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PASADENA Hyperpolarized ^{13}C Phospholactate

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ABSTRACT: We demonstrate that potassium 1-^{13}C-phosphoenolpyruvate becomes hyperpolarized potassium 1-^{13}C-phospholactate with ^{13}C T_1 = 36 s after molecular hydrogenation by PASADENA (Parahydrogen and Synthesis Allows Dramatically Enhanced Nuclear Alignment). This proof-of-principle study was conducted with a fully protonated molecular precursor. ^{13}C was polarized to a level of 1%, corresponding to nearly 4000-fold sensitivity enhancement at 3 T. The relevant homo- and heteronuclear spin−spin couplings are reported.

Hyperpolarized magnetic resonance (MR) of ^{13}C and ^{15}N exogenous metabolic contrast agents is a rapidly developing field, which progressed from proof-of-principle studies in the early 2000s[1,2] to the first clinical trial in men.[3] The underlying driving force stems in part from the demonstrated capability to detect abnormal metabolic pathways in cancer[4−7] and other diseases with impaired metabolism.[8] Hyperpolarization of long-lived nuclear spin states increases sensitivity by 4−6 orders of magnitude[2,9] with nuclear spin polarization approaching the order of unity. Hyperpolarized metabolic agents such as 1-^{13}C-pyruvate can report on metabolic status of tumors similarly to positron emission tomography (PET) tracers.

One of the main bottlenecks for preclinical and clinical application of hyperpolarized MR is the development and validation of relevant compounds that can probe biochemical pathways in vivo. Dynamic nuclear polarization (DNP)[10] has been most widely used to date with the main drawback of long (∼1 h) polarization cycles. Parahydrogen-induced polarization (PHIP) offers significantly faster preparations with hyperpolarization cycles as short as 1 min. However, it is limited by the availability of the required unsaturated molecular precursors that are necessary for molecular addition of parahydrogen,[11,12] which acts as a source of spin order. An additional requirement for increased relaxation times of the hyperpolarized ^{13}C or ^{15}N site is the absence of directly attached protons allowing for in vivo spin−lattice relaxation time T_1 as long as 43 s[13] and 2 min,[14] respectively. As a result, the smallest PHIP moiety consists of an unsaturated C═C or C≡C bond adjacent to a labeled ^{13}C or ^{15}N site. For ^{13}C-hyperpolarized compounds, this represents a three-carbon limitation successfully exemplified by acrylate moiety by a number of groups.[15,16] However, hydrogenation of acrylate results in propionate, which turns out to be useful only for angiographic applications.[1,15] In contrast, a leading DNP-hyperpolarized metabolic agent, 1-^{13}C-pyruvate, is also a three-carbon molecule carrying an extra oxygen atom in addition to the three-carbon skeleton of propionate, Figure 1. Hyperpolarized 1-^{13}C-pyruvate is not amenable by PHIP.

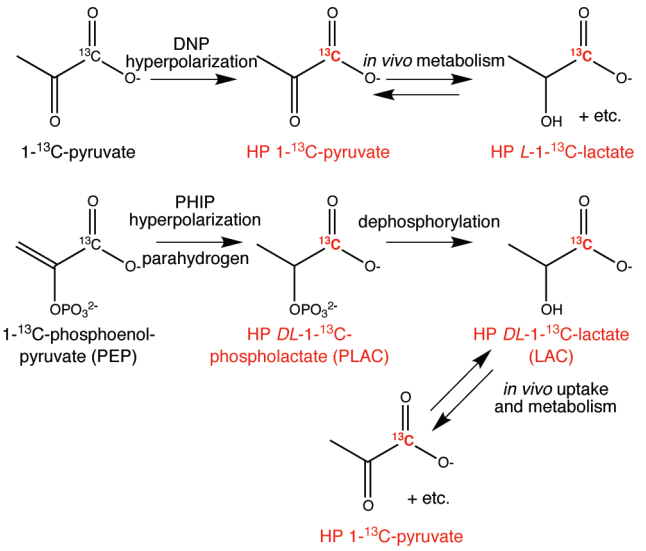

Figure 1. Molecular diagrams of PHIP and DNP hyperpolarization of 1-^{13}C-pyruvate and 1-^{13}C-phospholactate (PLAC) and their metabolism for application as metabolic hyperpolarized (HP) agents.

