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Article *in* NMR in Biomedicine · November 2013

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Received: 10 January 2013, Revised: 5 June 2013, Accepted: 5 June 2013, Published online in Wiley Online Library: 25 July 2013

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Automated transfer and injection of hyperpolarized molecules with polarization measurement prior to *in vivo* NMR

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Hyperpolarized magnetic resonance via dissolution dynamic nuclear polarization necessitates the transfer of the hyperpolarized molecules from the polarizer to the imager prior to *in vivo* measurements. This process leads to unavoidable losses in nuclear polarization, which are difficult to evaluate once the solution has been injected into an animal. We propose a method to measure the polarization of the hyperpolarized molecules inside the imager bore, 3 s following dissolution, at the time of the injection, using a precise quantification of the infusate concentration. This *in situ* quantification allows for distinguishing between signal modulations related to variations in the nuclear polarization at the time of the injection and signal modulations related to physiological processes such as tissue perfusion. In addition, our method includes a radical scavenging process that leads to a minor reduction in sample concentration and takes place within a couple of seconds following the dissolution in order to minimize the losses due to the presence of paramagnetic polarizing agent in the infusate. We showed that proton exchange between vitamin C, the scavenging molecule and the deuterated solvent shortens the long carboxyl ^{13}C longitudinal relaxation time in [1-^{13}C]acetate. This additional source of dipolar relaxation can be avoided by using deuterated ascorbate. Overall, the method allows for a substantial gain in polarization and also leads to an extension of the time window available for *in vivo* measurements. Copyright © 2013 John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this paper

Keywords: DNP; hyperpolarization; ^{13}C MRS; *in vivo*; rodents; vitamin C; radical scavenging; proton exchange

INTRODUCTION

The tremendous gain in signal-to-noise ratio resulting from hyperpolarization techniques and in particular dissolution dynamic nuclear polarization (DNP) opened new perspectives in biomedical NMR research (1–3). A large effort has so far been devoted to the optimization of the sample preparation (e.g. choice of radicals (4), solvents (5), adjunction of Gd(III) complexes (6,7)) and hardware developments (1,8–10), to maximize the solid-state nuclear spin polarization. However, little has been done to minimize the polarization losses once the sample is dissolved. Losses due to relaxation can be essentially minimized by, on one hand, alleviating relaxation pathways through cancellation of some interactions between the nuclear spins of interest and their environment and, on the other hand, reducing the time delay between the dissolution step and the injection into the subject under investigation. The use of deuterated solvents to dissolve the samples can significantly reduce the dipolar relaxation, but in most cases the residual paramagnetic centers (polarizing agents and Gd ions used during the solid-state polarization process) will be the main source of relaxation. It has already been shown that nitroxyl radicals that are used for DNP can be scavenged by ascorbate (11), but the proposed method leads to strongly reduced concentrations of hyperpolarized molecules following dissolution since the frozen droplets of ascorbate solution placed inside the polarizer take a large part of the available sample space. It is thus not optimal for biomedical applications. Note that another proposed solution to reduce the nuclear spin polarization losses due to relaxation is to create singlet states which have a much longer lifetime but it can only be applied to specific types of molecule (12–16).

To quantify and possibly minimize the losses, it is necessary to accurately measure the polarization following the transfer from the polarizer to the imager. The *in vivo* signal enhancement is

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Abbreviations used: *DNP, dynamic nuclear polarization; TEMPO, 2,2,6,6-tetramethyl-1-piperidinyloxy; TTL, transistor–transistor logic; NaAsc, sodium ascorbate; AA, ascorbic acid.*

