Effect of ionic interaction between a hyperpolarized magnetic resonance chemical probe and a gadolinium contrast agent for the hyperpolarized lifetime after dissolution

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Abstract

In hyperpolarization of ^{13}C -enriched magnetic resonance chemical probes in the solid-state, a trace amount of gadolinium (Gd) contrast agent **can be** used to maximize polarization of the ^{13}C nuclear spins. Here, we report systematic measurement of the spin-lattice relaxation time (T_1) and enhancement level of ^{13}C -enriched chemical probes in the presence of various Gd contrast agents in the liquid-state after dissolution. Using two different ^{13}C probes having opposite electric charges at neutral pH, we clearly show the T_1 of hyperpolarized ^{13}C was barely affected by the use of a Gd complex that displays repulsive interaction with the ^{13}C probe in solution, whilst T_1 was drastically shortened when there was ionic attraction between probe and complex.

Hyperpolarization is a promising technique to enhance the sensitivity of nuclear magnetic resonance (NMR) [1]. The hyperpolarized chemical probe, which is a low molecular mass compound (MW ~200) labeled with an NMR-positive nuclei like ^{13}C (**Fig. 1A**), is dissolved in a glassing agent (*e.g.* glycerol/ H_2O) doped with a stable radical such as trityl OX063. The sample mixture is conditioned in a polarizing system (*e.g.* 3.35 T, 1.4 K, 2.8 mbar), and irradiation of the unpaired electrons with microwaves (*e.g.* 94 GHz, 100 mW) transfers the spin polarization from the electrons to the nuclei. Using superheated solvent (~473 K), the polarized sample mixture is dissolved by warming up to around biological temperature, and then transferred for NMR/MRI acquisition. The chemical shift change of NMR-positive nuclei, which is induced upon metabolism of the chemical probe, can be observed in real time depending on the enhanced NMR signals. Following the development of functional hyperpolarized chemical probes and surrounding options, this technology is currently being applied to non-invasive metabolic MR imaging of various animal models and patients with cancer [2-4].

During the polarization of NMR-positive nuclei in the solid-state, the addition of an appropriate concentration range of gadolinium (Gd) contrast agent (**Fig. 1B**) was reported to maximize the enhancement levels by improving the excitation energy transfer via the reduction of the electronic Zeeman relaxation time (T_{1Z}) of the free radical paramagnetic electron [5-7]. However, in the liquid-state after dissolution, the paramagnetic Gd ion is predicted to shorten the spin-lattice relaxation time (T_1) of hyperpolarized nuclei when in close proximity [8, 9], whose concept is explained by the Solomon-Bloembergen-Morgan (SBM) theory of relaxation [10, 11]. According to the theory, relaxation is dependent on the Larmor frequency, magnetic moment as well as frequency of molecular motions and the accessibility of spin nucleus to the paramagnetic ions. Among the molecular motions (rotation, translation and vibration), rotations depend much on the solution temperature and significantly overlaps the MHz region of frequencies, thereby largely affects the spin relaxations. The solvent pH is also an important factor to determine the electric charge of functional group and whole molecule in the solution, which may allow access of chemical probe to Gd ions and enhance their complex formation and spin relaxations. Therefore, a better

understanding of these **factors in dissolution liquids** may contribute to not only optimizing the experimental conditions, but also facilitating the rational design of chemical probes in advance that can circumvent **the hyperpolarized nuclear spin relaxation (T_1 shortening effects)** brought about by the **paramagnetic** Gd ion.