Here, we describe hyperpolarization of 1-^{13}C-phospholactate by PHIP. While both 1-^{13}C-propionate and 1-^{13}C-phospholactate are carboxylic acids with three-carbon backbones, 1-^{13}C-phospholactate has an extra phosphate-protected oxygen. The latter makes it similar to pyruvate, as it can be potentially metabolized, Figure 1. This is a proof-of-principle study demonstrating (i) the feasibility of molecular addition of parahydrogen with established and commercially available Rh-based water-soluble catalyst and resulting in ^{13}C hyperpolarization, (ii) sufficiently long lifetime of this potentially new metabolic contrast agent, and (iii) complete characterization of homo- and heteronuclear spin−spin couplings. The latter are necessary for optimization of PHIP polarization efficiency especially for perdeuterated unsaturated precursors, which represent a three-spin system consisting of two nascent parahydrogen protons and a ^{13}C or ^{15}N nucleus. In addition, perdeuterated precursors may result in hyperpolarized compounds with longer relaxation times.[17] Deuteration minimizes the number of nearby protons, which have significantly greater magnetic moments compared to that of deuterons. As a result,

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^{13}C T_1 decrease due to dipolar relaxation mechanism may be somewhat minimized.[17]

The double C═C bond is stable with a phosphate moiety in the second position, Figure 1, allowing for molecular hydrogenation and PHIP. The unsaturated precursor is nontoxic 1-^{13}C-phosphoenolpyruvate (PEP, Sigma-Aldrich #589,462), which becomes ^{13}C-hyperpolarized racemic 1-^{13}C-phospholacate (PLAC) with *D*- and *L*-forms. The protection by a phosphate group allows stabilizing the carbon−carbon double bond adjacent to the oxygen atom. While other protecting groups can potentially be used, the phosphate moiety could be quickly removed enzymatically[18] by a suitable choice of phosphatase to yield hyperpolarized 1-^{13}C-lactate (LAC), Figure 1. Moreover, phosphate ions are biologically compatible and therefore do not require additional purification steps other than removal of phosphatase prior to in vivo administration.

Experimental polarization procedures and equipment were identical to those described earlier.[9,19] ^{13}C PHIP polarization of PLAC, Figure 2a, was conducted in a 0.0475 T polarizer[9] with 2.5 mM water-soluble Rh-based catalyst[19,20] using the Goldman polarization transfer sequence.[16] D_2O, 99.8% (Sigma-Aldrich-Isotec # 617,385) was used as a medium, pH = 11 using 18 mM phosphate buffer, for 4-s long hydrogenation of 1.8 mM PEP for high-resolution ^{13}C studies using an 11.7 T Bruker NMR spectrometer equipped with a 5 mm X−H RF probe, Figure 2. Following the PHIP procedure, hyperpolarized solution was injected in a 5-mm NMR tube. The NMR tube was inserted into the NMR spectrometer and the solution degassed during a ∼30 s delay. The degassing step is necessary to eliminate residual parahydrogen−nitrogen bubbles and their associated susceptibility gradients and broad lines. ^{1}H-decoupled ^{13}C spectra, Figure 2b, were acquired approximately 60 s or 3 × T_1 after PHIP hyperpolarization, resulting in hyperpolarization decay by 20-fold. Nevertheless, a 50-fold signal enhancement of PLAC was observed, Figure 2b, when compared to the ^{13}C signal recorded from the same sample at Boltzmann levels of ^{13}C polarization, Figure 2c. This 50-fold enhancement corresponds to a ^{13}C polarization of 0.05% at 11.7 T and 298 K. Taking into account 20-fold polarization loss during ∼60 s delivery and degassing procedure, polarization of 1% was produced in the polarizer. The conversion of PEP (^{13}C δ = 171.9 ppm) to PLAC (^{13}C δ = 182.1 ppm) was only 25% due to nonoptimal catalyst preparation. The Rh-based catalyst was further optimized for low-field in situ NMR studies at 0.0475 T by titration of bis(norbornadiene)rhodium(I) tetrafluoroborate (Strem # 45-0230, Newburyport, MA) and 3, 3′-(1,4-butanediylbis(phenylphosphinidene))bispropanesulfonic, disodium salt (Sigma-Aldrich-Isotec #Q36333-SPEC), Shchepin, R.V., unpublished results.