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however difficult to estimate since the thermal equilibrium signal needed to determine the polarization is too small to be detected once the solution has been injected. Measuring the polarization *ex situ* by inserting part of the hyperpolarized solution into a dedicated NMR setup (sometimes referred to as "polarimeter") is also inaccurate since the hyperpolarized solution is subject, during the transfer between polarizer and imager on one side, and polarizer and polarimeter on the other, to unequal dramatic time variations in magnetic field, which may strongly affect nuclear spin relaxation. A precise quantification of the polarization at the time of the injection would allow for distinguishing between signal modulations related to variations in the nuclear polarization prior to injection and signal modulations related to physiological processes such as tissue perfusion. For instance, a localized acquisition in necrotic or ischemic tissue will lead to reduced signal as compared with healthy tissue as a consequence of the altered substrate uptake, and a precise knowledge of the polarization at the time of each injection will allow assessment of the difference in cellular uptake across experiments. This is particularly relevant when multiple injections are performed in the same animals at different time points. If a substrate such as ^{13}C-urea or ^{13}C-tert-butanol is co-injected to normalize the metabolite signals in order to take into account the variable tissue perfusion, it is also necessary to have an accurate measurement of the polarization of all substrates to be able to have comparable ratios across experiments.

We herein propose a method based on a home-built infusion pump allowing us (a) to reduce the transfer time between the polarizer and the pump to 2 s, (b) to scavenge the radicals inside the infusion pump and (c) to measure, *in situ*, the polarization of the hyperpolarized substrate inside the imager bore at the time of the infusion using the spectrometer of the imager. The whole procedure is remotely controlled and automated and lasts 3 s. Since the injection starts 3 s after dissolution and lasts up to 9 s, the relaxation inside the infusion pump is minimized following the scavenging process. We demonstrate the applicability of this method for *in vivo* real-time metabolic studies in rodents using hyperpolarized [1-^{13}C]acetate, a biomolecule that has previously been proven to be an interesting substrate for probing energy metabolism (17,18).

EXPERIMENT

All animal experiments were conducted according to federal and local ethical guidelines, and the protocols were approved by the local regulatory body of the Canton Vaud, Switzerland (Service de la Consommation et des Affaires Vétérinaires, Affaires Vétérinaires, Canton de Vaud, Switzerland). Male Sprague Dawley® rats (275–325 g) were anesthetized with 1.5% isoflurane in a 30:70% O_2:N_2O mixture. A catheter was placed into the femoral vein for intravenous delivery of the hyperpolarized acetate solution. The respiration rate and temperature were monitored and maintained during all animal experiments.

Separator/infusion pump and NMR probes

Compared with the design presented in an earlier publication (9), the new all-plastic separator/infusion pump has two vent ports instead of three. It was modified to allow very fast transfer between the polarizer and the pump (see below). The wall thickness of the main compartment in which the liquid is collected inside the separator/infusion pump (cylinder volume) was also reduced to 1.5 mm in order to place NMR coils with maximized filling factor ($V_{sample}/V_{coil} \cong 0.7$). Two coaxial solenoidal coils with an identical diameter of 19 mm were wound around the main compartment (see Fig. 1): one coil was tuned to ^{13}C frequency and the other one, interleaved with the ^{13}C coil, was tuned to ^{1}H frequency. The ^{13}C probe quality factor was set to a low enough value (~200) in order to minimize its sensitivity to coil loading, in particular with respect to the sample salt concentration that may vary between experiments. The separator/infusion pump, which was screwed on the NMR insert next to the animal holder, was placed at a horizontal distance Δ away from the isocenter of a 31 cm horizontal bore actively shielded 9.4 T imager (Magnex Scientific, Abingdon, UK) coupled to a Direct Drive spectrometer (Agilent, Palo Alto, CA, USA) (see Fig. 1). To determine the maximum distance Δ at which the field homogeneity $\Delta B/B_0$ was high enough to make NMR measurements inside the pump, the insert was moved along the magnet bore axis and the ^{13}C signal of a 1 M [1-^{13}C]acetate solution (2.5 mL) was measured each centimeter for Δ values between −12 and +12 cm. The field homogeneity over the volume of interest was determined from the ^{13}C spectral line width, and it was observed that it is nearly constant for all Δ between −9 cm and +9 cm (see Fig. 1 inset). For larger Δ it was not possible to adequately compensate the field inhomogeneity within the volume of interest with the imager shim coils. For *in vivo* skeletal muscle metabolic studies the distance Δ between the axis of the ^{13}C coil wound around the pump (filled circles) and the animal ^{13}C surface coil center was set to 6 cm.

