Quantitation of a spin polarization-induced nuclear Overhauser effect (SPINOE) between a hyperpolarized $^{13}$C-labeled cell metabolite and water protons

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The spin polarization-induced nuclear Overhauser effect (SPINOE) describes the enhancement of spin polarization of solvent nuclei by the hyperpolarized spins of a solute. In this communication we demonstrate that SPINOEs can be observed between [1,4-$^{13}$C$_2$]fumarate, hyperpolarized using the dissolution dynamic nuclear polarization technique, and solvent water protons. We derive a theoretical expression for the expected enhancement and demonstrate that this fits well with experimental measurements. Although the magnitude of the effect is relatively small (around 2% measured here), the SPINOE increases at lower field strengths, so that at clinically relevant magnetic fields (1.5–3 T) it may be possible to track the passage of a bolus containing a hyperpolarized $^{13}$C-labeled substrate through the increase in solvent water $^1$H signal. © 2014 The Authors. Contrast Media & Molecular Imaging published by John Wiley and Sons, Ltd.

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1. INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy of nuclei other than protons has been limited by a lack of sensitivity. With the development of dissolution dynamic nuclear polarization (DNP), which increases the signal-to-noise ratio in solution-state $^{13}$C experiments more than 10 000 times (1), this limitation can to some extent be overcome. The DNP process involves transferring polarization from free radical electrons, at ~1 K and in a high magnetic field (usually 3.35 T), to the nuclear spin of interest using microwave irradiation. The electron spins return to equilibrium and the thermodynamically coupled nuclear spin polarization is enhanced. The sample is then rapidly dissolved to physiological temperatures while retaining high levels of nuclear spin polarization (up to ~50%) in the liquid state. However, once in the liquid state the polarization decays according to the longitudinal spin–lattice relaxation time, $T_1$, and moreover any sampling of this polarization is destructive.

The nuclear Overhauser effect (NOE) is due to polarization transfer by cross-relaxation from one nuclear spin population to another following perturbation of one of the spins (e.g. by application of RF pulses). SPINOE (spin polarization-induced NOE) describes the enhancement of solvent spin polarization by dissolved solute spins that have been hyperpolarized previously (by DNP or other methods). The inefficient cross relaxation between solute and solvent spins, owing to the relatively low concentration of the solute spins, is overcome by their high levels of polarization. In addition, since the polarization of the solute spins is already perturbed from thermal equilibrium, it does not require the application of RF pulses. Navon et al. were the first to demonstrate transfer of hyperpolarization from optically pumped $^{129}$Xe gas to solution phase proton spins without the need for radio-frequency irradiation (2). They found that the magnitude of the SPINOE enhancement depended on the proximity and relative motion of the molecules and therefore was concentration, diffusion and field-dependent.

In this communication, we demonstrate that a SPINOE can be observed in solutions of [1,4-$^{13}$C$_2$]fumarate that have been hyperpolarized using the DNP technique. We derive a theoretical expression for the expected enhancement and demonstrate that this fits well with experimental measurements. Although the magnitude of the effect is relatively small (~2% for 20 mM [1,4-$^{13}$C$_2$]fumarate hyperpolarized to 36%), the SPINOE is field-dependent (2), and therefore at clinically relevant field strengths (1.5–3 T) it may be possible to track the passage of a bolus of hyperpolarized $^{13}$C-fumarate in the body through the increase in $^1$H signal of solvent water. For example, in a 3.4 T magnet the enhancement is predicted to be ~14%.

2. THEORETICAL

2.1. Intermolecular SPINOE enhancement

In a two-spin system, where the two spins $S$ and $I$ are in different molecules and relax owing to translational diffusion, such as a...
Hyperpolarization of the solvent water, and assuming that $Sz = \gamma$, where $\gamma$ is the gyromagnetic ratio, $h/2\pi$, $k_B$ is the Boltzmann constant, $T$ is the temperature of the sample and $B_0$ is the magnetic field strength.

Inserting eqns (3) and (7) into eqn (6), accounting for the exponential decay of the polarization from the moment it is measured to the time of maximum enhancement, and with hyperpolarization of the $S$ spin ($^{13}$C), $S_2(t_{pol}) > S_0$, one gets the maximum enhancement in terms of independently measurable parameters:

$$\eta_{\text{max}} = -T_1 J_S \gamma S(S + 1) \left( e^{(t_{pol} - t_{\text{max}})\gamma I} \right) P(t_{pol}) P_0$$

where $S$ and $I$ are the spin quantum numbers of the nuclei. For an extended mathematical description the reader is referred to the literature (2,5,6).