Low-field in situ detection of ^{13}C hyperpolarization was conducted with 2.0 mM PEP in aqueous medium with double deionized water, pH = 9.8 using 22 mM phosphate buffer resulting in 99+% chemical conversion to PLAC, pH = 8.8. While excellent PEP chemical conversion is achieved, in vivo use of the hyperpolarized contrast agent would require additional pH neutralization and catalyst removal. It should also be noted that *D*-isomers may not be metabolized in vivo and may be toxic. The signal intensity of a single-scan ^{13}C-hyperpolarized spectrum, 0.007 mmol of PLAC, Figure 3b, was compared to that of a ^{13}C spectrum acquired using 170 mmol of sodium 1-^{13}C-acetate at Boltzmann polarization at 0.0475 T and 308 K, Figure 3a.[9] One percent polarization was achieved corresponding to ^{13}C signal enhancement ε = 240,000 fold at

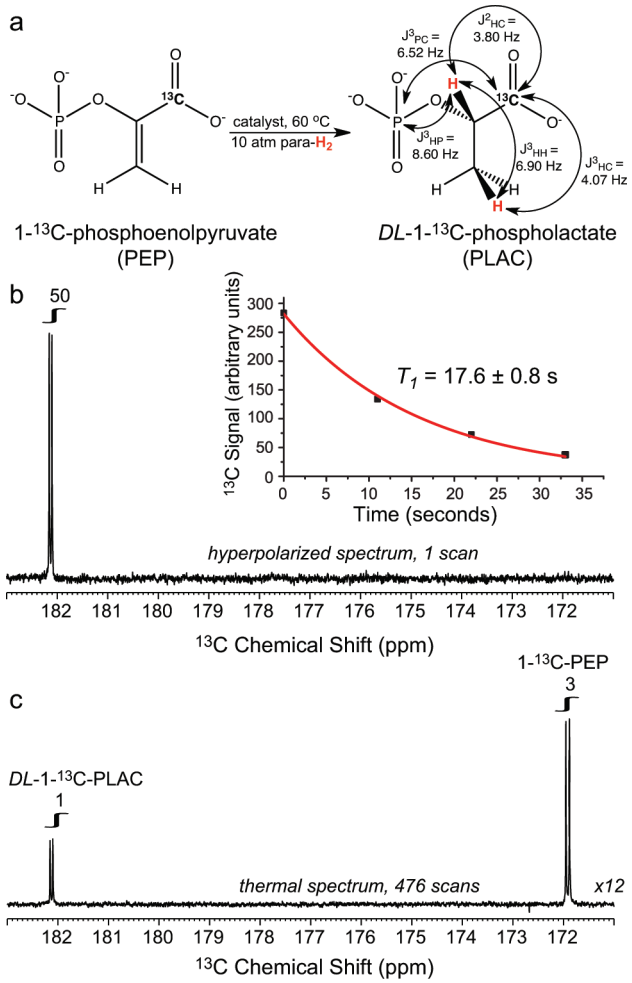

Figure 2. ^{13}C PASADENA (parahydrogen and synthesis allows dramatically enhanced nuclear alignment) hyperpolarization of potassium 1-^{13}C-phospholactate. (a) Diagram of molecular hydrogenation of potassium 1-^{13}C-phosphoenolpyruvate (PEP) yielding 1-^{13}C-phospholactate (PLAC). (b) Proton-decoupled single-scan ^{13}C spectrum of hyperpolarized *DL*-1-^{13}C-PLAC. (Inset) T_1 decay of ^{13}C hyperpolarized signal of PLAC measured with 15° excitation RF pulses. All ^{13}C spectra are acquired on a 500 MHz Bruker spectrometer equipped with X−H dual-tuned RF coil and identical acquisition parameters. (c) Proton-decoupled ^{13}C spectrum of Boltzmann polarized mixture of *DL*-1-^{13}C-PLAC and unreacted 1-^{13}C-PEP using the same sample as in spectrum 2b except for nuclear spin polarization; note the doublet peak appearance due to $J^3_{PC} \approx 6.5$ Hz, a signature of carbon−phosphorus spin−spin couplings in PLAC and PEP. The conversion of PEP (^{13}C δ = 171.9 ppm) to PLAC (^{13}C δ = 182.1 ppm) was only 25% due to nonoptimal catalyst preparation.