The *in vivo* measurements were performed with a home-built dual ^{1}H/^{13}C probe consisting of two 10 mm diameter ^{1}H coils in quadrature and a 10 mm diameter ^{13}C coil placed over the hind leg of the rat to selectively excite and detect skeletal muscle tissue. The animal leg was placed at the isocenter of the imager.

^{13}C probe switching scheme

Both ^{13}C probes were connected to the same spectrometer channel via a radiofrequency (RF) network allowing switching between the probe located on the separator/infusion pump for *in vitro* measurements and the probe placed on the animal for

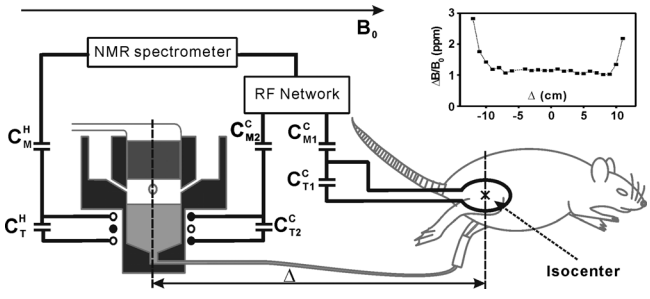

Figure 1. Sketch of the experimental setup inside the 31 cm diameter magnet bore. The distance between the axis of the ^{13}C coil wound around the pump (filled circles) and the animal ^{13}C surface coil center was set to $\Delta = 6$ cm for *in vivo* skeletal muscle metabolic studies. A ^{1}H coil (open circles) was added to perform the shimming procedure required to reduce the magnetic field inhomogeneity inside the separator/infusion pump. The field homogeneity $\Delta B/B_0$ inside the pump as a function of the distance from the magnet isocenter is shown in the inset. The ^{1}H coils used for shimming the volume of interest of the animal are not drawn for simplicity.

in vivo measurements (see supporting information). A highly isolated TTL-triggered RF broadband switch (Minicircuits, Munich, Germany) was placed in front of the two power amplifiers used to generate the high-power RF pulses for the animal coil (350 W amplifier; Varian, Palo Alto, CA, USA) and for the pump coil (100 W amplifier; Tomco, Stepney, Australia). The NMR signals were preamplified through two different 30 dB low-noise amplifiers (Varian) before being fed into the spectrometer receiver through a second identical TTL-triggered RF switch. The time required to switch the electronics paths from one probe to the other was less than 5 μs. Due to the switches' insertion losses (0.8 dB at 100.67 MHz) and the losses in the additional coaxial cables (1–2 dB), RF pulse power had to be adjusted for both coils following the insertion of the RF network. The presence of the coil on the infusion pump had no influence on the surface coil used for the *in vivo* detection and the high isolation (−78 dB) of the RF switches strongly limited signal contamination between the two ^{13}C electronics paths. The insertion loss of the RF switch incorporated in front of the preamplifier, however, led to a typical SNR reduction of 0.5 dB. The RF network is described in detail in the supplementary material.

Hyperpolarization process, transfer and *in vitro* measurements

300 μL of 4.5 M sodium [1-^{13}C]acetate solution in the form of glassy frozen beads (2:1 D_2O:d_6-ethanol with 33 mM TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) radical) were dynamically polarized at 5 T and 1.02 ± 0.03 K using the hardware described in earlier publications (9,10). All chemicals were bought from Sigma-Aldrich, Buchs, Switzerland. The ^{13}C solid-state polarization was monitored by applying a 5° flip angle pulse every 5 min using a circuit and a protocol previously described (see Fig. 4(A) below) (19). Once the polarization level had reached the targeted value, the sample was rapidly dissolved in 5 mL of superheated D_2O using a procedure similar to the one developed by Ardenkjaer-Larsen *et al.* (1) Immediately after dissolution, a pressure differential was created for 2 s across a 5 m long and 2 mm inner diameter PTFE tube using pressurized helium gas (see below for detailed parameters). The hyperpolarized solution was collected in the separator/infusion pump connected at the end of the tube and positioned inside the imager bore. A 1 s delay between the end of the application of the high-pressure gas and the injection was set to allow the solution to settle at the bottom of the pump, where the ^{13}C coil and the injection port are located (see Fig. 1). The ^{13}C signal from the hyperpolarized solution was measured inside the separator/infusion pump by applying 5° flip-angle RF pulses. T_1 measurements inside the pump were performed with series of 5° pulses separated by a delay of 3 s. For *in vivo* experiments, a single 5° flip angle pulse was applied 250 ms before the beginning of the infusion (the acquisition was set to 250 ms), i.e., 2.75 s after dissolution (see Fig. 4(B) below).