Cross-relaxation owing to intermolecular dipole–dipole interactions is caused by the diffusion of solvent water and solute molecules. Since:

$$\sigma_{IS} = \frac{\rho_{IS}^d}{2}$$

and

$$\sigma_{IIS} = \frac{N_S}{N_I} \sigma_{IS}$$

(6) the cross-relaxation rate constant in the absence of molecular binding can be extended to nuclei with different spin quantum numbers as:

$$\sigma_{IS} = h\gamma_S \gamma I \left( \frac{2\pi N_S(S + 1)}{15D_0} \right)$$

$$\sigma_{IIS} = \frac{1N_S(S + 1)}{2N_I(l + 1)\rho_{IS}^d}$$

where $D_0$ is the mean diffusion coefficient of the two species, $d$ is the minimum distance between the two spins, $N_I$ and $N_S$ are the concentrations of nuclear spins in the sample and $\rho_{IS}^d$ is the intermolecular contribution to the spin–lattice relaxation rate constant. Equations (10) and (11) were derived under the extreme narrowing limit, $\omega_{IS} < 1$ which is the case for water protons at room temperature and will also hold for hyperpolarized metabolites in solution (7).

Finally, setting $l = 1 = S = 1/2$, the SPINOE enhancement of the solvent proton spins ($I$) owing to hyperpolarized solute $^{13}$C spins ($S$) is given by:

$$\eta_{\text{max}} = -T_1 J_S \gamma S \left( e^{(t_{pol} - t_{\text{max}})\gamma I} \right) P(t_{pol}) P_0$$

The sign of the enhancement depends on the sign of $\gamma_I$ and $\gamma_S$: if they are both positive (which is the case for $^{13}$C and $^1$H), then the sign of $\eta(t)$ is opposite to that of the polarization of $S$ (6).

### 2.2. Determination of $\rho_{IS}^d$ from measurable parameters

The spin–lattice relaxation time constants for the magnetically equivalent $^{13}$C$_1$ and $^{13}$C$_4$ in 20 mM solutions of $[1,4-^{13}$C$_2]$fumaric acid in 90% $^2$H$_2$O and 10% $^1$H$_2$O were measured (see Experimental section). The relaxation rate constant for $S$ is influenced by intermolecular dipole–dipole interactions with solvent protons ($\rho_{IS}^d$) and deuterons ($\rho_{IS}^{d_d}$), and also intramolecular interactions with fumarate protons and other relaxation mechanisms that contribute to $\rho_{IS}^d$ (8). The spin–lattice relaxation time constants were also measured in 20 mM solutions of $[1,4-^{13}$C$_2]$fumaric acid in 100% $^2$H$_2$O. In this case the relaxation of the $S$ spins is dependent only on intermolecular relaxation with solvent deuterons ($\rho_{IS}^{d_d}$) and on $\rho_{IS}^d$.

The relaxation rate constants owing to the different mechanisms add linearly, resulting in relaxation rate constants that one can measure:

$$\rho_{IS}^d(90\%^2\text{H}_2\text{O}) = \rho_{IS}^d + \rho_{IS}^{d_d}$$

$$\rho_{IS}^d(90\%^2\text{H}_2\text{O}) = \rho_{IS}^d + 0.9\rho_{IS}^{d_d} + 0.1\rho_{IS}^{d_d}$$

Subtracting the two relaxation rate constants in eqns (13) and (14), and using eqns (10) and (11), we derive an equation...
dependent only on parameters that can be measured experimentally:

\[
\rho_{S,I}^{\text{ad}} = \left( \frac{\rho_S(90\%\text{H}_2\text{O}, 10\%\text{D}_2\text{O}) - \rho_S(\text{D}_2\text{O})}{0.9} \right) \frac{1}{1 - \frac{D_S + D_I}{D_S + D_K}}
\]  

(15)

We make the assumptions that, in the same volume of solution, there are the same numbers of protons in 90/10% \(\text{H}_2\text{O}/\text{D}_2\text{O}\) and 100% \(\text{D}_2\text{O}\) solution \((N_1 = N_k)\) and that the minimum approach distance \((d)\) between these nuclei and the carbon spins \((S)\) is the same, \((d_1 = d_0)\). For this calculation we used the ratio

\[
\frac{D_S + D_I}{D_S + D_K} = 1.125
\]

obtained using the known values of the diffusion coefficients of \(\text{H}_2\text{O}\) and \(\text{D}_2\text{O}\) at 30 °C \((2.6 \times 10^{-9} \text{ and } 2.1 \times 10^{-9} \text{ m}^2/\text{s})\), respectively) \((9)\) and, as \(D_S\) is unknown, we take the average value of the ratio in the limit when \(D_S < D_I D_K\) and the limit when \(D_S > D_I D_K\). The degree of enhancement was shown to be relatively insensitive to the value used for \(D_S\) with a maximum difference of 2% in the results obtained between these two extreme cases.

