PARACEST Agents: Modulating MRI Contrast via Water Proton Exchange

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ABSTRACT

Scientific interest in optimizing the properties of gadolinium (III) complexes as MRI contrast agents has led to many new insights into lanthanide ion coordination chemistry in the last two decades. Among these was the surprising observation that water exchange in lanthanide (III) derivatives of DOTA can be modulated dramatically by judicious choice of ligand side chain and Ln^{3+} ionic radii. This resulted in the discovery of paramagnetic CEST agents for altering MRI image contrast based upon the chemical exchange saturation transfer mechanism. The goal of this article is to review the factors that govern water molecule and water proton exchange in these complexes and to compare the potential sensitivity of PARACEST agents versus Gd^{3+}-based T_1 relaxation agents for altering tissue contrast.

Introduction

Scientific interest in lanthanide ion coordination chemistry has grown substantially over the past 25 years, driven largely by the emergence of magnetic resonance imaging (MRI) as a preeminent tool in clinical medicine.[1] Gadolinium (III) is most often used as a "contrast agent" for MRI because it is the most efficient of all lanthanide ions at relaxing bulk water protons.[2,3] The remaining paramagnetic lanthanide ions are much less efficient at relaxing water protons but can induce sizable NMR hyperfine shifts in nearby NMR nuclei.[4] Hyperfine shifts can provide useful quantitative structural information or may simply be used to "resolve" resonances of NMR active nuclei in biological compartments. This feature has been particularly attractive for separating the NMR signals of intra- and extracellular Na^+ in isolated cells, organs, and tissues in vivo.[5,6] The frequency shifting ability of the remaining lanthanide ions has not found an application in proton imaging because, unlike Gd^{3+}-based relaxation systems where rapid water exchange is considered imperative, fast water exchange effectively reduces water proton hyperfine shifts to near zero (weighted average of a small shifted pool and a large bulk pool). Very recently, however, several slow water exchange lanthanide complexes have been reported,[7−15] and in these systems highly hyperfine shifted Ln^{3+}-bound water resonances are often seen by high-resolution NMR. As we shall see, this feature once again offers an opportunity to take advantage of the variable hyperfine shifting ability of the lanthanide ion series.[16]

An alternative way to introduce contrast into a MR image would be to alter the total water signal detected in the experiment. This was demonstrated by Balaban and co-workers[17] when they used a presaturation pulse to saturate the broad water signal that lies beneath the sharper bulk water signal in many tissues. This broad resonance is thought to reflect water tightly associated with tissue proteins, lipids, and/or subcellular structures and appears to change with progressive diseases such as multiple sclerosis, ALS, etc. Upon saturation, exchange

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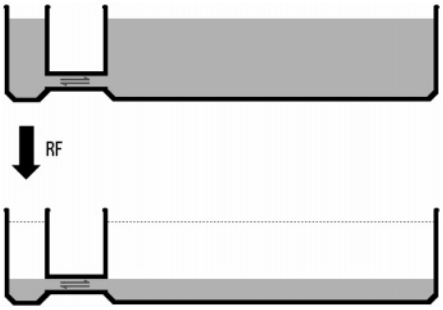

FIGURE 1. A scheme of a two pool CEST model. By selective saturation of the small pool (the exchanging −OH or −NH site), the large pool (bulk water) will decrease also if the chemical exchange kinetics are appropriate.

occurs between the bound water pool and the larger bulk water pool resulting in a decrease in bulk water signal intensity (Figure 1). This technique is now commonly known as magnetization transfer (MT) imaging.[18] More recently, this same group demonstrated that low molecular weight compounds with slowly exchanging −NH or −OH protons may also be used to alter tissue contrast via chemical exchange saturation transfer (CEST) of presaturated spins to bulk water.[19] The molecules first used to demonstrate the CEST effect were diamagnetic and thereby had proton chemical shifts not much different from that of bulk water (−NH and −OH groups are typically well within 5 ppm of bulk water). Paramagnetic analogues of such compounds were not known until the discovery of lanthanide complexes having unusually slow water exchange kinetics.[16,20] In this Account, we review the impact of lanthanide ion size and ligand stucture on water exchange and demonstrate that large hyperfine shifting lanthanide ions such as Dy^{3+}, Tb^{3+}, Tm^{3+}, and Yb^{3+} offer advantages for introducing MRI contrast via the CEST mechanism. Such slow water exchange paramagnetic complexes are referred to herein as PARACEST agents.