0.0475 T and 308 K. It is in good agreement with our high-field studies. ^{13}C T_1 was measured by monitoring ^{13}C hyperpolarization decay using a time series of ^{13}C spectra acquired every 20 s with 30° excitation pulses, Figure 3c. The T_1 of 1-^{13}C at 0.0475 T was 36 ± 2 s, which is twice as long as the T_1 measured at 11.7 T in D_2O. The T_1 decay was simulated as an exponential decay taking into account the effect of RF pulses on ^{13}C magnetization. A likely explanation for longer T_1 at low magnetic field is a reduced chemical shift anisotropy, which is expected to dominate T_1 relaxation at high magnetic fields.[21] A similar field effect was reported for the ^{13}C T_1 in 2-hydroxyethyl-1-^{13}C-propionate-2,3,3-d_3.[9]

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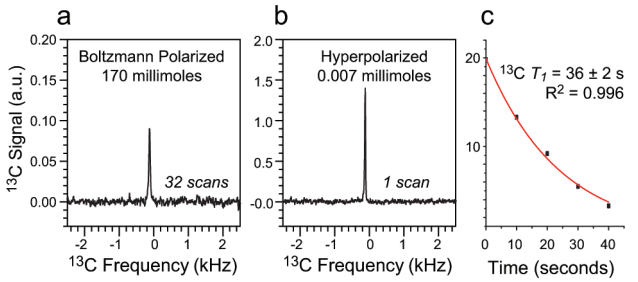

Figure 3. ^{13}C NMR spectroscopy conducted at 0.0475 T. (a) ^{13}C reference spectrum of 170 mmol (14 g in 50 mL D_2O) of sodium 1-^{13}C-acetate, Boltzmann polarization at 308 K, 32 averages. (b) ^{13}C spectrum of 0.007 mmol, 1.5 mg in 3.6 mL H_2O at pH = 9.0, hyperpolarized potassium *DL*-1-^{13}C-PLAC, polarization P = 1.0% corresponding to signal enhancement by 240,000-fold measured by comparing ^{13}C signal intensity of hyperpolarized 0.007 mmol of PLAC with that of 170 mmol of sodium 1-^{13}C-acetate at Boltzmann polarization at 0.0475 T and 308 K (in a).[9] (c) Decay of ^{13}C hyperpolarized PLAC signal measured with 30° excitation pulses. The T_1 decay was simulated (red trace) as an exponential decay taking into account the effect of RF pulses on ^{13}C magnetization.

The solution used in PHIP low-field studies was reduced in volume using a rotary evaporator, dissolved in 99.8% D_2O, and used for high-resolution studies of PLAC spin−spin couplings, Figure 4. The combination of heteronuclear spectra, Figure 4, overdetermines the problem of complex multiplets and allows for accurate measurements of the spin−spin coupling magnitudes shown in Figure 2a.: J^3_{HH} = 6.90 Hz, J^3_{HC} = 4.07 Hz, J^2_{HC} = 3.80 Hz, J^3_{PC} = 6.52 Hz, J^3_{HP} = 8.60 Hz. The knowledge of spin−spin couplings is critical for optimal performance of PHIP polarization transfer sequences that convert spin order of nascent parahydrogens to detectable ^{13}C magnetization.

