Journal of Magnetic Resonance 270 (2016) 157–160

Contents lists available at ScienceDirect

Journal of Magnetic Resonance

journal homepage: www.elsevier.com/locate/jmr

Communication

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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ARTICLE INFO

Article history:
Received 6 June 2016
Revised 25 July 2016
Accepted 25 July 2016
Available online 27 July 2016

Keywords:
Hyperpolarization
Chemical probe
Spin-lattice relaxation time
Gadolinium
NMR

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

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http://dx.doi.org/10.1016/j.jmr.2016.07.015
1090-7807/

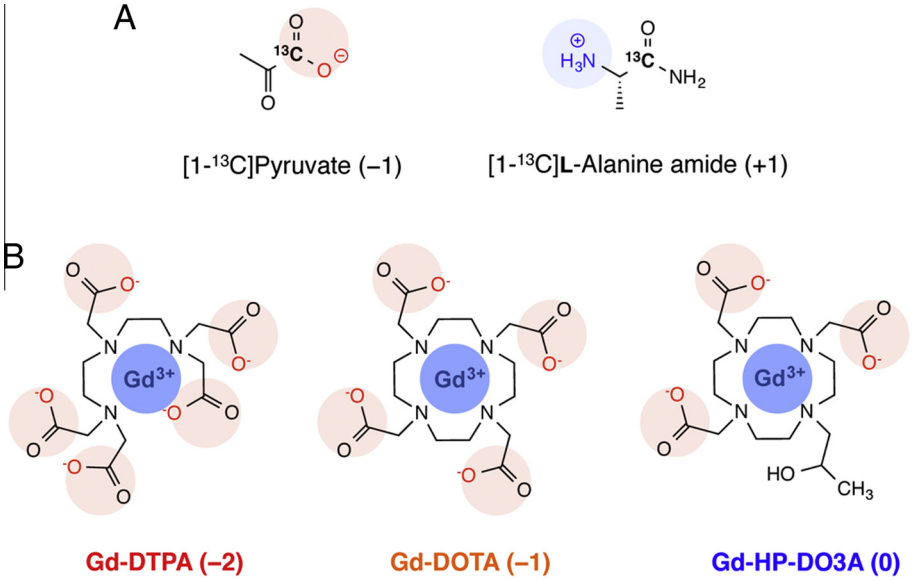

Fig. 1. The structure of molecules used in this study. (A) ^{13}C-enriched magnetic resonance chemical probes. (B) Gadolinium contrast agents in clinical use. The number in parenthesis indicates the net valency of ionic charge in each complex.

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-^{13}C]pyruvate (pK_a 2.5) and [1-^{13}C]**L**-alanine amide (Ala-NH_2, $pK_a \simeq 8$, Suppl. Fig. 1), which were chosen because they have opposite ionization at neutral pH (−1, +1, respectively) (Fig. 1A). [1-^{13}C]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-^{13}C]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].

Fig. 2A shows microwave spectra (94 GHz, HyperSense™, Oxford Instruments) of 100 μl samples containing 1.4 M [1-^{13}C]pyruvate (T_1: 58.4 s, 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 [P(+); from 93.96 to 93.975 GHz] and downfield of negative peak [P(−); from 94.05 to 94.035 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-^{13}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 s, over 96% decay) via its T_1 shortening effect. By contrast, T_1 decrease remained at 58% (T_1: 33.7 s) 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 s) and, even a linear chain structure of Gd-DTPA (T_1: 54.0 s). These results indicate that the presence of chelators in the Gd complex prevent the T_1 shortening of [1-^{13}C]pyruvate elicited by the paramagnetic Gd ions in the complex.

To further evaluate the influence of the ionic interaction, we tested the [1-^{13}C]Ala-NH_2 probe (T_1: 28.3 s, 9.4 T) of monovalent positive charge at pH 7 (Suppl. Fig. 1) *i.e.*, opposite charge to that of [1-^{13}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-^{13}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 s, 53%) as 1-^{13}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 s) and linear chain structure of Gd-DTPA with divalent negative charge (T_1: 6.3 s). 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 s, 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.

Fig. 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-NH_2 (+1) facilitated the attraction of the Gd complex with