Tuning the Bound Water Lifetime in Ln^{3+} Complexes

During early development of Gd^{3+}-based MRI contrast agents, it was commonly assumed that water exchange between Ln^{3+}-bound, inner-sphere coordination sites and bulk solvent was so fast that it effectively had no impact on the relaxation characteristics of those complexes. However, more recent studies have shown that this is not always the case. For example, it is now known that the bound water lifetime (τ_M) in two clinically used MRI agents, $GdDTPA^{2-}$ and $GdDOTA^{-}$ (both have one inner-sphere bound water, $q=1$), is ~240 ns,[21,22] ~100-fold longer than the inner-sphere water lifetime of the Gd^{3+} aqua ion.[23−25] Solomon−Bloembergen−Morgan theory of paramagnetic relaxation indicates that water exchange in $GdDTPA^{2-}$ and $GdDOTA^{-}$ lowers the relaxivity of these complexes somewhat below their theoretical optimum, but this is irrelevant in terms of their clinical efficacy. τ_M is about 10-fold longer (~2 μs) in bis-amide derivatives of DTPA, like GdDTPA-BMA[26,27] and longer still (~20 μs) in tetra-amide derivatives of DOTA such as $GdDTMA^{3+}$.[7,28] Clearly, complexes with even slower water exchange are detrimental if ones goal is to optimize relaxivity but this same feature does allow one to consider using these complexes as PARACEST agents.

Factors Affecting τ_M: Lanthanide Coordination Geometry

Early NMR studies of the $LnDOTA^{-}$ complexes showed that two slowly interconverting species are present in aqueous solution and that the populations of these two species are sensitive to the size of the central Ln^{3+} cation.[29] These two coordination isomers have since been assigned to the square antiprism (commonly referred to as the M isomer) and twisted square antiprism (commonly referred to as the m isomer) structures, related to each other by rotation of the side-chain acetate groups. Interestingly, it was subsequently discovered that these two species have quite different bound water lifetimes, the m isomer showing much faster exchange compared to the M isomer. For example, τ_M is 120 μs for the M isomer of $EuDTMA^{3+}$ but only 2 μs for the m isomer.[7,8,10]

Factors Affecting τ_M: Identity of the Ligand Side Chains

As noted above, the bound water lifetimes for LnDOTA−tetra(amide) complexes tend to be considerably longer than for the corresponding $LnDOTA^{-}$ complexes. The shapes of ^{17}O curves of Figure 2 immediately show that water exchange is quite different in these complexes and hence sensitive to the number of amide substitutions. Although a detailed analysis is beyond the scope of this review,[30] the maxima in each of these curves (near 328 K for the mono-amide, near 355 K for the bis-amide, and above 370 K for the tetra-amide) show that the bound water lifetimes are in the order GdDOTA−4AmP > GdDOTA−2AmP > GdDOTA−1AmP. A complete fitting of these data to theory gives τ_M values of 26, 6.2, and 1.3 μs, respectively, for the M isomers in each complex. The bound water lifetimes in the m isomers are also much shorter (~6−20 ns) and differ in this series. This illustrates that even one amide side chain appears to have a significant impact on the bound water lifetime.

The chemical characteristics, i.e., charge and polarity, of the extended amide side-chain arms also play a role in determining τ_M. For the series of EuDOTA−tetraamide complexes shown in Figure 3, it has been found that τ_M varies considerably and this has been correlated with a calculated solvent accessibility parameter. Given that the mechanism of water exchange in these tetra-amide complexes is thought to be dissociative,[15] greater solvent accessibility to the Eu^{3+}-bound water may aid in dissociation of the bound water by hydrogen bonding. In general, for EuDOTA−tetra(amide) complexes, the bound water lifetime correlates with polarity of the ligand side chains, i.e., phosphonate ester ≥ carboxylate ester ≫ alkyl groups ≥ simple amides. For example, τ_M's decrease in order of