The exact [1-^{13}C]acetate concentration of the infusate was determined in all experiments by comparing the [1-^{13}C]acetate and [1-^{13}C]pyruvate ^{13}C NMR signals in a solution containing 100 μL of infusate (a residual volume is left inside the pump after injection), mixed in 500 μL of 20 mM sodium [1-^{13}C]pyruvate aqueous solution (D_2O) containing 0.4 mM Gd agent Omniscan®. The measurements were made in a 9.4 T high-resolution NMR spectrometer (Bruker BioSpin SA, Fällanden, Switzerland).

Scavenging nitroxyl radicals inside the separator/ infusion pump

The nitroxyl radical scavenging process took place inside the separator/ infusion pump, at the bottom of which 1 mL of L-ascorbate sodium (NaAsc) or L-ascorbic acid (AA) (Sigma-Aldrich) aqueous (D_2O) solution at different concentrations was loaded prior to dissolution. The temperature inside the separator/ infusion pump was monitored and maintained by blowing hot air into the imager bore.

In vivo measurement protocol

Once the animal was positioned inside the imager magnet, ten axial 1 mm thick slices were acquired using a gradient echo sequence (T_R = 50 ms, T_E = 3 ms, field of view = 30 × 30 mm^2, matrix = 128 × 128, flip angle = 30°), from which the volume of interest was selected. The static magnetic field was shimmed in a 600 μL (6 × 10 × 10 mm^3) voxel to reduce the localized proton line width to 20 Hz using the FASTESTMAP shimming protocol (20). A volume of 1.6 mL of the dissolved hyperpolarized solution was injected within 9 s. Series of single-pulse ^{13}C acquisitions were sequentially recorded starting 6 s after dissolution using 30° adiabatic RF pulses (BIR4) applied every 3 s with ^{1}H decoupling during acquisition (WALTZ) (a typical spectrum is shown in Fig. 4(C) below). The adiabatic pulses' offset and power were set so as to ensure a homogeneous 30° excitation of substrate and metabolite resonances within the entire volume of interest. Since the pump and the animal were located at a distance Δ from one another, two different sets of shim parameters determined and saved prior to the dissolution and injection were used to perform the consecutive NMR measurements in the pump and *in vivo* respectively. The shim current values were changed during the injection (this was performed in a few seconds by the imager control electronics).

In vitro T_1 measurements

The ^{13}C T_1 in thermally polarized 0.2 M sodium [1-^{13}C]acetate aqueous (D_2O) solutions containing various concentrations of vitamin C were independently measured in a vertical 9.4 T high-resolution system (Bruker BioSpin SA) using a standard saturation recovery pulse sequence (filled symbols in Fig. 2). The solutions did not contain nitroxyl radicals and they were deoxygenated prior to the measurements by blowing nitrogen gas at the bottom of the 5 mm NMR tubes for 15 min. The same measurements were also made in solutions containing respectively 0.4 M and 0.8 M of partially deuterated NaAsc (filled triangles in Fig. 2; see supplementary material for details on the deuteration process). In addition, the ^{13}C T_1 of sodium [1-^{13}C]acetate in a 0.4 M partially deuterated NaAsc aqueous (D_2O) solution was measured in the pump as described above following dissolution DNP and mixing with 1 mL of a 1 M partially deuterated NaAsc solution preloaded inside the separator/infusion pump (open triangle in Fig. 2).

