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PASADENA Hyperpolarization of Succinic Acid for MRI and NMR Spectroscopy

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One of the major shortcomings of NMR is poor sensitivity, which limits imaging of molecular concentrations *in vivo* to the millimolar level. Both DNP (dynamic nuclear polarization)[1] and PASADENA (parahydrogen and synthesis allow dramatically enhanced nuclear alignment)[2,3] have recently[4–11] been demonstrated to reach spin polarization of order unity on ^{13}C sites with spin-lattice relaxation time T_1 in the range of tens of seconds. This is a signal enhancement by a factor of ~100 000 on currently utilized MRI scanners. Exploring the potential for fast *in vivo* ^{13}C imaging and spectroscopy is a principal motivation in these studies. While DNP was recently successfully applied to several metabolically relevant compounds including 1-^{13}C-pyruvic acid,[9,11] PASADENA at comparable polarization was demonstrated only with the ether 2-hydroxyethyl-propionate, which has no metabolic relevance. Recently, we have demonstrated with NMR and fast MRI the use of disodium 1-^{13}C-acetylenedicarboxylate (ADC) as a precursor to hyperpolarized1-^{13}C-succinate[5] via the molecular addition of parahydrogen. However, that chemical approach suffered from (i) a short spin-lattice relaxation time T_1 of 6 s, (ii) toxicity of the precursor ADC and intermediate maleate, which is relevant if hydrogenation is incomplete, and (iii) polarization of only several percent.[5] The system described here overcomes these shortcomings.

We used 1-^{13}C-fumaric acid-d_2 as the unsaturated PASADENA precursor for the molecular addition of dihydrogen (Figure 1). In order to break the magnetic equivalence and simplify the spin dynamics, the ^{13}C label is confined to only one carboxyl site (C1) in this otherwise symmetric molecule. The choice of an unprotonated carbon maximizes T_1 for the hyperpolarized product species. Deuteration of the precursor at the positions 2 and 3 in the present approach (Figure 1) enables the increase of the crucial product T_1 from 6 s (no deuteration) to 27 s (pH 3 and pH 7). A further increase to 39 s (pH = 3) and 56 s (pH = 7) was observed when the solvent was D_2O. After molecular addition of parahydrogen, an efficient transfer of spin order from the nascent protons to a third spin[2,7,8] requires precise information about the *J*-couplings among the three nuclei of succinate highlighted in red in Figure 1. A strong dependence of the couplings on pH has been reported[12] due to the changing probabilities of the interconverting neutral, anion, and dianion forms. We were motivated to revisit this problem with ^{1}H coupled ^{13}C spectroscopy of natural abundance succinic acid, since spectra needed to extract the couplings and dephasing of the three spins of interest here were not reported. The fits of ^{13}C multiplet patterns of C1 and C2 carbons (Figure 2) provide sufficient spectroscopic information for elucidation of these couplings. We also report in Table 1 the

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distinct $^3J_{HaHc}$ coupling present in the absence of the deuteration of Figure 1. The simulated spectra were calculated by GAMMA,[13] and the fits were optimized by MATLAB using the Levenberg-Marquardt algorithm. The fitting of $^1J_{CH}$ and $^2J_{CH}$ couplings to C2, $2J_{CH}$ and $3J_{CH}$ couplings to C1, and the two $^3J_{HH}$ couplings between protons was performed simultaneously. Distinct dephasing times T_2 for C1 and C2 were allowed in the fitting. We find (Figure 2) that the C1 spectrum of the dicarboxylic succinic acid shows line broadening characteristic of intermediate chemical exchange, precluding accurate extraction of the *J* couplings at a pH near the two pK_a values. As a result of this finding we choose to transfer spin order at pH ≪ pK_a, where the *J*-couplings necessary for PASADENA are best resolved.