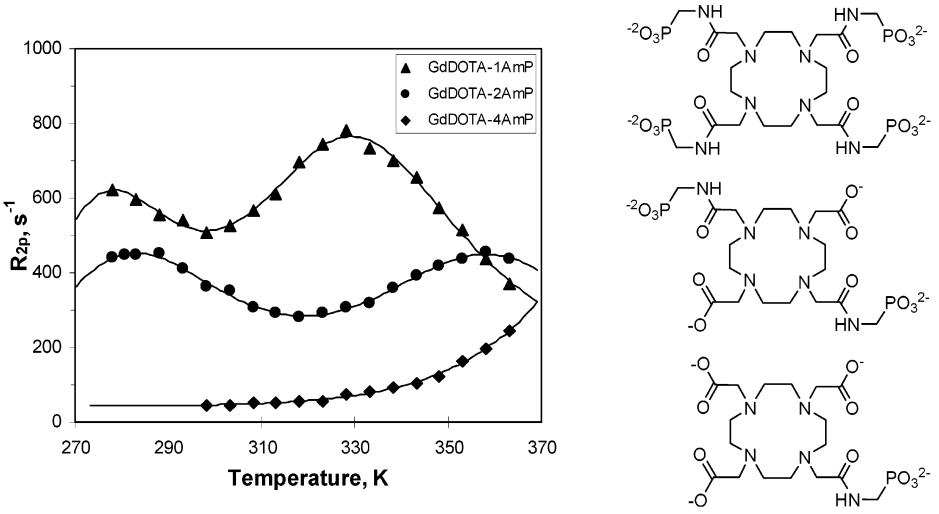

FIGURE 2. Plots of the ^{17}O transverse relaxation rate vs temperature for Gd^{3+} complexes of tetra-, bis-, and mono-amide derivatives of DOTA (unpublished data).

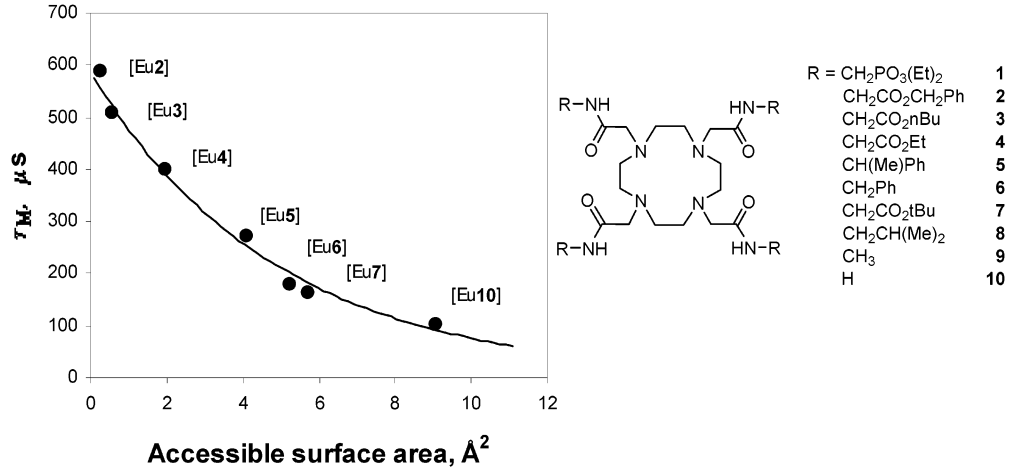

FIGURE 3. Relationship between coordinated water molecule lifetimes in Eu-tetraamide complexes vs the calculated solvent accessible surface around the bound water (adapted from ref 15). Although solvent accessible surface areas have not been reported for compounds **1**, **8**, and **9**, their structures are included here because they are referred to in the text.

Eu(**1**)$^{3+}$ at 1.3 ms[12] > Eu(**4**)$^{3+}$ at 780 μs[11] ≫ Eu(**5**)$^{3+}$ at 278 μs[9] > Eu(**8**)$^{3+}$ at 146 μs (unpublished) ∼ Eu(**9**)$^{3+}$ at 156 μs[8] > Eu(**10**)$^{3+}$ at 120 μs[7] (all for M isomers measured in wet acetonitrile). We have also observed that Eu^{3+}-bound water lifetimes for complexes that can be measured in more than one solvent are about 2-fold shorter in aqueous solution than in wet acetonitrile[11] and complexes with negatively charged side chains have shorter τ_M values than their neutral counterparts. For example, the τ_M found for EuDOTA−4AmC$^-$ (300 μs, pH 7, aqueous) is somewhat shorter than the τ_M for the corresponding ester, EuDOTA−4AmCE^{3+} (382 μs, pH 7.4, aqueous).[11,31] Both observations suggest that there is proton exchange contribution to τ_M in aqueous media.