RESULTS

Transfer and radical scavenging

A pressure of 6 bar was applied to rapidly transfer the hyperpolarized solutions through the 2 mm inner diameter PTFE tube without creating an excessive overpressure in the separator/

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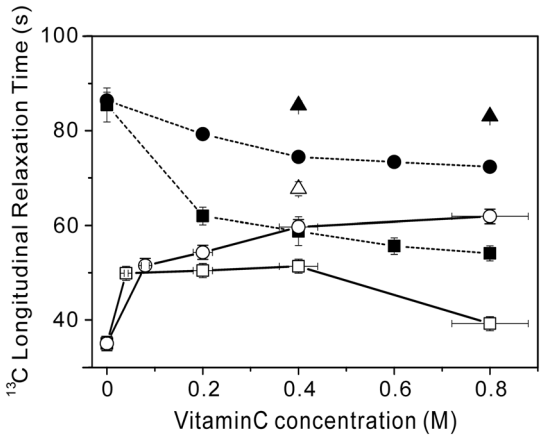

Figure 2. *In vitro* ^{13}C T_1 measurements of sodium [1-^{13}C]acetate in aqueous (D_2O) solutions at 33 °C as a function of NaAsc (circles), AA (squares) or deuterated NaAsc (triangles) concentration. Experiments with 0.25 ± 0.05 M hyperpolarized acetate solutions containing 2 ± 0.3 mM TEMPO were performed following dissolution and mixing with vitamin C in the separator/infusion pump placed in the 9.4 T imager (open symbols). The vertical error bars originate from the 5 °C uncertainty on the solution temperature. The thermally polarized 0.2 M acetate solutions (without added nitroxyl radicals) were measured in a vertical 9.4 T high-resolution system (filled symbols).

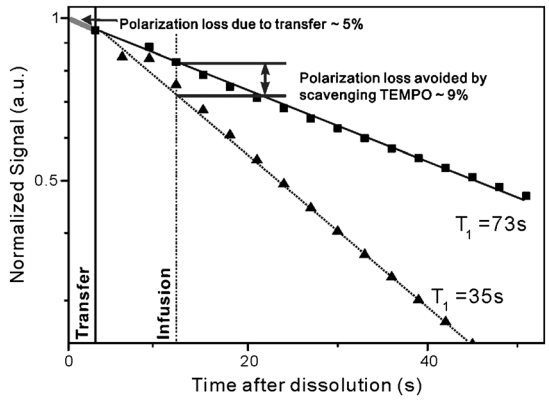

Figure 3. ^{13}C signal decay of hyperpolarized sodium [1-^{13}C]acetate with (■) and without (▲) deuterated NaAsc (1 M); the polarization was about 9% larger at the end of the infusion when the nitroxyl radicals were scavenged inside the pump as proposed herein.

infusion pump. Test experiments showed that lower pressures led to longer transfer times and higher pressures to losses through the vent ports of the pump. At 6 bar, 4 ± 0.2 mL was transferred into the pump through 5 m of tube in 2 s but only 2.4 ± 0.1 mL of the total volume was located in the pump main compartment (the rest was pushed out into the two vent ports). The concentration of the vitamin C (NaAsc and AA) aqueous (D_2O) solutions introduced inside the separator/infusion pump prior to dissolution experiments ranged from 0.1 M to 2 M. The temperature of the separator/infusion pump was stabilized at 33 °C. Following the transfer of the hyperpolarized solution and after rapid mixing with the aqueous solution introduced inside the pump, the final vitamin C concentration of the infusate ranged from 40 mM to 800 mM. It was observed that the temperature of the infusate is always within 5 °C of the pump temperature at the end of the transfer and consecutively stabilizes at the pump temperature within 3 ± 0.5 min. The longitudinal relaxation times of [1-^{13}C]acetate were deduced from fitting the ^{13}C signal decays measured in the infusion pump with a mono-exponential decaying function after having corrected for the effect of the RF pulses on the signal intensity. The ^{13}C T_1 values were measured for several vitamin C infusate concentrations (open symbols in Fig. 2).