2.3. Time dependence of the enhancement

Equations (4) and (5) predict that the evolution of \(\eta\) with time follows a biexponential function. Following the same reasoning as presented above, a time-dependent expression for \(\eta\) can be expressed in terms of \(\eta_{\text{max}}\). Here it is assumed that \(S_2(0) > S_0\) and \(S_2(t_{\text{max}}) < S_0\):

\[
\eta(t) = \eta_{\text{max}} \frac{\rho_1}{\rho_1 - \rho_S} e^{\max \rho_1(t - e^{-\rho_2})}
\]

(16)

Equation (16) is a biexponential function of the type \(\eta(t) = A e^{-Bt} - e^{-Ct}\), where \(A = \eta_{\text{max}} \frac{\rho_1}{\rho_1 - \rho_S} e^{\max \rho_1}, B = \rho_S\) and \(C = \rho_1\).

The time-point at which the absolute value of the enhancement is at a maximum, \(t_{\text{max}}\), is dependent on the relaxation rate constants of the two species \(l\) and \(S\):

\[
t_{\text{max}} = \frac{\ln(\rho_1/\rho_S)}{\rho_1 - \rho_S}
\]

(17)

An exact result, obtained without making any approximations, is given by Song \((6)\).

It can be seen from eqn (16) that, when intermolecular relaxation is not an important contributor to the relaxation of the \(S\) spin, that is, the difference in the \(\text{C}^{13}\) relaxation rate constants in \(\text{H}_2\text{O}\) and \(\text{D}_2\text{O}, |\rho_S(\text{H}_2\text{O}) - \rho_S(\text{D}_2\text{O})|\), is small, then a smaller enhancement will be observed. The time to reach maximum enhancement \(\eta_{\text{max}}\) also depends on \(T_{1,5}\); the faster the \(S\) spins relax, the earlier the maximum enhancement will be reached (Fig. 1).

3. RESULTS AND DISCUSSION

Measurements of \(\text{C}^{13}\) spin lattice relaxation time constants \((T_{1,5})\) for \([1,4-\text{C}^{13}]\text{fumarate}\) showed that the \(\text{C}^{13}\) nuclei relaxed faster in 90/10% \(\text{H}_2\text{O}/\text{D}_2\text{O}\) than in 100% \(\text{D}_2\text{O}\), that is, the intermolecular dipole–dipole interaction is a strong relaxation mechanism for this molecule (Table 1).

| \(T_{1,5}\) (\(\text{H}_2\text{O}\) (s)) | \(T_{1,5}\) (\(\text{D}_2\text{O}\) (s)) | \(\eta_{\text{max}}\) | \(B^0\) (T) |
|---|---|---|---|
| 27.6 ± 0.3 | 30.8 ± 0.8 | -0.020 ± 0.005 | 9.4 |
| 41.0 ± 1.3 | 66.6 ± 7.5 | -0.14 ± 0.03 | 3.4 |
| 34.3 ± 5.5 | 49.5 ± 1.2 | -0.450 ± 0.238 | 0.001 |

With these values of \(T_{1,5}\) the maximum enhancement of the water proton signal with 20 mM \([1,4-\text{C}^{13}]\text{fumarate}\) hyperpolarized to 36% and dissolved in \(\text{H}_2\text{O}\) was calculated to be ~2% at 9.4 T and ~14% at lower fields [calculated using eqns (12) and (15)].

The water proton \(T_{1,5}\) used for the predicted enhancement was ~2.8 s, which was measured at 9.4 T. Since water protons have a very short correlation time constant (~10^{-11} s), their spin–lattice relaxation time is independent of the magnetic field strength (10). In tissue the \(T_1\) of water protons decreases with increasing magnetic field strength (11) and would need to be accounted for in calculation of the SPINOE. However, in an injected bolus, the \(T_1\) is expected to be approximately the same as that in pure water. Although a gadolinium chelate was used in the DNP process, the concentration of this paramagnetic ion after dissolution was too small (~8 μM) to have an effect on the water proton \(T_1\) (12,13).

