Hyperpolarized 89Y Complexes as pH Sensitive NMR Probes

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INTRODUCTION

Dynamic nuclear polarization (DNP) can significantly increase NMR sensitivity by a factor of 10,000 times or more,[1] but one fundamental limitation is the decay in magnetization due to T_1 relaxation. Among the NMR active nuclei, ^{89}Y has one of the longest T_1's in the periodic table (some Y(III) complexes have a T_1 of 600 s).[2] ^{89}Y has a spin of ½, a narrow linewidth (3-5 Hz) and is 100% natural abundant so hyperpolarized ^{89}Y becomes an attractive tool for *in vivo* imaging and spectroscopy. The long T_1 not only dramatically expands the window over which hyperpolarized ^{89}Y compounds can be delivered and consequently imaged but it also makes ^{89}Y an ideal choice for use in fly-back imaging techniques where transverse magnetization is restored along the longitudinal axis between acquisitions for resampling. The relatively long T_2's also render ^{89}Y highly suitable for fast imaging methods including FSE or SS-FSE sequences where numerous phase encodes can be conducted without significant loss of coherent transverse magnetization.[3] Furthermore, Y(III) is a pseudo-lanthanide and the sensitivity of the chemical shift of ^{89}Y(III) to its chemical environment could be exploited in the design of sensitive probes to image and map physiological parameters such as pH. Here, we report initial results for Y(III) complexes with two ligands, DOTP and DO3A-NTs (Figure 1). These ligands were chosen because other Ln complexes with these ligands have been reported to exhibit pH dependent properties in the physiologically relevant pH range between 5 and 8.[4,5]

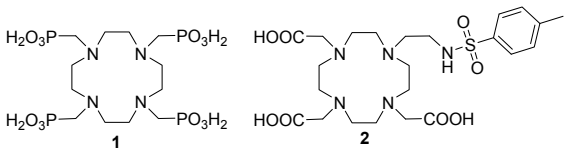

Figure 1. Ligands used in this work

METHODS

Hyperpolarized NMR spectra were collected at 9.4T and 25°C using a Varian VNMRS console and a 10mm probe tuned to 19.6 MHz for ^{89}Y. Samples were polarized using an Oxford DNP HyperSense operating at 1.4K and 3.35T, subject to 94.125 GHz of CW irradiation at 100 mW power. 160 μL samples consisting of either 176 mM YDOTP or 143 mM YDO3A-NTs plus 15 mM OX63 trityl radical dissolved in a 75/25 H_2O/glycerol mixture were hyperpolarized. To ensure proper glassing of the mixture, necessary to achieve optimum polarization levels, samples were pre-frozen outside of the HyperSense in liquid N_2.

RESULTS

Of the 4 mL of hyperpolarized YDOTP solution that was ejected from the HyperSense, 3.6 mL was divided evenly into six 10 mm NMR tubes, each of which contained a previously prepared 400 μL 1M buffer solution of pH 4, 5, 6, 7, 8, and 9. Figure 2 shows the chemical shift dependence on these pH values for YDOTP and YDO3A-NTs, where apparent pK_a values of 7.64 and 5.77, respectively, were estimated from the fit[6]. The ^{89}Y chemical shift of YDOTP gradually decreases from ~150 ppm to ~140 ppm between pH 5 and 8 as the electronic shielding of the ^{89}Y nucleus decreases with increasing protonation of the complex at the non-coordinated phosphonate oxygen atoms. The ^{89}Y chemical shift dispersion of YDO3A-NTs shows the opposite trend, increasing from ~132 ppm at pH 4 to ~157 ppm at pH 9. The pH sensitivity of this complex originates from deprotonation of the tosyl-amide proton which results in coordination of the tosyl N-atom to the Y(III) at higher pH values. Surprisingly, no hyperpolarized ^{89}Y signal for YDO3A-NTs could be observed at any pH between about 5 and 7. ^1H NMR studies on this complex (not shown) indicate that this arises from exchange averaging of intramolecular on- and off-coordination of the tosyl N-atom which exchange-broadens the Y(III) signal. Figure 3 shows the hyperpolarized magnetization decay of YDOTP as a function of time; a fit[7] of these data gives T_1's of 202 s and 57 s at pH 4 and 9, respectively. The origin of this T_1 pH dependence is unknown but one plausible explanation may involve strong ionic interactions between the highly charged $[YDOTP]^{5-}$ anion and multiple quadrupolar $^{23}Na^+$ ions above pH 8 when the complex is fully deprotonated. This could provide an additional relaxation pathway for the ^{89}Y nucleus. While we originally reported[2] only modest signal enhancement for YDOTP (298-fold over thermal equilibrium at 310 K), we managed to achieve considerably higher values (~3000-fold) by optimizing the sample preparation (as described in methods).

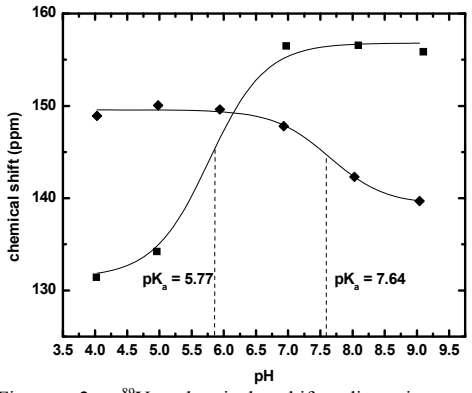

Figure 2. ^{89}Y chemical shift dispersion of hyperpolarized YDOTP (♦) and YDO3A-NTs (■) measured at 9.4 T and 25°C, as a function of pH. Data is referenced to YCl_3 (0 ppm). No signal was observed for YDO3A-NTS at pH 5.5, 6, or 6.5 presumably due to line broadening (see text).

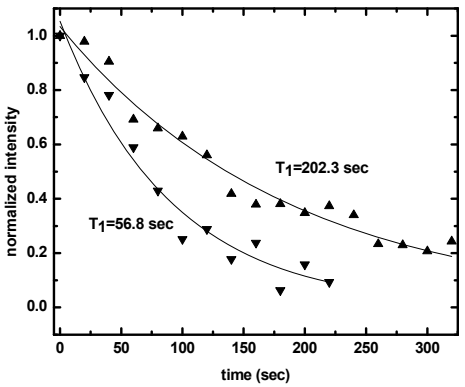

Figure 3. T_1 decay of hyperpolarized YDOTP magnetization, measured at pH 4 (▲) and 9 (▼), along with the corresponding fits.

CONCLUSIONS

We have demonstrated that hyperpolarized ^{89}YDOTP can be used as a pH sensor. The chemical shift dispersion of this complex covers about 10 ppm in the pH range of 5 to 9 due to the protonation of the non-coordinating phosphonate oxygen atoms. Interestingly, the T_1 values of YDOTP are also pH dependent - 202.3 and 56.8 seconds at pH 4 and 9, respectively. Although the ^{89}Y chemical shift dispersion of YDO3A-NTs is even more favorable, over 20 ppm, the ^{89}Y signal for this complex could not be detected at physiologically relevant pH values apparently due to intramolecular intermediate exchange processes. Finally, we managed to achieve significant signal enhancements by optimizing the sample preparation, clearly important for future *in vivo* spectroscopy and imaging applications.

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