1-^{13}C-Phospholactate is suitable for measurement of spin−spin couplings due to ^{13}C enrichment. The PHIP product of perdeuterated 1-^{13}C-PEP, PLAC-d_2, could lengthen ^{13}C T_1 somewhat and yield higher polarization, with the caveat that the extra deuterons in PLAC would pose synthetic challenges and complicate the spectra of ^1H, ^{13}C, and ^{31}P, making spin−spin coupling measurements more arduous. An alternative would be to use natural abundance PEP similarly to the strategy utilized for ^{13}C-hyperpolarized succinate,[17] but this would require recording ^{13}C spectra at natural abundance levels of ^{13}C, resulting in many hours of acquisition time. If PEP is perdeuterated, the spin system participating in polarization transfer dynamics would be reduced to three spins: ^{13}C and two nascent parahydrogens in PLAC. Since Goldman's polarization transfer[16] is designed for three-spin systems and is not expected to perform well for systems with more than three spins, it is likely to result in a significantly greater hyperpolarization using perdeuterated PEP compared to the five-spin system used here.[17,22]

While the metabolic relevance of PLAC itself for biomedical imaging is yet to be seen, the corresponding relevance of LAC as an imaging agent is certainly well-established.[23] One possibility to produce LAC from PLAC would be to enzymatically cleave the phosphate moiety.[18] It is also possible that phosphatases of blood could cleave phosphate moiety after intravenous injection of PLAC. Fast hyperpolarization of LAC by the PHIP method would present a clear advantage over the DNP method and would make this promising contrast agent available to a broader range of biomedical scientists for cancer

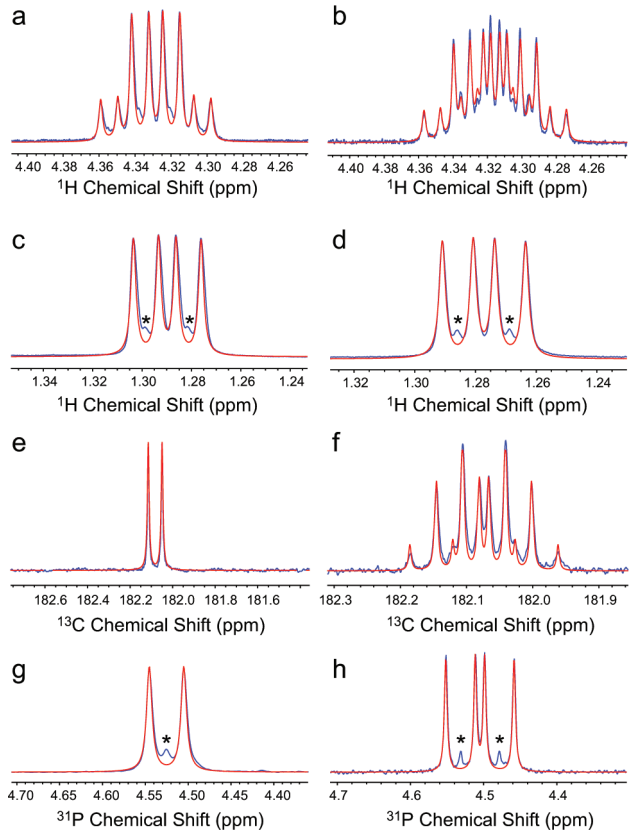

Figure 4. Multinuclear high-resolution 11.7 T NMR spectroscopy (blue trace) and simulations (red trace) of PLAC at pH = 8.8. ^1H spectra (a) methine peak and (c) methyl peak with ^{31}P decoupling; (b) methine peak and (d) methyl peak without ^{31}P decoupling. ^{13}C spectra (e) with ^1H decoupling and (f) without ^1H decoupling. ^{31}P spectra (g) with ^1H decoupling and (h) without ^1H decoupling. The resonances marked with * correspond to peaks from natural abundance PLAC without 1-^{13}C enrichment.

and cardiovascular research.[7] Moreover, if successful, this strategy of using phosphate-protected −C═C−O− motifs and subsequent cleavage could be extended to hyperpolarization of other metabolic contrast agents including ^{15}N-choline.

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Notes

The authors declare no competing financial interest.

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