The aim of this study was to systematically evaluate the spin-lattice relaxation time (T_1) and the enhanced ^{13}C NMR signal in the liquid-state after dissolution. In particular, we focused on the ionic charge of Gd complexes and chemical probes at neutral pH. Here, we chose three types of Gd contrast agent [Gd-DTPA (Magnevist®), Gd-DOTA (MagneScope®) and Gd-HP-DO3A (ProHance®)] in clinical use where the Gd^{3+} ion and the type of chelators make a difference in the net valency of ionic charge (-2 , -1 and 0). As ^{13}C -enriched chemical probes, we used $[1\text{-}^{13}\text{C}]\text{pyruvate}$ (pK_a 2.5) and $[1\text{-}^{13}\text{C}]\text{L-alanine amide}$ (Ala-NH_2 , $pK_a \approx 8$, **Suppl. Fig. 1**), which were chosen because they have opposite ionization at neutral pH (-1 , $+1$, respectively) (**Fig. 1A**). $[1\text{-}^{13}\text{C}]\text{pyruvate}$ is the most commonly used hyperpolarized chemical probe for detecting tumors where the metabolic enzyme lactate dehydrogenase (LDH) activity is elevated by Warburg effect [12, 13]. By contrast, $[1\text{-}^{13}\text{C}]\text{Ala-NH}_2$ is a newly developed amino-acid-based probe targeting alanine aminopeptidase N (APN) that plays an important role in the maintenance of basic renal functions and tumor angiogenesis [14].

Figure 2A shows **microwave spectra (94 GHz, HyperSense™, Oxford Instruments) of 100 μl samples containing 1.4 M $[1\text{-}^{13}\text{C}]\text{pyruvate}$ (T_1 : 58.4 sec, 9.4 T NMR, Japan Redox) in a 1:1 glycerol/ H_2O glassing matrix doped with 15 mM trityl radical OX063.** As compared with OX063 alone, the samples doped with 2.3 mM of various types of Gd complex or GdCl_3 induced 15 MHz of upfield shift of the positive polarization peak $[\text{P}(+); \text{from } 93.96 \text{ to } 93.975 \text{ GHz}]$ and downfield of negative peak $[\text{P}(-); \text{from } 94.05 \text{ to } 94.035 \text{ GHz}]$, as reported previously [5]. As shown in **Fig. 2B** and **D**, polarization of each Gd-doped sample with 93.975 GHz microwave achieved approximately 2.6~2.8-fold polarization enhancement than that of normal sample, regardless of the type of Gd complex. Each hyperpolarized sample was dissolved in a superheated neutral pH buffer (100 mM Tris-HCl, 100 mg/L EDTA, pH 7.0) and signal decay in liquid-state was monitored after dissolution

(24.4 mM [$1\text{-}^{13}\text{C}$]pyruvate, 54 μM Gd complex). As shown in **Fig. 2C** and **E**, GdCl_3 drastically reduced the polarization lifetime of ^{13}C nuclear spins (T_1 : 2.6 sec, over 96% decay) via its T_1 shortening effect. By contrast, T_1 decrease remained at 58% (T_1 : 33.7 sec) by the use of macrocyclic Gd-HP-DO3A with neutral charge. Such an effect was minimal in the case of macrocyclic Gd-DOTA with negative charge (T_1 : 52.2 sec) and, even a linear chain structure of Gd-DTPA (T_1 : 54.0 sec). These results indicate that the presence of chelators in the Gd complex prevent the T_1 shortening of [$1\text{-}^{13}\text{C}$]pyruvate elicited by the paramagnetic Gd ions in the complex.

To further evaluate the influence of the ionic interaction, we tested the [$1\text{-}^{13}\text{C}$]Ala- NH_2 probe (T_1 : 28.3 sec, 9.4 T) of monovalent positive charge at pH 7 (**Suppl. Fig. 1**) *i.e.*, opposite charge to that of [$1\text{-}^{13}\text{C}$]pyruvate under the same conditions. During the solid-state polarization, Gd ion and its complexes induced a similar DNP spectra shift (**Fig. 2F**) and polarization enhancement (1.6~2.0-fold) by irradiating with 93.975 GHz-microwave radiation (**Fig. 2G, I**). In the liquid-state after dissolution (24.0 mM [$1\text{-}^{13}\text{C}$]Ala- NH_2 , 54 μM Gd complex), non-ionic Gd-HP-DO3A showed the same extent of T_1 decay (T_1 : 15.1 sec, 53%) as $1\text{-}^{13}\text{C}$ -pyruvate. By contrast, T_1 of the hyperpolarized ^{13}C nuclear spins in Ala- NH_2 was further shortened proportionally by the use of macrocyclic Gd-DOTA with monovalent negative charge (T_1 : 9.3 sec) and linear chain structure of Gd-DTPA with divalent negative charge (T_1 : 6.3 sec). Intriguingly, unlike pyruvate, the polarized NMR signal of the positively charged Ala- NH_2 was retained even in the presence of GdCl_3 with no chelator. The T_1 value was evaluated to be 7.5 sec, which was longer than that in the presence of Gd-DTPA. These results, together with those of pyruvate, suggest that ionic interaction between chemical probe and Gd ion or its complex is directly associated with the extent of the shortening of hyperpolarized ^{13}C lifetime, possibly due to the regulation of the distance between ^{13}C nuclei and Gd ion in the complex. In addition, it seems likely that the structure of the chelator (cyclic or linear) is irrelevant to this effect. The parameters obtained from each sample are summarized in **Table 1**.

