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Kinetics of Yttrium−Ligand Complexation Monitored Using Hyperpolarized ^{89}Y as a Model for Gadolinium in Contrast Agents

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At room temperature, the magnetic resonance of low-gamma nuclear spins with $I = {}^1/_2$ often suffers from a lack of sensitivity, owing to the low Boltzmann polarization, the weak induction of low-frequency signals, and acoustic ringing. Moreover, slow nuclear spin−lattice relaxation rates $R_1 = 1/T_1$ make use of long pulse intervals mandatory. Dynamic nuclear polarization[1] (DNP) provides a way to enhance the nuclear spin polarization $P = (P_\alpha - P_\beta)/(P_\alpha + P_\beta)$ at low temperatures and medium magnetic fields (typically at 1.2 K and 3.35 T). Frozen samples can be rapidly dissolved and warmed up to room temperature while retaining much of the polarization that has been built up in the solid state, in the manner of the so-called "*dissolution*-DNP" technique invented in 2003 by Ardenkjaer-Larsen et al.[2] Dramatic sensitivity enhancements, up to 10 000-fold, have been reported for different spin $I = {}^1/_2$ isotopes such as ^{13}C[3], ^{15}N[4], and ^{31}P[5] and for a spin $I = 1$ isotope with a small quadrupole moment, ^{6}Li[6]. Merritt et al.[7] applied dissolution-DNP to yttrium ^{89}Y (100% natural abundance, $I = {}^1/_2$, $\gamma = -1.3163$ rad·T^{-1}·s^{-1}, i.e., 14.67 MHz at $B_0 = 7.05$ T where protons resonate at 300 MHz) using a "Hypersense" polarizer (Oxford Instruments). The longitudinal relaxation time was determined to be $T_1(^{89}\mathrm{Y}) > 600$ s for 15 mM YCl_3 dissolved in D_2O.[7] Such long spin−lattice relaxation times make use of hyperpolarization attractive for ^{89}Y NMR. Yttrium allows one to determine crystal defects in pyrochlores such as $Y_2Ti_{2-x}Sn_xO_7$ (a model for uranium and other actinide storage materials) by ^{89}Y magic-angle spinning (MAS) NMR.[8] The radioisotope ^{90}Y can be used for cancer therapy.[9]

Golman et al.[10] demonstrated that the metabolic conversion of [1-^{13}C]pyruvate into [1-^{13}C]lactate and [1-^{13}C]alanine could be monitored in real time *in vivo* after infusion of hyperpolarized [1-^{13}C]pyruvate in rodents. Recently, we have shown that the long-lived ^{15}N spin states in choline derivatives could also be utilized for the storage of magnetization and subsequently transferred to ^{1}H to increase both sensitivity and spectral resolution.[11] We show herein that the complexation kinetics of Y^{3+} with a ligand can be monitored "on the fly" by means of DNP-enhanced ^{89}Y NMR. We take as an example free yttrium Y^{3+} being complexed with 1,4,7,10-tetrakis(acetamido)-1,4,7,10-tetraazacyclododecane (DOTAM) to form $[Y(DOTAM)(H_2O)]^{3+}$. The yttrium ion Y^{3+} can be used as a model for paramagnetic Gd^{3+} in various complexes, since the coordination chemistry and the ionic radii of these rare earth elements are very similar (Gd^{3+}: 1.053 Å, Y^{3+}: 1.019 Å). It was shown that complexes of both ions are isostructural with chelating ligands used in MRI contrast agents such as DOTA[12] and DTPA-type ligands.[13] Recent studies have raised concerns about the safety of such gadolinium chelates.[14] Several cases of nephrogenic systemic fibrosis have been reported and related to the administration of gadolinium chelate-based contrast agents. The toxicity of these chelates might be related to their tendency to undergo transmetalation with endogenous cations such as Zn^{2+} or Ca^{2+}. Replacing paramagnetic Gd^{3+} by hyperpolarized diamagnetic Y^{3+} should allow one to characterize such exchange processes.[15] NMR of ^{89}Y enhanced by DNP has the advantage that it may be used *in vivo* (in as far as it is compatible with H_2O or serum environment), in contrast to methods such as relaxivity or spectrophotometry.

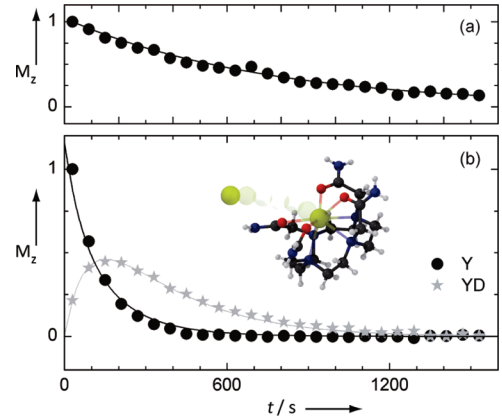

Figure 1. Experimental NMR signal integrals of free yttrium ions Y (black circles) and complexed YD where D = DOTAM (gray stars) in D_2O with 0.5% d_8-glycerol, 54 mM hyperpolarized $^{89}Y^{3+}$, 100 mM DOTAM, and 0.4 mM TEMPO. The spectra were obtained at 60 s intervals with 15° radio frequency (*rf*) pulses at 14.76 MHz (7.05 T). (a) Decay of M_z-magnetization of YCl_3 dissolved in D_2O fitted with $T_1 = 1277 \pm 51$ s (black curve). (b) Complexation of free yttrium by DOTAM. The simulations were calculated for $[Y]_0 = 54$ mM, $[D]_0 = 100$ mM, $[YD]_0 = 0$, $T_{1Y} = 1277$ s, $T_{1YD} = 300$ s and $k = 0.095$ s^{-1}·M^{-1}.