Instrumentation for parahydrogen addition and transfer of spin order to ^{13}C in an auxiliary low field magnet has been previously described.[6,8,10] Here we used a modified version in which the antechamber, chemical reactor, sprayer, and all connecting tubing were replaced by acid resistant parts eliminating any possible direct contact of chemical mixture with stainless steel or copper to prevent corrosion. The hydrogenation catalyst, 2.5 mM Rh norbornadiene bisphosphine, was prepared on site using a 5% molar excess of 1,4-bis[(phenyl-3-propanesulfonate)phosphate butane disodium salt (Sigma-Aldrich/Isotec, Miamisburg, OH) relative to bis(norbornadiene)rhodium(I) tetrafluoroborate (Strem Chemicals, Newburyport, MA, 96% purity) in ultrapure water (Millipore Super-Q Plus System). Degassed catalyst solution was mixed with 1-^{13}C-fumaric acid-d_2 (Cambridge Isotope Laboratories, 96% d, 99% 13C) and acidified with sodium phosphate buffer to pH = 2.9 with a 50 mM final buffer concentration. Hydrogenation was conducted as previously described[5,6,8,10] at 62 °C by spraying 1-^{13}C-fumaric acid-d_2 in aqueous catalyst solution into 10 bar of H_2 (97% para) resulting in >95% chemical conversion as shown by ^{13}C solution NMR spectroscopy at 14 T. The ^1H decoupling pulses were applied for 4 s to preserve spin order in the singlet state while the reaction occurs.[7,14] The spin order was transferred from ^1H nuclei to ^{13}C at 1.76 mT in the reactor by the heteronuclear pulse sequence described by Goldman and Jóhannesson[7] using $^2J_{CH}$ =−7.15 Hz, $^3J_{CH}$ = 5.82 Hz, and $^3J_{HH}$ = 7.41 Hz to determine the pulse timing. The resulting hyperpolarized product was automatically delivered to an NMR/MRI scanner and detected at 4.7 T 25 s after production. Figure 3 demonstrates the ^{13}C spectrum of hyperpolarized 1-^{13}C-succinate-d_2 at pH = 2.9 (resonance at ~180 ppm). A reference spectrum from natural abundance ethanol (insert of Figure 3) was used to calibrate the polarization. A T_1 value of 26.6 ± 1 s was measured by both inversion recovery method of 1-^{13}C-succinate-d_2 at Boltzmann polarization and using hyperpolarized material and small angle excitation pulses of ~5°. A ^{13}C hyperpolarization at the output of the reactor of 15–20% was consistently achieved (Figure 3).

The achievement of hyperpolarization of order unity in this metabolically relevant system is encouraging for further development of PASADENA which is unique among hyperpolarization methods in its combination of effectiveness, speed, and the simplicity of the apparatus. Under the present conditions, the signal arises from those product molecules that are formed in 4 s in the low field reactor, as evidenced by the nearly complete loss of hyperpolarization if the low field pulse sequence is delivered off resonance. In principle, the theoretical limit is nearly 100% for ^{13}C hyperpolarization in this system, and it is a promising one for elucidating the interplay of coherent and incoherent spin evolution and chemical reaction, providing a quantitative understanding and optimization of the several steps.

The present strategy of using low pH to minimize relaxation by chemical exchange and partial deuteration of both metabolite and solvent to increase T_1 demonstrates a route to efficient PASADENA hyperpolarization applicable to many carboxylic acids. The ^{13}C label introduced as 1-^{13}C-succinate was shown to be a biomarker, distinguishing a brain tumor, with its compromised blood-brain barrier, from normal tissue.[5] Thus, the location and status of a tumor can be potentially interrogated *in* situ with hyperpolarized succinate. The long T_1 suggests