Figure 3A shows the relaxation rate of ^{13}C nuclear spin in each sample doped with Gd contrast agent. As compared with the chemical probes alone (relaxation rate: 0), the relaxation rate was around 8.0 per mM of Gd in both chemical probes in the presence of non-ionic Gd-HP-DO3A (0).

By contrast, the relaxation enhanced by Gd-DOTA (-1) or Gd-DTPA (-2) varied depending on their valency and the charge of the ^{13}C -enriched chemical probe. The Gd-induced relaxation of ^{13}C nuclear spin in pyruvate (-1) was minimized due to their ionic repulsion (relaxation rate: 2.0 for Gd-DOTA, 1.4 for Gd-DTPA), whereas positively charged Ala-NH₂ (+1) facilitated the attraction of the Gd complex with negative charge, thereby increasing contact between the hyperpolarized ^{13}C nuclei and the Gd ions, and thus dramatically shortening the hyperpolarized lifetime (relaxation rate: 12.4 for Gd-DOTA, 14.4 for Gd-DTPA). Indeed, this trend of relaxation correlated well with those from inversion recovery measurements under thermal equilibrium (Fig. 3B), supporting the accuracy of the T_1 values from decay curve fitting (Suppl. Info.) and the fact that there is ionic interaction of ^{13}C chemical probe with the Gd complex, which directly influences the T_1 shortening of the ^{13}C nuclei in solution [15]. Thus, careful choice of the type of Gd contrast agent or dissolution pH can minimize the T_1 relaxation by Gd ions for hyperpolarized ^{13}C nuclear spins in solution. These findings may be useful for measurement of metabolic profiles that can be monitored using Gd-enhanced hyperpolarized signals. Furthermore, this information in turn maximizes the rational design of ^{13}C -enriched magnetic resonance chemical probes or Gd complexes in advance, which may circumvent the undesirable T_1 shortening effect in the liquid-state after dissolution.

Conclusions

Our systematic measurement of hyperpolarized NMR has clearly shown that ionic interaction between ^{13}C -enriched magnetic resonance chemical probe and Gd contrast agent is directly associated with the T_1 shortening of hyperpolarized ^{13}C nuclear spins by Gd ions after dissolution. The T_1 shortening effect brought about by Gd ions was repressed minimally in the case of ionic repulsion between Gd complex and hyperpolarized ^{13}C chemical probe. When molecules show ionic attraction, however, the hyperpolarized ^{13}C lifetime was drastically shortened by Gd ions in close proximity to the probe, thereby reducing the enhanced NMR signals. This information provides an experimental basis for solution measurements of hyperpolarized samples enhanced by a Gd contrast agent in the solid-state in order to achieve minimal T_1 shortening even after dissolution. Consideration of these findings will also encourage the rational design of ^{13}C chemical probes and Gd complexes that can circumvent the T_1 shortening in advance.