The resulting T_1 values are reported in Fig. 2 (triangles). The ^{13}C signal integral decay measured in a 0.4 M deuterated NaAsc solution after the dissolution DNP experiment is reported in Fig. 3 along with the ^{13}C signal integral decay measured following a dissolution DNP experiment performed without radical scavenging molecules. A loss of polarization resulting from the transfer between the polarizer and the imager is unavoidable, but our fast transfer scheme limits it to 2–5% for hyperpolarized [1-^{13}C] acetate solutions. Once the solution has been transferred into the imager bore, the polarization losses can be strongly reduced by scavenging the radicals inside the separator/infusion pump. In the case of hyperpolarized [1-^{13}C]acetate, we showed that, if the pump was prefilled with a 1 M deuterated NaAsc solution, we could reduce the ^{13}C polarization losses by roughly 1% per second, corresponding to a gain of about 9% of polarization by the end of the 9 s long injection (see Fig. 3). Note that the significant gain in polarization also leads to an extension of the time window available for *in vivo* measurements.

In situ polarization quantification and *in vivo* measurements

The ^{13}C polarization after 2 h of microwave irradiation (30 mW at 140.22 GHz) was estimated to be 12 ± 1% in all experiments (n = 5) by comparing the polarized ^{13}C signal with the 1 K ^{13}C thermal equilibrium signal of the same sample measured separately. The ^{13}C solid-state DNP polarization build-up time constant of [1-^{13}C]acetate was 1900 ± 50 s. The liquid-state polarization was determined by comparing the hyperpolarized signal (Fig. 4(B)) with either the signal from a thermally polarized reference sample of known concentration when the solution was injected, or the thermal equilibrium signal when the solution was kept inside the pump to make T_1 measurements. We measured an enhancement factor of 15 500 ± 1000, which corresponds to a polarization of 12 ± 1%.

The *in vivo* acquisition started 3 s after the beginning of the 9 s long injection. The large [1-^{13}C]acetate signal was readily detected and, in agreement with Jensen *et al*. (17), we observed the formation of the metabolic product [1-^{13}C]acetylcarnitine 8.7 ppm upfield (see Fig. 4(C)). A characteristic *in vivo* decay time of around 16 ± 1 s was determined for acetate, and the *in vivo* metabolite acetylcarnitine in the skeletal muscle was observed over a time period of 30 s. A full study of the metabolism of [1-^{13}C]acetate in the rat skeletal muscle can be found in (21).

DISCUSSION

In-line *in situ* polarization measurements

Throughout the *in vivo* hyperpolarized NMR experiments presented herein, the ^{13}C signal of the same polarized [1-^{13}C]acetate sample was measured at three different stages (see Fig. 4): in the solid state, about 1 min prior to dissolution, in the liquid state, 2.75 s after dissolution after having been transferred inside the imager bore, and finally *in vivo* in skeletal muscle, a few seconds after the injection started. Since the *in vitro* liquid-state measurement is made at the beginning of the injection, lasts less than 1 s,

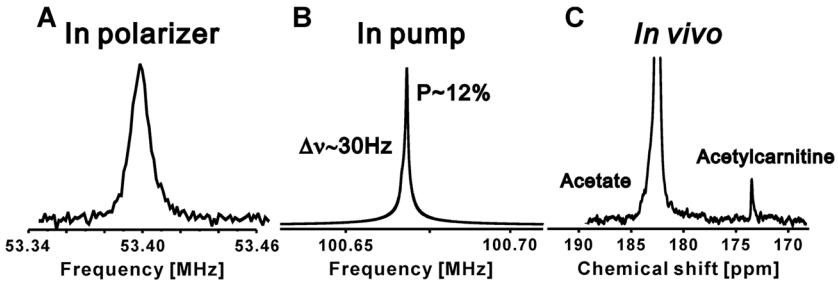

Figure 4. Three consecutive single-shot low-flip-angle ^{13}C measurements during a metabolic study. (A) 5 T/1 K solid-state signal in the polarizer. (B) Liquid-state signal inside the pump at the time the infusion started. (C) *In vivo* [1-^{13}C]acetate and [1-^{13}C]acetylcarnitine signals in the rat skeletal muscle.

and can be performed with a very low-flip-angle RF pulse, it has no substantial effect on the *in vivo* measurements. Most notably, this additional NMR measurement does not increase the delay between dissolution and injection and negligibly affects the *in vivo* signal intensity.