Factors Affecting τ_M: Molecular Exchange versus Prototropic Exchange

Aime et al.[32] recently published a short review paper on the relaxivity contributions from the prototropic exchange versus the water molecule exchange in Gd^{3+} complexes. It has been observed that prototropic exchange is accelerated in complexes such as these by acid and/or base and that an increase in prototropic exchange results in an increase in relaxivity below pH ∼2 and above pH ∼9−10.[28] This early observation prompted us to examine more closely the water relaxivity of GdDOTA−4AmP as a function of pH. In this water-soluble complex, τ_M as measured by ^{17}O NMR was ∼26 μs over the entire pH range, 6−9.5; yet the water ^1H relaxivity steadily increases between pH 8.5 (r_1 = 4.5 $mM^{-1}s^{-1}$) and pH 6 (r_1 = 9.8 $mM^{-1}s^{-1}$).[33] Thus, upon protonation of the extended phosphonate groups (four pK_a's ranging from 8.5 to 6), the protons on a single Gd^{3+}-bound water undergo prototropic exchange with bulk solvent. Such pH-dependent relaxivity is not a feature of current clinical Gd^{3+}-based CAs where water exchange is relatively fast (τ_M < 240 ns), so the slow water exchange feature of GdDOTA−4AmP makes this an attractive agent for mapping extracellular pH in tissues by MRI.[34]

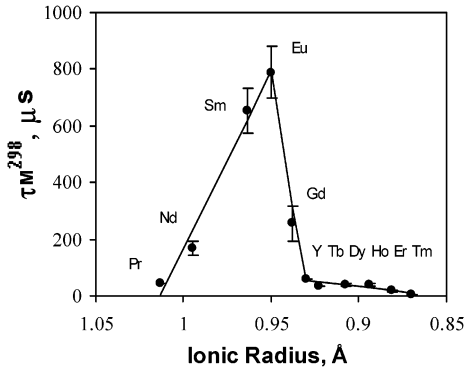

FIGURE 4. Plot of experimental τ_M vs Ln^{3+} ionic radii for the LnDOTA−4AmCE complexes. The τ_M values were obtained by fitting the temperature dependence of either the ^{17}O or ^1H NMR bound water line widths (from ref 14).

Factors Affecting τ_M: Lanthanide Cation Size

Merbach et al.[27] found for series of LnDTPA−BMA complexes (Ln^{3+} = various lanthanide cations) that the Ln^{3+}-bound water lifetimes decreased along the series from 1.88 μs (Nd^{3+}) to 0.16 μs (Ho^{3+}) and suggested that this reflected a change in water exchange mechanism, from interchange (I_a) for the larger cations to dissociative (D) for the smaller cations. More recently, Parker et al.[9] reported that the bound water lifetime of a EuDOTA−tetra(amide)$^{3+}$ might be as much as 500 times that of the corresponding Yb^{3+} complex. We subsequently found that the bound water lifetimes of EuDOTA−4AmCE[11] and EuDOTA−4AmPE[12] are about 2-fold longer than those of corresponding Gd^{3+} complexes, even though Eu^{3+} and Gd^{3+} complexes are usually considered isostructural. To help clarify the relationship between τ_M and Ln^{3+} ionic radius, we determined τ_M values for the remaining complexes in this series and found that τ_M is highly sensitive to ionic radii for those ions nearest Eu^{3+} (Figure 4).[14] Although the data appear to separate into three distinct subgroups, two crystal structures (the Eu^{3+} and Tb^{3+} complexes) and a lanthanide induced shift (LIS) analysis of paramagnetic ^1H shift indicate that the entire series is isostructural (all largely the **M** isomer). Therefore, the sharp changes in τ_M versus Ln^{3+} radii (Figure 4) must be ascribed to altered water exchange mechanisms along the series.

Characterization of PARACEST Agents

Two-Site Exchange Theory. The basic physical process is that of chemical exchange between two pools of protons. Pool A is bulk water, and pool B is water bound to the chelated lanthanide ion. Pool B is chemically shifted by the paramagnetic lanthanide away from pool A. The basic NMR experiment is to apply RF irradiation at the Larmor frequency of pool B for a time sufficiently long so as to reach a steady-state in which the magnetizations in the rotating frame are time-independent.

The NMR behavior of the two exchanging pools of nuclei is described by the modified Bloch equations that include the effects of chemical exchange on the X, Y, and Z spin magnetizations of both pools of nuclei. These equations are given below:[35,36]

$$\frac{dM_X^a}{dt} = -(\omega_a - \omega)M_Y^a - k_{2a}M_X^a + C_bM_X^b \quad (1)$$

$$\frac{dM_X^b}{dt} = -(\omega_b - \omega)M_Y^b - k_{2b}M_X^b + C_aM_X^a \quad (2)$$

$$\frac{dM_Y^a}{dt} = (\omega_a - \omega)M_X^a - k_{2a}M_Y^a + C_bM_Y^b - \omega_1M_Z^a \quad (3)$$

$$\frac{dM_Y^b}{dt} = (\omega_b - \omega)M_X^b - k_{2b}M_Y^b + C_aM_Y^a - \omega_1M_Z^b \quad (4)$$

