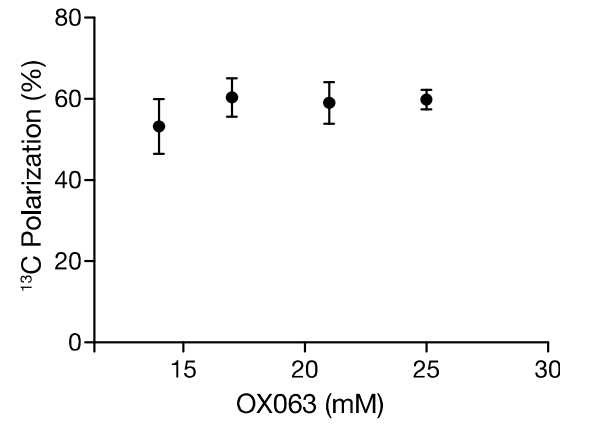

Figure 3. Post-dissolution and transfer ^{13}C polarization of [1-^{13}C]pyruvate polarized with OX063 trityl radical at 7 T and 1.0 K as a function of radical concentration. Error bars indicate standard deviation of three independent experiments (two experiments for 14 mM OX063).

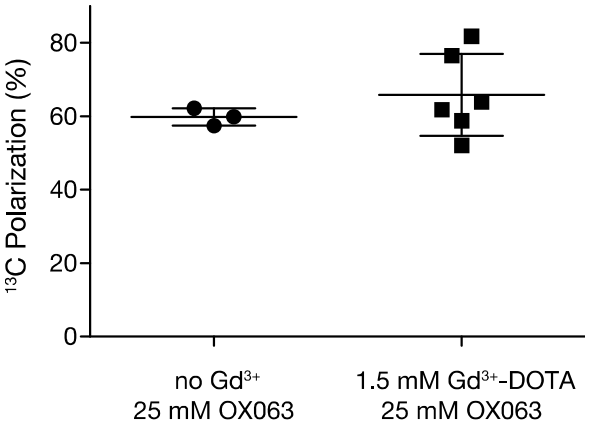

Figure 4. Post-dissolution and transfer ^{13}C polarization of [1-^{13}C]pyruvate polarized with 25 mM OX063 trityl radical at 7 T and 1.0 K in the absence and presence of 1.5 mM gadoteric acid. Error bars indicate standard deviation.

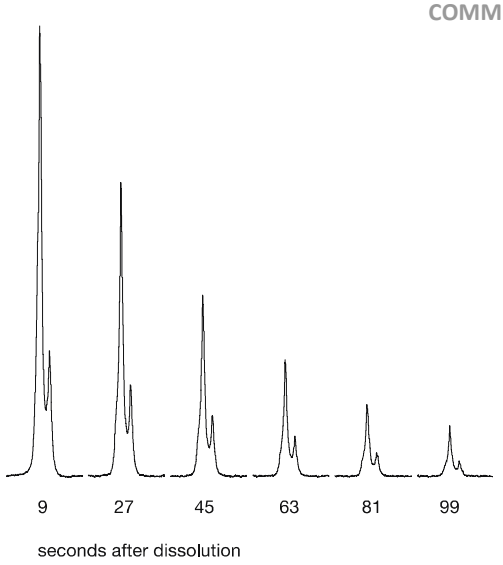

Figure 5. Evolution of [1,2-$^{13}C_2$]pyruvate doublet asymmetry. Doublet of the ketone ^{13}C (at natural abundance) at 207.7 ppm is strongly asymmetric, reflecting the high degree of negative polarization of the enriched carboxylate ^{13}C. Numbers under spectra indicate time in seconds after dissolution. The asymmetry remains approximately constant as the bulk polarization decays.

Solution-state polarization

The pyruvate ^{13}C polarization after dissolution and rapid (3 s) transfer to a 9.4 T spectrometer was high and exceeded previously reported levels. Unlike its effect on the polarization buildup rate, maximum polarization level was not strongly affected by radical concentration (Figure 3). The highest polarization levels, 60.4 ± 4.7%, were observed using 17 mM OX063 (Figure 3), while 21 mM and 25 mM also yielded polarization in the same range. With 14 mM OX063, a slightly lower polarization was measured (53.2 ± 6.8%). The solution-state polarization levels were not significantly different across the tested range of radical concentrations (one-way ANOVA, p=0.41). Since polarization is not adversely affected, higher radical concentrations offer a practical solution by largely overcoming the slower polarization rates seen at higher field and lower temperature.

