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Hyperpolarized ^{89}Y Complexes as pH Sensitive NMR Probes

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Dynamic nuclear polarization (DNP) of an NMR sample can significantly increase sensitivity by creating nuclear spin polarization levels that are much higher than ambient temperature Boltzman levels. DNP is based on the transfer of electron spin polarization from a stable free radical to coupled nuclear spins by microwave irradiation in a frozen glass matrix at low temperatures (~1 K). The method gained practical importance when it was demonstrated that compounds hyperpolarized in the solid state could be dissolved and transferred into an NMR magnet for spectrum acquisition with negligible loss of polarization.[1] Liquid state DNP NMR offers dramatic signal enhancements, 10 000-fold or more, for some ^{13}C and ^{15}N enriched compounds. Such increases in sensitivity have made it possible to perform molecular/functional imaging of nuclei other than ^{1}H. Hyperpolarized ^{13}C labeled substrates, particularly [1-^{13}C]-pyruvate, have successfully been used to study metabolism in various normal and diseased tissues.[2] While the advantages of ^{13}C labeled metabolites as imaging agents are obvious, the typical longitudinal relaxation time (T_1) of ^{13}C nuclei (few seconds to ~1 min) limits the metabolic processes that can be studied by hyperpolarized ^{13}C compounds. The time constraint emerging from the inevitable decay of polarization motivates the search for long T_1 agents. Among the NMR active nuclei, ^{89}Y in its diamagnetic 3+ oxidation state has one of the longest T_1 relaxation times known (600 s or longer).[3] This very long T_1 combined with a favorable spin quantum number ($^1/_2$), sharp NMR line width (3−5 Hz), and 100% natural abundance makes hyperpolarized ^{89}Y attractive as a potential *in vivo* imaging and spectroscopy probe.[3]

In addition, Y(III) is a pseudolanthanide so ligand systems developed for Gd(III)-based MRI contrast agents can be used to safely chelate Y(III) for biomedical applications. Most importantly, the sensitivity of the chemical shift of ^{89}Y(III) to its chemical environment could be exploited in the design of probes to image physiological parameters such as pH, temperature, and other indices of metabolism *in vivo*. Among the physiological parameters that describe a particular state of an organism, pH is considered to be one of the most important. Extracellular pH is tightly regulated, and even small deviations from normal are indicative of a metabolic abnormality. For example, the acidic microenvironment of various tumors due to the Warburg effect is well documented.[4] Imaging of extracellular pH around a tumor could provide relevant information for tumorogenesis and therapy; however, current methods based on microelectrodes, ^{31}P or ^{1}H NMR, are invasive, have poor spatial resolution, and/or offer a poor signal-to-noise ratio (SNR).[5] The successful application of hyperpolarized ^{89}Y(III) chelates as pH responsive agents requires complexes that exhibit a significant change in the coordination environment around the Y(III) ion as a function of pH in the physiologically relevant pH range (pH 5 to 8). We have selected two ligands, DOTP (**1**) and DO3A-NTs (**2**) (Figure 1), because Ln(III) complexes of these ligands have been reported to exhibit pH-dependent properties in the desired pH range.[6,7] The ^{31}P chemical shift of several LnDOTP complexes show a marked pH dependence as a result of the protonation of the noncoordinating phosphonate oxygens, while GdDO3A-NTs exhibits pH-dependent relaxivity due to the intramolecular coordination of the tosyl nitrogen side arm.

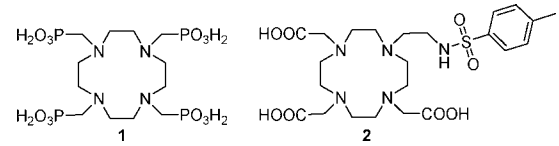

Figure 1. Ligands studied in this work.

Because of the combined effects of a low γ, low sensitivity, and long T_1, acquiring ^{89}Y NMR data on thermally polarized samples is impractical, often requiring days even for concentrated solutions. Our preliminary experiments have shown that various chelated forms of Y(III) including YDOTP can be polarized with currently available commercial hardware using the trityl radical (OX63) as a polarizing agent.[3] While only a modest signal enhancement was observed previously for YDOTP (298-fold over thermal equilibrium at 310 K), we are now able to routinely achieve much higher enhancements (~3000-fold) by optimizing the sample preparation prior to DNP (see Supporting Information).

The ^{89}Y chemical shift of hyperpolarized ^{89}YDOTP as a function of pH is shown in Figure 2. Given the long T_1 of YDOTP, the entire ^{89}Y chemical shift versus pH data set was collected using a single batch of hyperpolarized YDOTP in ~3 min. In comparison, collecting this entire titration curve on thermally polarized samples would have required 24 h for each pH value. A single ^{89}Y(III) resonance was seen at all pH values showing that the protonated and deprotonated species are in fast exchange. The ^{89}Y chemical shift gradually decreases from 150 ppm to 140 ppm between pH 5 and 8 following a reverse sigmoid curve as the electronic shielding of the ^{89}Y nucleus decreases with increasing protonation of the complex at the noncoorodinating phosphonate oxygens.[6] The apparent macroscopic protonation constant of the complex could be determined by fitting this curve ($pK_a = 7.6$). Thermally polarized ^{31}P NMR spectra of YDOTP were also run as a function of pH to confirm the hyperpolarized Y(III) results. The ^{31}P chemical shift dispersion followed a similar trend (Figure S1), and fitting the data gave a protonation constant (pK_a) of 5.7. While this value is in agreement with the average pK_a's of the four protonation steps reported previously for other LnDOTP complexes,[6] the pK_a value obtained from the ^{89}Y chemical shift dispersion is significantly higher and reflects the fact that the coordination environment of