feasible delivery of hyperpolarization also to cells and tissue which normally take up and metabolize succinate. Information on the first minutes of metabolism is scarce but encouraging. If we multiply the rate of succinate uptake and metabolism per unit volume of rat kidney[16] by the C1 T_1 measured here in H_2O, we obtain 2 mM as a semiquantitative measure of the concentration of hyperpolarized molecules available for detection. The signal from this pool of intracellular molecules will still be enhanced by ~10^4 and thus detectable without signal averaging in diverse situations. The details of their fate may be influenced by the fact that certain enzymatic reactions which transfer hydrogen atoms are often substantially slower when transferring deuterium,[17] but this difference in kinetics for some reactions does not prevent the metabolism of deuterated molecules serving as a fingerprint of the state of cells or tissues. Further work is needed to estimate the time-dependent spectrum of daughter molecules, but the present results and analysis suggest that the signal-to-noise ratio will be adequate to follow the early steps of metabolism *in vivo* with unprecedented spatial and time resolution.

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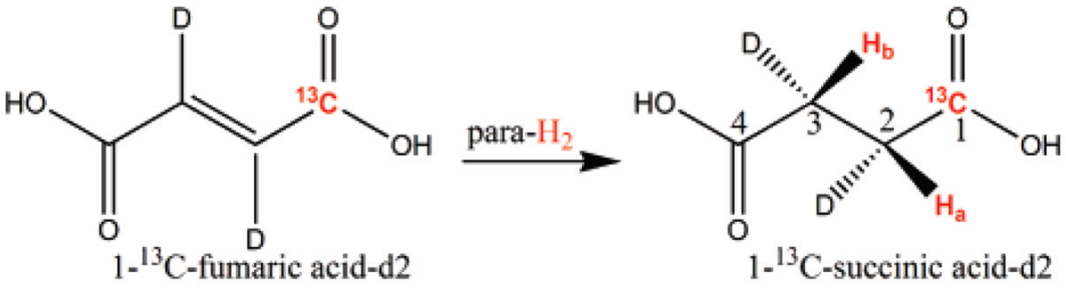

Figure 1.

Cis molecular addition of parahydrogen to 1-^{13}C-fumaric acid-d_2 to produce 1-^{13}C-succinic acid-d_2. The catalytic reaction was carried out at 62 °C in H_2O or D_2O with substrate concentrations of 1–5 mM.