Accurately measuring the nuclear polarization prior to injection can greatly facilitate the interpretation of *in vivo* results. If the *in vivo* signal intensity in a specific experiment is substantially different from the average value, the polarization measurement will provide a way to decouple issues linked to the hyperpolarization and transfer processes from problems related to the injection or animal physiology. The variations in substrate and metabolite signal intensity are linked to differences in tissue perfusion, and a quantitative comparison across subjects should be possible if the intensities are rescaled by the polarization level measured at the time of the injection. Accurate *in situ* polarization measurements are also of great importance when several substrates are polarized and injected at the same time (22). To be meaningful, the relative intensities between the different substrates and their metabolites have to be weighted by the polarization level at the time of infusion. This is particularly essential if the low-field T_1 is dramatically different for the different substrates, as is the case for [1-^{13}C]pyruvic acid (~50 s at 1 mT (23)) and [^{13}C]urea (~10 s in pure H_2O and ~130 ms in pure H_2O containing residual nitroxyl radicals at 1 mT (24)).

We demonstrated the applicability of our method to *in vivo* hyperpolarized NMR experiments in the framework of a skeletal muscle metabolic study. The method is also compatible with other types of *in vivo* application such as cerebral or cardiac metabolic studies, although the pump might have to be placed farther away from the isocenter due to the animal and surface coil location. The field homogeneity in the 9.4 T imager used for the present studies was sufficient up to about 10 cm away from the isocenter and additional shim coils could be added around the separator if larger distances were required.

Radical scavenging

The strong influence of TEMPO on the ^{13}C carboxyl T_1 of acetate has already been determined in a previous publication (25). Therefore, as expected (11), the addition of vitamin C in [1-^{13}C] acetate aqueous (D_2O) solutions containing 2 ± 0.3 mM TEMPO led to an increase in ^{13}C T_1 (see Fig. 2, open symbols). To increase the kinetics of the scavenging process, large concentrations (M range) need to be used. The increase in ^{13}C T_1 with increasing vitamin C concentration observed in the dissolution DNP experiments indeed demonstrates the strong influence of the scavenger concentration on the kinetics, and the near-unity Pearson's R values of the fitted ^{13}C T_1 relaxation decay curves ($R > 0.999$) observed for all vitamin C concentrations larger than 0.2 M (see example in Fig. 3) show that a large fraction of the radicals are rapidly scavenged. However, the concentrations used in our dissolution DNP experiments do not seem to be large enough to completely cancel out the effect of the radicals on the ^{13}C relaxation, since even with the largest concentrations (0.8 M) the observed T_1 values do not quite match the values measured in thermally polarized solutions (see Fig. 2). Our results also show that the addition of large quantities of vitamin C lead to a substantial shortening of the ^{13}C T_1 values. The effect was clearly more pronounced with AA than with NaAsc, as confirmed by the T_1 measurements made on thermally polarized solutions (see Fig. 2, open symbols). Following the observation that T_1 was not substantially affected by the pH of the solution (data not shown), we deduced from the comparison between the ^{13}C longitudinal relaxation obtained in solutions containing either AA, NaAsc, deuterated AA (data not shown) or deuterated NaAsc that the concentration of exchangeable protons added to the solutions through the incorporation of vitamin C was the cause of the additional relaxation. This was confirmed by measuring the ^{13}C T_1 of [1-^{13}C]acetate in aqueous (D_2O) solutions (0.2 M) containing either 1 M AA and 1 M NaCl or 1 M NaAsc and 1 M HCl (both solutions had the same pH of 3.4). The carboxyl ^{13}C T_1 of acetate was identical in both solutions (45 ± 1 s). As already observed in [1-^{13}C]glycine aqueous solutions (26), the stronger dipolar fields induced by protonated water as compared with deuterated water strongly reduce the long T_1 of the carboxyl ^{13}C spins. Since vitamin C is a diprotic acid with pK_a values of 4.21 and 11.79, the amount of protons exchanged with the solvent within the time course of an experiment is much larger in AA solutions than in NaAsc solutions (see Fig. 5).