$$\frac{dM_Z^a}{dt} = \frac{M_0^a}{T_{1a}} - k_{1a}M_Z^a + C_bM_Z^b + \omega_1M_Y^a \quad (5)$$

$$\frac{dM_Z^b}{dt} = \frac{M_0^b}{T_{1b}} - k_{1b}M_Z^b + C_aM_Z^a + \omega_1M_Y^b \quad (6)$$

where

$$k_{1a} = \frac{1}{T_{1a}} + C_a \quad (7)$$

$$k_{2a} = \frac{1}{T_{2a}} + C_a \quad (8)$$

and ω_a is the Larmor frequency of nuclei in pool A. In these definitions, T_{1a} is the spin−lattice relaxation time of A, T_{2a} is the transverse relaxation time of A, and C_a is the transition rate of A nuclei in leaving A and is equal to $1/\tau_a$. Similar definitions apply to B nuclei; $C_b = 1/\tau_M$. Since exchange is a detailed-balance phenomenon, $\tau_a = \tau_M/(M_0^b/M_0^a)$. It is convenient to use the RF intensity B_1 as expressed in Hertz: $B_1 = \omega_1/2\pi$.

A mathematical expression for M_z^a/M_0^a was obtained from a solution of this set of equations under steady-state conditions in which the time derivatives of all the nuclear magnetizations are zero. The result is a complicated function of the seven NMR parameters (T_{1a}, T_{2a}, T_{1b}, T_{2b}, M_0^b, M_0^a, τ_M, and the Larmor frequency difference $\Delta\omega = \omega_a - \omega_b$) and the two experimental variables (B_1 and irradiation frequency).

The maximum reduction in M_z^a/M_0^a caused by chemical exchange occurs when the last two terms in eq 5 are zero. This situation occurs when (a) the B nuclei are completely saturated so that $M_z^b = 0$ and (b) there is no direct excitation of pool A nuclei by the B_1 irradiation that excites B nuclei. Condition a is met when B_1 is sufficiently large. Condition b is met when the relative Larmor frequency shift of A is very large so that $\Delta\omega/\omega_1 \gg 1$. Under these conditions, the steady-state solution of eq 5 gives

$$\frac{M_Z^a}{M_0^a} = \frac{\tau_a}{\tau_a + T_{1a}} = \frac{1}{1 + \frac{cq}{55.5}\frac{T_{1a}}{\tau_M}} \quad (9)$$

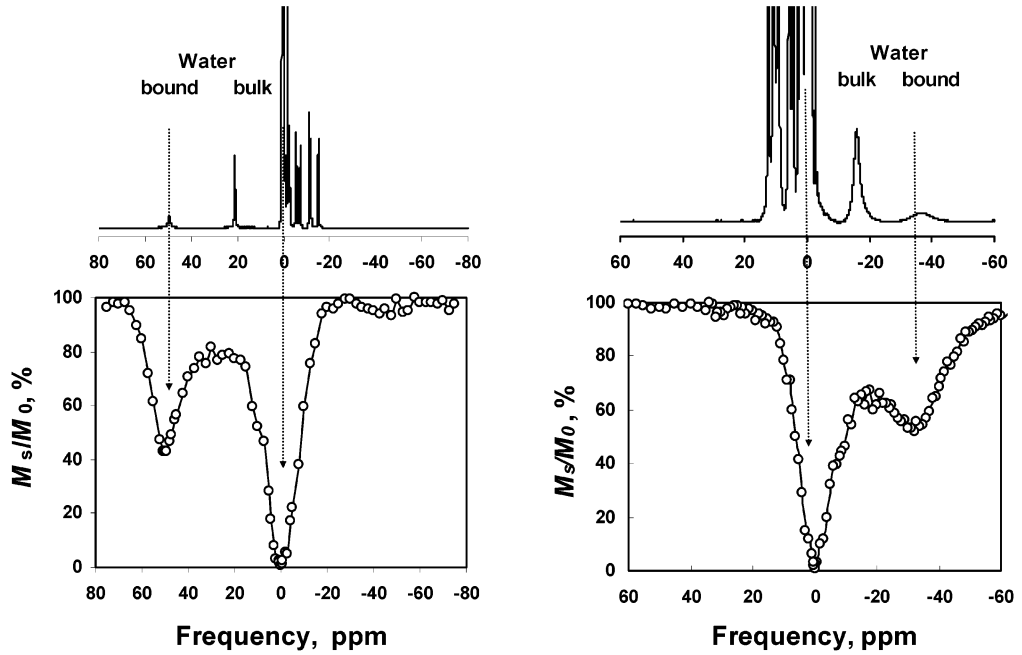

FIGURE 5. Z-spectra for EuDOTA−4AmCE (left) and NdDOTA−4AmCE (right) at 4.7 T obtained by using a 1 s presaturation pulse. The high-resolution ^{1}H spectra (500 MHz) of the complexes show bound water resonances at +50 ppm (Eu^{3+}) and −32 ppm (Nd^{3+}) (from refs 16 and 38).

where c is the concentration of PARACEST agent and q is the number of bound water molecules per PARACEST complex.