We dissolved YCl_3·$6H_2O$ (Sigma Aldrich) to a concentration of 1 M in 40/60 *v*/*v* glycerol-d_8/D_2O (Cambridge Isotopes) with 30 mM TEMPO free radical (Sigma Aldrich) as a polarizing agent. The solution was rapidly frozen in liquid nitrogen to form 10 μL beads that were further cooled to 1.15 K in a 3.35 T home-built polarizer.[16,17] The ^{89}Y nuclei were then dynamically polarized. The hyperpolarized sample was subsequently rapidly dissolved, and 0.25 mL was transferred into a 5 mm NMR tube (see Supporting Information for experimental details) prefilled with 0.25 mL of a 0.2 mol·L^{-1} D_2O solution of DOTAM at pH 5.01 with 1 M urotropine as buffer and 1 M KCl to maintain constant ionic strength. The DOTAM ligand was synthesized in our laboratory by the method of Maumela et al.[18] The purity was checked by LCMS as well as ^{1}H and ^{13}C NMR. The solution was maintained at 296 ± 0.5 K using a 270 L·h^{-1} air flow with controlled temperature. After an interval of ∼5 s, the solution can be assumed

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to be homogeneous, containing 0.5/99.5 *v*/*v* glycerol-d_8/D_2O, 54 mM of $^{89}Y^{3+}$ (at this point, the polarization was still $P = 3\%$), 100 mM DOTAM, and 0.4 mM TEMPO.

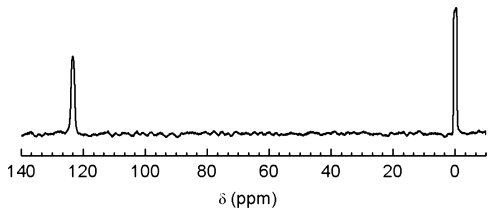

Figure 2. Hyperpolarized ^{89}Y NMR signals recorded 150 s after mixing the two solutions (54 mM YCl_3, 100 mM DOTAM, 0.4 mM TEMPO) at 296 K (23 °C). The spectrum clearly shows two well-resolved peaks of free $^{89}Y^{3+}$ (left) and ^{89}Y-DOTAM (right). The spectrum was collected at 14.76 MHz in a 7.05 T magnet using a single 15° excitation pulse.

Figure 1 shows the time-dependence of the signals arising from the free and complexed forms Y and YD of hyperpolarized $^{89}Y^{3+}$. The two species are well resolved with a chemical shift difference of 123 ppm (1.812 kHz at $B_0 = 7.05$ T) and line widths of 0.04 ppm (0.6 Hz) and 0.4 ppm (6 Hz) for Y and YD respectively. The width of the YD peak may be attributed to intermediate exchange between different conformations of the DOTAM ligand.[19] Figure 2 shows the ^{89}Y NMR spectrum, acquired 150 s after injection, excited with a 15° pulse. Boltzmann's law gives a polarization of $P(^{89}Y) = \tanh(\hbar\omega/2k_BT) = 1.18 \times 10^{-6}$ at 296 K and 7.05 T. Given the very small Boltzmann polarization, the low $^{89}Y^{3+}$ concentration (54 mM) and the long $T_1(^{89}Y) = 1277$ s, such NMR spectra could not be observed without DNP. We estimated the polarization to be enhanced by more than a factor of 4000 by comparing signal integrals with a solution of 4.1 M YCl_3 in D_2O doped with 0.1 M $CuCl_2$ as a relaxing agent to shorten the $T_1(^{89}Y)$ to <10 s.

The complexation of free yttrium Y and DOTAM D obeys second-order kinetics: [Y] + [D] $\rightleftarrows$ [YD]. Because the stability constant is very high for such complexes ($\log K > 10$),[18] the decomplexation rate can be neglected. With the knowledge of initial concentrations [Y_0], [D_0], and [YD_0], the second-order complexation rate constant k can be extracted from the time-dependence of the signals shown in Figure 1. One must distinguish the spin−lattice relaxation rates $1/T_{1Y}$ and $1/T_{1YD}$ (which can be measured independently) and take into account the partial depletion of the longitudinal magnetization by the *rf* pulses with 15° nutation angles. The equations (see Supporting Information) that describe the evolution of the magnetization (without assuming pseudo-first-order two-site chemical exchange[20]) were solved numerically (Mathematica). Fitting gives a second-order complexation rate constant $k = 0.095\ s^{-1}\cdot M^{-1}$. This is very close to $k = 0.097\ s^{-1}\cdot M^{-1}$ measured by Baranyai et al.[21] using spectrophotometry for gadolinium DOTAM complexation under similar conditions.

In conclusion, ^{89}Y hyperpolarization can overcome sensitivity issues and opens the way to monitor very slow complexation processes "on the fly". Other phenomena such as the *in vivo* release of gadolinium (using yttrium as a model) from a ligand via transmetalation with cations such as Zn^{2+} that are present under physiological conditions could be studied in a similar manner. Metals other than ^{89}Y with a spin $I = {}^1/_2$ like ^{57}Fe, ^{77}Se, $^{107,109}Ag$, and $^{123,125}Te$ are potential candidates for DNP enhanced NMR. For example, antitumor and antimicrobial properties of Ag^{1+} (which can react with proteins or bind to bacterial DNA and RNA[22]) could also be observed.

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Supporting Information Available: Experimental procedures and equations used to fit the NMR data to obtain complexation rates. This material is available free of charge via the Internet at http://pubs.acs.org.

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