We deduced that the optimal aqueous (D_2O) scavenging solution to be inserted inside the separator/infusion pump should contain 1–2 M of deuterated NaAsc (much higher concentrations are not possible due to the solubility threshold of sodium ascorbate in D_2O (~2.5 M at 25 °C)), which corresponds to a final infusate concentration of 0.4–0.8 M. A signal intensity gain of 9% was observed at the end of the 9 s infusion in a 0.4 M solution. This corresponds to a blood concentration of 35 ± 5 mM, a dose that is much lower than the reported lethal dose for rats (oral LD_{50} = 11 900 mg/kg, intravenous LD_{50} = 4000 mg/kg (27)), and plasma concentrations as large as 30 mM were used in previous human studies (28). The gain in signal would be even larger for longer injection times.

Similar T_1 measurements were made at 17 °C, which is the base temperature of the separator/infusion pump inside the imager if the hot air is turned off. Although the same behavior was observed when the concentration of vitamin C was increased (see Fig. 2 of supplementary material), the T_1 values were significantly lower at 17 °C. Besides the significant increase

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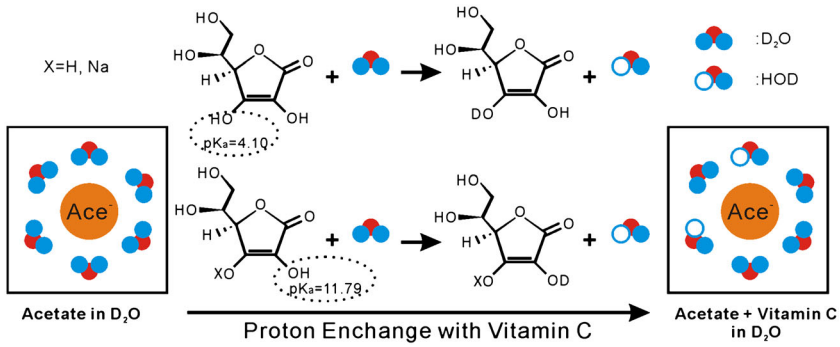

Figure 5. Effect of proton exchange between vitamin C and water molecules on the magnetic environment of acetate molecules in aqueous (D_2O) solutions.

in T_1, two additional reasons lead to the use of hot air to maintain the infusion pump at 33 °C: first the scavenging reaction kinetics is faster at higher temperature, and second the temperature of the hyperpolarized solution is nearly at body temperature at the time of injection, since it is within 5 °C of the pump temperature after its transfer into the pump.

In summary, the method presented herein allows us (a) to precisely determine the infusate ^{13}C polarization inside the imager at the time of the injection without additional delay between dissolution and injection, (b) to scavenge the radicals used as polarizing agents without the cost of reducing the final infusate concentration and (c) to start the injection of hyperpolarized solutions into live animals as rapidly as 3 s following dissolution. This automated protocol leads to highly reproducible experiments and can facilitate the quantitative analysis of *in vivo* hyperpolarized NMR experiments while minimizing the signal losses.

Acknowledgements

We are deeply grateful to Dr. Jacques van der Klink for his invaluable advice. We would also like to thank Mr Martial Rey for his help with the high-resolution NMR measurements, Mr Yves Pilloud, Dr Arthur Magill and Dr. Hongxia Lei for their help with the integration of the RF network, and Chen Shiyu and Professor Christian Heinis for helping with the lyophilization process. This work was supported by the Swiss National Science Foundation (grants 200020_124901 and PP00P2_133562), the National Competence Center in Biomedical Imaging (NCCBI), the Centre d'Imagerie BioMédicale (CIBM) of the UNIL, UNIGE, HUG, CHUV, EPFL, and the Leenards and Jeantet Foundations.

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