The enhancement \((\eta(t))\) was determined by measuring changes in the proton signal integral following injection of a hyperpolarized sample of \([1,4-\text{C}^{13}]\text{fumarate}\) into \(\text{H}_2\text{O}\) and was plotted as:

\[
\eta(t) = \frac{l(t) - l_{\text{non-DNP}}(t)}{l_{\text{non-DNP}}(t) - l_{\text{DNP}}(t)}
\]

(18)

where \(l_{\text{non-DNP}}\) was measured in an experiment using a non-hyperpolarized solution of fumarate. Figure 2 shows the observed enhancement in water protons at 9.4 T after the addition of 20 mM...
[1,4-13C]fumarate that had been hyperpolarized to 36%. The enhancement curves shown in Fig. 2 were calculated using the biexponential function $\{A(e^{-T_1} - e^{-T_2})\}$ defined in eqn (16) and the relaxation rate constants reported in Table 1 (dashed line). $\eta(t)$ is the best fit to the experimental data (solid line).

Figure 2. Measured SPINOE effect curve for solvent protons (water) at 9.4 T following the addition of 20 mM [1,4-13C]fumarate that had been hyperpolarized to 36% at the time of injection ($n = 2$). Error bars are the standard deviation on the mean. $\eta(t)$ was calculated using eqn (16) and the experimental values shown in Table 1 (dashed line). $\eta(t)$ is the best fit to the experimental data (solid line).

5. EXPERIMENTAL

5.1. $T_1$ measurements

All chemicals were acquired from Sigma, unless stated otherwise. Samples of [1,4-13C]fumaric acid (99% 13C, Cambridge Isotope Laboratories) were prepared in 40 mM phosphate buffer, pH 7.4, containing 50 mM NaCl, 40 mM NaOH, 100 mg/L EDTA and 4 mM sodium 3-trimethylsilylpropionate d$_4$ (TSP), as a chemical shift and intensity standard. The buffer was made with either 90/10% H$_2$O/H$_2$O or 100% H$_2$O. 13C $T_1$ ($\rho_3$), and 1H $T_1$ ($\rho_1$) values were measured at room temperature using an inversion-recovery pulse sequence in a vertical 9.4 T magnet. Additional 13C $T_1$ measurements were carried out (a) in a vertical 3.4 T magnet using an inversion-recovery pulse sequence, and (b) in the stray field of the magnet room (~1 mT). For the latter measurement, 0.5 ml of hyperpolarized sample, dissolved in buffer made with either 90/10% H$_2$O/H$_2$O or 100% H$_2$O, was injected into eight 10 mm NMR tubes containing 2 ml of buffer made with either 90/10% H$_2$O/H$_2$O or 100% H$_2$O, respectively, that were maintained in the NMR laboratory background field of ~1 mT at room temperature and then inserted sequentially into the 9.4 T spectrometer magnet at intervals of ~30 s. The first spectrum of each tube was acquired using a flip angle of 6°. The area under the fumarate peak (from both the 13C1 and 13C4 resonances) was integrated and used to determine the error in the prediction, taking into account the error on each of the parameters employed in eqn (15) for prediction of enhancement values in solvent water protons using eqn (12). Propagation of errors, $u^2 = \sum \left(\frac{\partial \eta}{\partial x_i}\right)^2 u^2_{x_i}$, was used to determine the error in the prediction, taking into account the error on each of the parameters employed in eqn (12).

5.2. Hyperpolarization of [1,4-13C]fumaric acid and observation of the SPINOE

13C-labeled fumaric acid was hyperpolarized as described previously (14). Briefly, [1,4-13C]fumaric acid (3.23 mmol) was dissolved in 8.74 mmol dimethyl sulfoxide containing 11.48 mmol of a trityl radical (~18.5 mM; AH111501; GE Healthcare, Amersham, UK) and 0.48 mmol of a gadolinium chelate (~0.8 mM; Gd-3 (15); GE Healthcare, Amersham, UK). The solution was sonicated and centrifuged, and a 40 mg aliquot was hyperpolarized at 3.35 T and ~1.2 K, with sample irradiation with a 94 GHz microwave source operating at 100 mW for 1 h. For some samples no microwave irradiation was used as a control. The solid sample was then rapidly dissolved in 6 ml of the buffer described above, which had been pressurized to 10 bar at 180°C. Half of the sample was injected into a 10 mm diameter NMR tube inside the magnet.
via a transfer line. The other half was used to determine the level of the polarization, at the time of injection, with a polarimeter. Proton signal was acquired with 6° flip-angle pulses every second. The zero time point was taken to be the moment of injection of the material into the NMR tube.

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