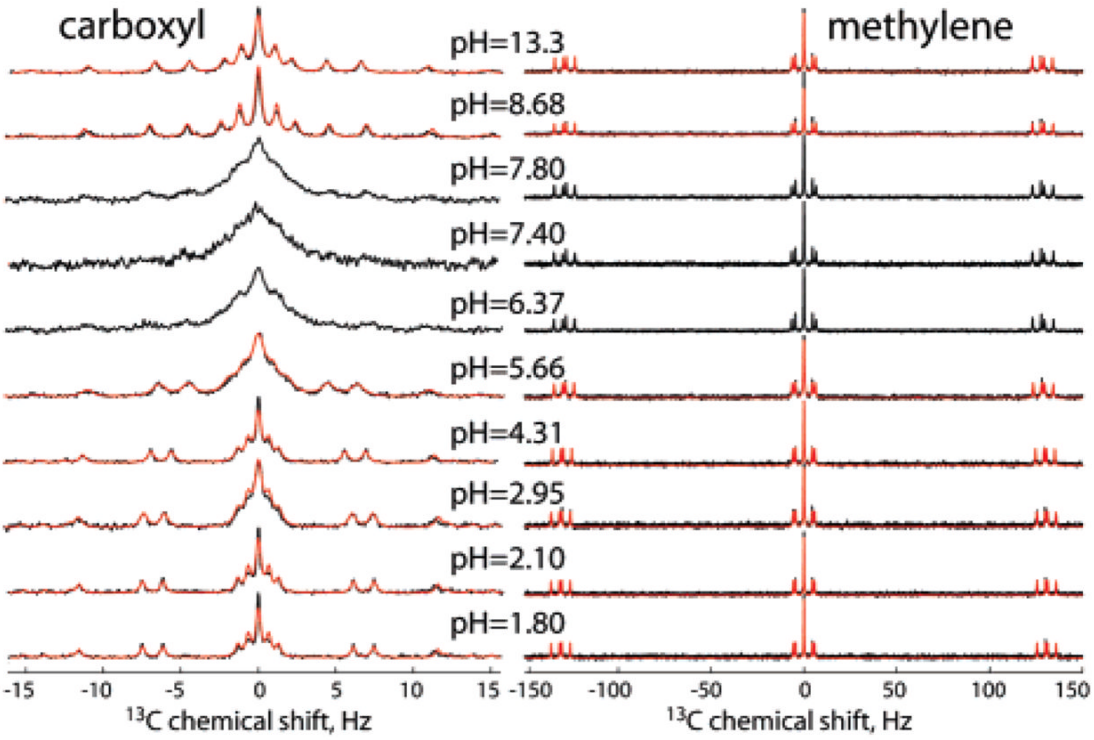

Figure 2.
^{13}C NMR multiplets (experimental in black and simulated fits in red) of natural abundance succinic acid at various pH. All spectra acquired at 14 T with 128–512 scans using Varian triple resonance H/C/N probe without ^{1}H decoupling.

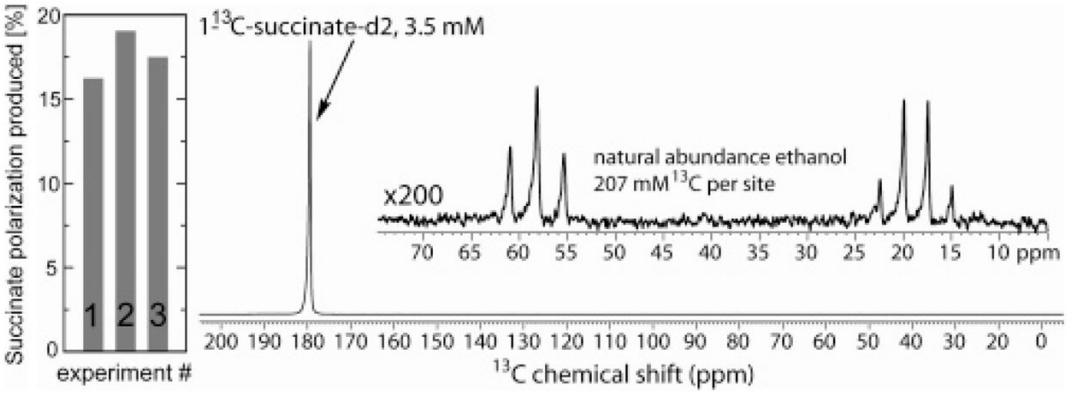

Figure 3.

Typical ^{13}C spectrum of 3.5 mM 1-^{13}C-succinate-d_2 hyperpo-larized at pH = 2.9. The chart on the left shows the typical reproducibility achieved; a series of three experiments was conducted within 30 min yielding 17.6 ± 1.4% polarization. Natural abundance ethanol with 207 mM of ^{13}C per carbon site is used as a reference. Spectra are acquired at 4.7 T with a Bruker Avance console and home-built double resonant probe.[15]

Table 1

J Couplings of ^{13}C1 and the Protons in Positions 2 and 3 in 1-^{13}C-succinic Acid; the Site Labels Are in Figure 1

pH	$2J_{CH}$ (Hz)	$3J_{CH}$ (Hz)	$3J_{HaHc}$ (Hz)	$3J_{HaHb}$ (Hz)
1.80	−7.15	5.84	5.43	7.45
2.10	−7.16	5.83	5.42	7.56
2.95	−7.15	5.82	5.62	7.41
4.31	−6.80	5.47	6.07	7.35
5.66	−6.41	4.48	7.55	7.17
8.68	−6.61	4.20	8.69	6.62
13.3	−6.32	4.13	8.51	6.68