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Table 1

[1- ¹³ C]Sodium pyruvate (Charge at pH 7.0: -1)							
Gadolinium		Net charge	Solid phase (build-up: 3 h)		Liquid phase		
			τ (min)	P (%)	T ₁ (sec) ^c	ε ^d	Soln. pH ^e
—		—	63.2	3.1	58.4	2730	7.0
Gd-DTPA	(Magnevist)	-2	70.1	8.7	54.0	6750	7.0
Gd-DOTA	(Magnescope)	-1	72.1	8.8	52.2	6020	7.0
Gd-HP-DO3A	(ProHance)	0	71.9	8.9	33.7	3490	7.0
GdCl ₃		+3	70.9	8.5	2.6 ^f	0 ^f	7.0

[1- ¹³ C]L-Alanine amide (Charge at pH 7.0: +1)							
Gadolinium		Net charge	Solid phase (build-up: 35 min)		Liquid phase		
			τ (min)	P (%)	T ₁ (sec) ^c	ε ^d	Soln. pH ^e
—		—	4.6	0.5	28.3	2340	7.2
Gd-DTPA	(Magnevist)	-2	5.1	0.8	6.3	2460	7.2
Gd-DOTA	(Magnescope)	-1	5.5	0.9	9.3	3800	7.2
Gd-HP-DO3A	(ProHance)	0	4.7	0.8	15.1	5200	7.2
GdCl ₃		+3	4.9	0.9	7.5 ^f	2870 ^f	7.1

Fig. 1 The structure of molecules used in this study. **A** ¹³C-enriched magnetic resonance chemical probes. **B** Gadolinium contrast agents in clinical use. The number in parenthesis indicates the net valency of ionic charge in each complex.

Fig. 2 Measurement of solid- and liquid-state polarization of $[1-^{13}\text{C}]$ pyruvate or $[1-^{13}\text{C}]$ Ala-NH₂ using the HyperSenseTM (Oxford Instruments) and 9.4 T NMR (Japan Redox). **A, F** Microwave DNP spectra of 100 μ l samples containing 1.4 M $[1-^{13}\text{C}]$ pyruvate (**A**) or 1.0 M $[1-^{13}\text{C}]$ Ala-NH₂ (**F**) in a 1:1 (pyruvate) or 85:15 (Ala-NH₂) glycerol/water glassing matrix doped with 15 mM trityl radical and Gd ion or its chelate complex (2.3 mM). **B, G** Polarization build-up curves (93.975 GHz microwave, 100 mW) in the presence or absence of Gd. **C, H** Decay curves of the hyperpolarized liquid-state NMR signal after dissolution using a buffer (100 mM Tris-HCl, 100 mg/L EDTA, pH 7.0) (24.4 mM $[1-^{13}\text{C}]$ sodium pyruvate (**C**) or 24.0 mM $[1-^{13}\text{C}]$ Ala-NH₂ (**H**), 54 μ M Gd). **D, I** Relative ^{13}C polarization level of $[1-^{13}\text{C}]$ pyruvate (**D**) or $[1-^{13}\text{C}]$ Ala-NH₂ (**I**) in the presence of each Gd complex or GdCl_3 in solid-state. **E, J** Relative enhancement level of ^{13}C nuclei in pyruvate (**E**) or Ala-NH₂ (**J**) in liquid-state after dissolution in the presence of each Gd complex or ion.

Fig. 3 Relaxation rate of [1-¹³C]pyruvate and [1-¹³C]Ala-NH₂ in the presence [Gd(+)] or absence [Gd(-)] of each Gd complex. **A** Relaxation rate calculated from the decay curve fitting of DNP-NMR data. **B** Relaxation rate from the inversion recovery at thermal equilibrium. Relaxation rate = $[1 - T_{1\text{Gd}(+)}/T_{1\text{Gd}(-)}] / [\text{Concentration of Gd complex (0.054 mM)}]$.

Graphical Abstract (TOC)

The ionic repulsion of a ^{13}C -enriched magnetic resonance chemical probe with a Gd complex, doped as an enhancer of preceding solid-state **polarization build-up**, prevented the T_1 shortening effect by paramagnetic Gd ions in the liquid-state after dissolution. Meanwhile, the ionic attraction between a probe and a complex allowed the Gd ions in close proximity to the ^{13}C nuclei and drastically shortened the hyperpolarized ^{13}C lifetime.