A "useful" reduction in M_z^a/M_0^a requires that τ_a be of the same order of magnitude or smaller than T_{1a}. Since $\tau_a = \tau_M/(M_0^b/M_0^a)$, short τ_M values are needed when the lanthanide concentration (M_0^b) is small. However, short τ_M values also broaden the Ln^{3+}-bound water protons; hence, larger $\Delta\omega$ values are required to avoid direct excitation of pool A. Such excitation obscures the actual CEST effect. Clearly, a paramagnetic complex with a large $\Delta\omega$ value is advantageous. Note that eq 9 makes predictions only for limiting cases while the complete solution to the set of Bloch equations enables one to predict the M_z^a/M_0^a values for any general case.

Z-Spectra (CEST Spectra). One practical way to characterize chemical exchange in such systems is to selectively presaturate the sample in incremental steps over a range of frequencies and plot the remaining bulk water signal, M_S/M_0, versus saturation frequency. This was originally referred to as a Z-spectrum[37] and more recently as a CEST spectrum.[19] Figure 5 illustrates two such Z-spectra for samples of EuDOTA−4AmCE and NdDOTA−4AmCE.[16,38] The peaks at 0 ppm in each Z-spectrum represent direct saturation of bulk water, while the peaks centered near +50 and −32 ppm reflect chemical exchange between the respective Eu^{3+}-bound and Nd^{3+}-bound water molecules in these complexes. The peak at +50 ppm in the Eu^{3+} plot is noticeably sharper than the peak at −32 ppm in the Nd^{3+} plot, consistent with slower water exchange in the former complex. This is also evident in the width of the bound water resonances in the high-resolution ^{1}H spectra shown plotted above the Z-spectra. Given that a peak due to chemical exchange should be detected in such Z-spectra for systems that meet the intermediate to slow exchange criteria, $\Delta\omega\cdot\tau_M \geq 1$, one can then predict which complexes might serve as useful PARACEST agents at various B_0 fields. These data, collected in Table 1, predict that most of the LnDOTA−4AmCE complexes should function as PARACEST agents at 11.75 T but only a few, Eu^{3+}, Tb^{3+}, Dy^{3+}, and Ho^{3+}, might be useable at the most common clinical MR imaging field strength, 1.5T. These data also illustrate that PARACEST agents will likely become more efficient at higher B_0 fields.

Table 1. ^{1}H Chemical Shifts and Lifetimes (τ_M) of Ln^{3+}-Bound Water in the LnDOTA−4AmCE Complexes[38]

complexes	observation of bound water	τ_M^{298} (μs)	$\delta(^1H)$ (ppm)	$\Delta\omega\cdot\tau_M$ 11.75 T	4.7 T	1.5 T
Pr^{3+}	Yes	20	−60	3.8	1.5	0.5
Nd^{3+}	Yes	80	−32	8.0	3.2	1.0
Sm^{3+}	Yes	320	−4	4.0	1.6	0.5
Eu^{3+}	Yes	382	50	60.0	24.0	7.7
Tb^{3+}	No	31	−600	58.5	23.4	7.5
Dy^{3+}	No	17	−720	38.5	15.4	4.9
Ho^{3+}	No	19	−360	21.5	8.6	2.8
Er^{3+}	No	9	200	5.7	2.3	0.7
Tm^{3+}	Yes	3	500	4.7	1.9	0.6
Yb^{3+}	Yes	3	200	1.9	0.5	0.2

One can further illustrate the impact of both $\Delta\omega$ and τ_M in determining the efficiency of a PARACEST agent by using predictions provided by the modified Bloch equations. Figure 6 shows 3D stacked plots of Z-spectra plotted as a function of bound water exchange rate ($1/\tau_M$) for two PARACEST agents, one having a Ln^{3+}-bound water molecule at +50 ppm ($\Delta\omega$ = 62 832 rad/s) and another having a Ln^{3+}-bound water at +800 ppm ($\Delta\omega = 1 \times 10^6$ rad/s). The predictions assume presaturation of the bound water using a B_1 field of 1500 Hz, a B_0 of 4.7 T, and 20 mM agent. These stacked Z-spectra illustrate several important