Effect of Gd^{3+} Doping

Doping with chelated gadolinium(III) can further enhance the maximum polarization level in the trityl-containing pyruvic acid preparations by approximately 50% and some other ^{13}C labeled compound preparation by as much as four-fold[10] in a HyperSense polarizer. This effect has been attributed to Gd^{3+} increasing the longitudinal relaxation rate of the radical electron,[24,25] while only modestly affecting the nuclear spin relaxation. The polarization enhancement was tested with Gd^{3+}-DOTA (1.5 mM in pyruvic acid with 25 mM OX063). On average, a slightly higher level of polarization (65.8 ± 11.2% vs. 59.8 ± 2.4%) was observed with the addition of Gd^{3+}-DOTA (Figure 4), but this difference is not statistically significant (*t*-test, p=0.41). The variability was much higher than in its absence. This may be due to slight variation in the Gd^{3+}-DOTA concentration, which may lead to either non-negligible modifications of the solid-state polarization dynamics, thereby affecting the maximum ^{13}C polarization, or to changes in the Gd^{3+}-induced ^{13}C relaxation during dissolution and transfer to the magnet; note however that the solution-state ^{13}C T_1 relaxation measured at 9.4 T was not found to be significantly different across all samples. In any case, in comparison to the HyperSense polarizer, the polarization enhancement conferred by Gd^{3+} doping is not anticipated to be as large at 7 T and 1.0 K, since the enhancement in solid state is approximately 10% at 4.64 T and 1.15 K.[14]

[1,2-$^{13}C_2$]pyruvate doublet asymmetry

A small signal from the minor [1,2-$^{13}C_2$]pyuvate isotopologue is apparent as a doublet (J_{CC} = 65 Hz) at 207.7 ppm. Assigned to the 2-carbon ketone, the doublet has a marked asymmetry, with a larger downfield peak (Figure 5), reflecting the high degree of negative polarization. Several methods have been proposed to use the information contained in the doublet asymmetry to determine the polarization of the 1-carbon.[26-29] Unlike what has been previously reported with positively polarized [1-^{13}C]pyruvate, where the asymmetry evolves and changes sign over the course of the series of low-flip-angle pulse-acquire experiments, in the current study the doublet asymmetry was observed to remain approximately constant, with the ratio of the downfield and upfield peaks ranging between 5.0 and 3.8. The solution-state ^{13}C NMR measurements are made in a phase separator / infusion pump, which receives a short column of liquid of variable volume from the polarizer, and the proximity of the liquid-air interface to the RF coil results in limited and variable spectral resolution. In order to ensure better resolution of the [1,2-$^{13}C_2$]pyruvate 2-carbon ketone resonance, polarization measurements were also performed after using the infusion pump to transfer the hyperpolarized pyruvate solution into a 10 mm NMR tube equipped with a ^{13}C RF coil. The transfer incurs an additional delay of 6 s, which

with the T_1 in excess of 60 s results in a polarization loss of less than 10% compared to measuring directly in the pump.

Implications for in vivo experiments with hyperpolarized ^{13}C labeled pyruvate

The high level of polarization observed here was measured in the same apparatus used for rapid intravenous infusion in an in vivo experiment. It should be noted that the polarization values reported are directly measured and not extrapolated back to the time of dissolution and therefore reflect the actual usable signal. A higher degree of polarization results in improved sensitivity, which can allow the detection of less abundant metabolite signals, as well as an extension of the time that the signals remain detectable. Alternatively, one can take advantage of the gain in polarization to administer smaller quantities of pyruvate, closer to physiological levels, and obtain the same signal. With further reductions in the cryostat temperature or increases in the static magnetic field, it should be possible to exceed the polarization levels reported here, but this would incur significantly higher costs and extend the already relatively long polarization times. One possible simple improvement is to polarize in the positive mode, since the positive maximum is higher than the negative maximum at 4.6 T;[14] however, there might not be an advantage as the magnitude of negative polarization appears higher at 5 T.[15] With the limited frequency range of the 197 MHz microwave source and the magnetic field optimized for using nitroxyl radicals, the positive polarization peak is just out of range (Fig 1), so the effect of positive polarization on the polarization level was not examined in this study.

Conclusions

Greater than 60% solution-state polarization of [1-^{13}C]pyruvate can be achieved upon dissolution and transfer into an MRI scanner following DNP at 7 T and 1.0 K. Combining polarization at low temperature, at a higher magnetic field strength with an optimal radical, together with the rapid transfer of the dissolved solution to the spectrometer magnet yields increased hyperpolarized signal with the potential to significantly improve sensitivity in metabolic NMR and spectroscopic imaging experiments.

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