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the central Y-ion is strongly affected by protonation of the first noncoordinating oxygen while further protonations have much less effect. This is likely the consequence of the relaxation of the coordination cage around the Y-ion occurring on protonation, as has been suggested for protonated LnDOTP comlexes.[6]

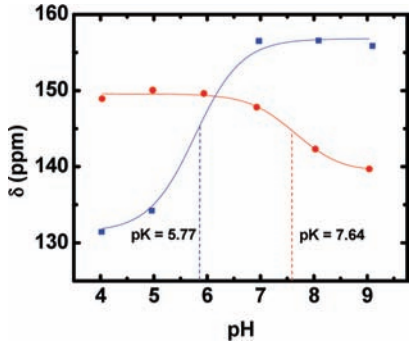

Figure 2. ^{89}Y chemical shift dispersion of hyperpolarized YDOTP (●) and YDO3A-NTs (■) as a function of pH (9.4 T and 25 °C). The YDOTP data were collected from a single sample adjusted to different pH values after hyperpolarization while the YDO3A-NTs data required collection on different hyperpolarized samples due to rapid relaxation.

The ^{89}Y chemical shift dispersion of YDO3A-NTs in the pH range of 4 to 9 followed an opposite trend and could be approximated by a sigmoid curve as it increased from 132 ppm at pH 4 to ~157 ppm at pH 9. This trend can be explained by the pH dependent intramolecular coordination of the tosyl N-atom whereby two metal bound water molecules are replaced by the sulfonamide pendant arm resulting in a decreased shielding of the ^{89}Y nucleus.[7] The apparent pK_a obtained from this curve (5.8) is in reasonable agreement with the pK_a value reported for EuDO3A-NTs (6.4) by luminescence measurements.[7]

Surprisingly, no hyperpolarized ^{89}Y signal for YDO3A-NTs could be observed at various pH values between pH 5 and 7. Likewise, we were unable to generate a signal from thermally polarized samples in this pH range. One possible explanation for this loss of signal could be that the two different chemical species present in solution, presumably the Y(III)-coordinated versus uncoordinated tosyl N-atom, are exchanging at a critical rate that results in extreme line broadening at these intermediate pH values. This hypothesis is supported by variable pH ^{1}H NMR studies in which exchange broadened spectra were recorded at pH 5, 6, and 7 indicating the presence of several species (Figure S2). These results are in agreement with the ^{1}H NMR studies performed on the Eu(III) complex of DO3A-NTs in which cooperative arm rotation was also observed in addition to the intramolecular ligation of the tosyl N-atom.[7]

Long longitudinal relaxation times are essential for *in vivo* imaging of hyperpolarized materials so the T_1 of YDOTP was measured at three different pH values to evaluate the sensitivity of T_1 to pH. Magnetization decay curves such as that shown in Figure 3 were fitted to give T_1 values of 202, 123, and 57 s at pH 4, 7, and 9, respectively. In human blood serum, the T_1 was found to be ~20% shorter (see Supporting Information). These data indicate that YDOTP has an adequately long T_1 at pH 7 for *in vivo* imaging. In comparison, the T_1 of hyperpolarized ^{13}C-bicarbonate ($H^{13}CO_3^-$), which has been used for imaging pH *in vivo*, is ~10 s.[8] At present we do not have a satisfactory explanation for the unexpectedly large changes in T_1 with pH, although a plausible explanation might involve the interaction of quadrupolar $^{23}Na^+$ ions with the highly charged $[YDOTP]^{5-}$ anion above pH 8, which may allow an additional relaxation pathway for the ^{89}Y nucleus. Ln-complexes of DOTP have been reported to bind Na^+ relatively strongly with a binding constant (log K) of ~2.6.[6]

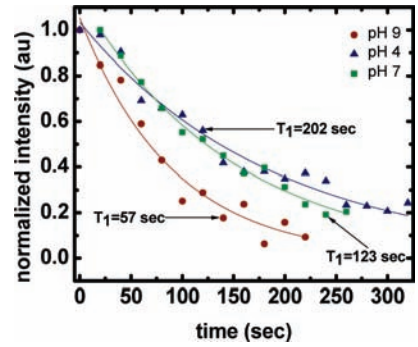

Figure 3. T_1 decay of hyperpolarized YDOTP magnetization at pH 4 (▲), 7 (■), and 9 (●), with $n = 2$ and an error of approximately ±10 s.

We were unable to measure the T_1 of YDO3A-NTs due to the rapid decay of the hyperpolarized signal. Although this makes YDO3A-NTs less attractive for *in vivo* imaging, it does provide insight into possible relaxation mechanisms that must be avoided in developing further long T_1 ^{89}Y agents.

In summary, we have demonstrated the potential of using hyperpolarized ^{89}YDOTP as a pH sensor. The chemical shift of this complex changes ~10 ppm over the pH range 5−9 due to protonation of the noncoordinated phosphonate oxygen atoms in the complex. Although the T_1 of ^{89}Y in YDOTP is also pH dependent, ranging from 202 s at pH 4 to 57 s at pH 9, it is sufficiently long for *in vivo* applications at physiological pH values. The pH dependent ^{89}Y chemical shift of YDO3A-NTs is even larger (20 ppm) over this same pH range but chemical exchange processes in this molecule cause both line broadening and rapid T_1 decay of the ^{89}Y signal making it unattractive for imaging applications. Finally, we managed to achieve significant signal enhancements (over 3000-fold) by optimizing the sample preparation, an important consideration for future *in vivo* spectroscopy and imaging applications.

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Supporting Information Available: Synthesis of the yttrium complexes; DNP sample preparation; and ^{1}H, ^{31}P, and ^{89}Y NMR experimental details and data. This material is available free of charge via the Internet at http://pubs.acs.org.

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