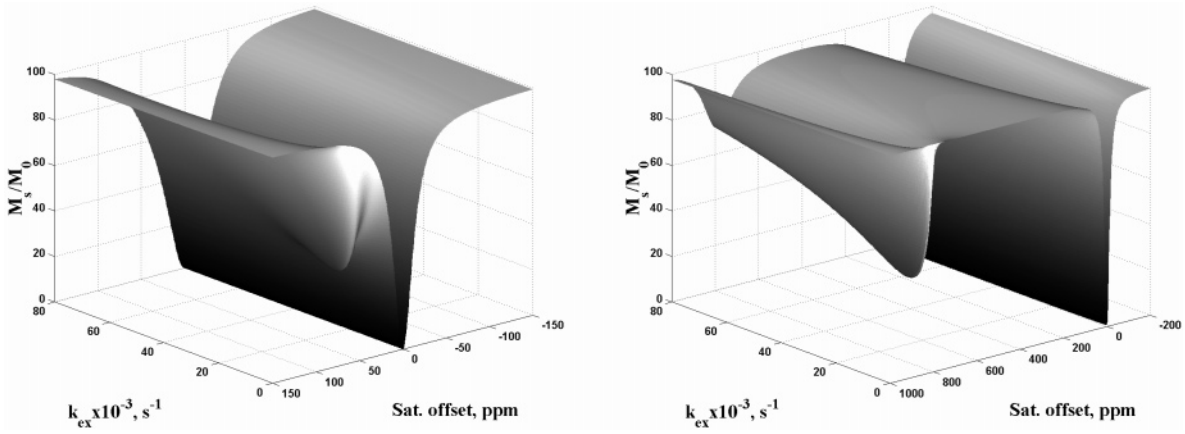

FIGURE 6. Stacked plots of Z-spectra derived from the modified Bloch equations for two-site exchange. The plots show M_S/M_0 (vertical axis) as a function of bound water exchanging rate ($k_{ex} = 1/\tau_M$) for two paramagnetic complexes, one having a bound water signal at 50 ppm (left) and one at 800 ppm (right). The same set of parameters was used to generate these two plots; $T_{1a} = 1.0$ s and $T_{2a} = 0.3$ s for bulk water, $T_{1b} = 0.1$ s and $T_{2b} = 0.03$ s for bound water, $B_0 = 4.7$ T, $B_1 = 1500$ Hz, and a concentration of 20 mM.

FIGURE 7. Plot of the fractional decrease in bulk water signal intensity expected for a PARACEST agent having a bound water lifetime (τ_M) of 3 μs and a bound water (^{1}H) paramagnetic shift of 500 ppm (based upon eq 9 by assuming $T_{1a} = 1.0$ s).

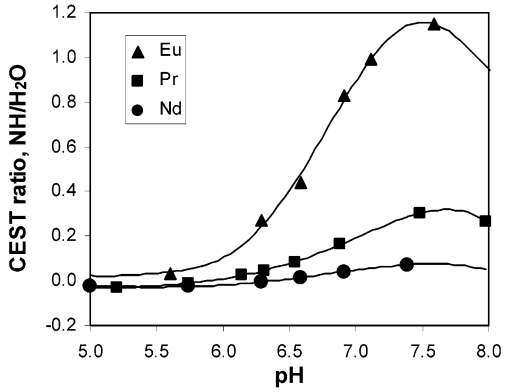

FIGURE 8. Ratiometric plots for 30 mM LnDOTA−4AmC complexes at 312 K and 7.05 T. These data were generated by applying a train of frequency-selective e-burp pulses (at train of 20 ms shaped pulses for the NH protons and 1 ms shaped pulses for the OH protons) (adapted from ref 46).

points: (1) M_S/M_0 falls off more rapidly with increasing exchange rate (shorter lifetime) for the 50 ppm agent than for the 800 ppm agent, (2) complexes with highly shifted bound water resonances are more efficient over a wider range of water exchange rates, and (3) the largest decrease in M_S/M_0 will be achieved by using an agent with optimal water exchange rate.

What is the lower detection limit for an optimized PARACEST agent? Figure 7 shows one result predicted by the Bloch equations for a PARACEST agent having a bound water resonance at 500 ppm and a τ_M of 3 μs. Assuming that a sufficiently strong B_1 field can be used to fully saturate the bound water signal, the Bloch equations predict that ~37% of the bulk water signal can be eliminated by using ~100 μM agent, and even 5% of the bulk water signal can be eliminated by using ~10 μM agent. The latter concentration is well below the detection limit of a low molecular weight Gd^{3+}-based contrast agent with a typical relaxivity (r_1) of 4−5 mM^{-1} s^{-1}. [39] These data suggest that PARACEST agents have the potential of being as sensitive as or even more sensitive than Gd^{3+}-based T_1 agents, assuming that all practical aspects can be solved. Just as one can increase the relaxivity of Gd^{3+}-based agents substantially by conjugation to a polymer or formation of an aggregate,[40−42] similar modifications of PARACEST agents could be done and this would easily extend the lower detection limit into the submicromolar range. This was recently demonstrated for a series of polymers containing large numbers of exchangeable −NH groups.[43]

pH Responsive PARACEST Agents. Balaban et al.[44] first demonstrated that diamagnetic CEST agents having more than one type of proton exchangeable site may be used to measure pH using a "ratiometric method". Aime et al.[45,46] used this same concept for PARACEST agents having both a slowly exchanging Ln^{3+}-bound water molecule and exchangeable −NH sites (Figure 8). For such complexes, exchange between the −NH sites and bulk water is quite sensitive to pH in the range 6−8, while proton and/or water molecule exchange is nearly independent over this same pH range. Hence, plots of the PARACEST effect expressed as a ratio for two different presaturation frequencies (Ln^{3+}−OH and Ln^{3+}−NH) removes the effect of agent concentration and provides a direct measure of solution pH. One should note that the PARACEST ratio will be even more sensitive to pH when using an agent with larger $\Delta\omega$ differences between the Ln^{3+}-bound water

and −NH resonances. The CEST efficiency at the two sites is field dependent (due to $\Delta\omega$) so the pH responsiveness also becomes field dependent.[31,47] For example, the CEST effect for the Eu^{3+}-bound water of EuDOTA−4AmC is independent of pH between pH 6 and 8 at 7.05 T[46] but not at 4.7 T.[31] Thus, one must establish ratiometric curves for each new compound and at each B_0 field before placing this technique into practical use.

Metabolite Responsive PARACEST Agents. One example of this was given by a recent report showing that a PARACEST agent could be used to detect and quantify the glycolytic end product, lactate.[48] For this purpose, a Ln^{3+}–tris-amide complex was used (a $q = 2$ complex) so that lactate could bind into the inner coordination sphere of the Ln^{3+} as a bidentate ligand. This resulted in a slow-exchanging ternary complex whose −NH resonances differed in chemical shift from the uncomplexed LnL −NH resonances by ∼5 ppm. Selective saturation of each −NH resonance (sequentially) resulted in a CEST effect at each site that was proportional to the concentration of each species, thereby allowing a quantitative comparison of their respective concentrations. It was shown the CEST ratio provides a direct readout of the lactate concentration in any equilibrium mixture that contains both LnL and LnL-lactate. Using this approach, one could envision the design of amide-based Ln^{3+} chelates having a topological surface that allows selective binding of any metabolite within a complex biological mixture. This offers the possibility of in situ monitoring of metabolites by transmitting this binding information to bulk water via the CEST effect.

Summary

Our general knowledge of lanthanide coordination chemistry was significantly advanced during the last two decades due to widespread scientific interest in Gd^{3+}-based MRI contrast agents. Many important and surprising discoveries, especially concerning the dynamics of lanthanide complexes, that have come from these basic investigations will likely result in an even broader range of applications of lanthanide complexes to medical science. For example, we now know that complexes can be made with Ln^{3+}-bound water lifetimes (τ_M) ranging from nanoseconds to milliseconds by simple modification of ligand side chain coordinating groups or by varying the central metal ion, solvent, or counterion. Complexes have been discovered wherein a single, bound water molecule is in such slow exchange with bulk water that a separate resonance appears in the high-resolution ^1H or ^{17}O NMR spectra of the complex, even at temperatures well above room temperature. This single feature alone already forms the basis of complexes that act as pH sensitive and metabolite specific MR agents. The recent demonstration that paramagnetic lanthanide complexes can act as efficient CEST agents offers the possibility of designing an array of new responsive agents for reporting biological events using conventional imaging technologies. Just as with Gd^{3+}-based relaxation agents designed to respond to enzyme activity,[49] pH,[50] Ca^{2+}, [51] Zn^{2+},[52] and sugars,[53] we believe that PARACEST agents may offer a new avenue for research into biologically "responsive" agents capable of sensing molecular exchange phenomena in tissue.

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