A Novel Europium(III)-Based MRI Contrast Agent

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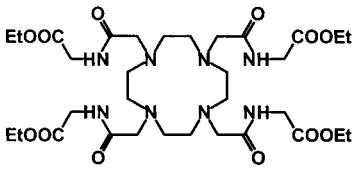
Chelates of gadolinium act as efficient contrast agents for magnetic resonance imaging (MRI) by shortening bulk water proton relaxation times through rapid exchange of an inner-sphere bound water molecule with bulk solvent.[1] Balaban and co-workers have recently reported a new class of MRI contrast agents based on chemical exchange saturation transfer (CEST)[2] between intrinsic metabolites such as amino acids, sugars, nucleotides, or other heterocyclics having exchangeable OH or NH groups that exchange protons with bulk water. They demonstrated that MR contrast can be switched on by applying a saturating irradiation pulse at an exchangeable site a few ppm away from the bulk water resonance. Magnetization transfer (MT) techniques have historically found many applications in chemical and biological systems.[3] Theoretically, the extent of observed MT depends on chemical exchange and relaxation:

$$\frac{M_t}{M_0} = \frac{1}{(1 + k_{obs}T_{1sat})} + \left[\frac{k_{obs}T_{1sat}}{(1 + k_{obs}T_{1sat})}\right] \exp\left[-\frac{(1 + k_{obs}T_{1sat})}{T_{1sat}} \times t\right] \quad (1)$$

Here, M_t and M_0 is the bulk water signal intensity with and without saturation at the exchanging site, respectively, k_{obs} is the pseudo-first-order exchange rate between bulk water and the exchanging protons, and T_{1sat} is the spin−lattice relaxation time of water protons during saturation at the exchangeable site. k_{obs} equals the concentration ratio of the exchanging site relative to water divided by the lifetime of the exchange site, τ_{CA}.[4] To observe a CEST effect, the system must be in the exchange limiting regime, $\tau_{CA}\Delta\omega_{CA} \geq 1$. Thus, one advantage of a paramagnetic complex that displays a large $\Delta\omega_{CA}$ is that faster exchange can take place (shorter τ_{CA}) without approaching the fast exchange limit.



Molecular structure of **1**

Recently, Aime et al.[5] reported that a ^{1}H NMR signal of bound water can be detected in EuDTMA^{3+} (where DTMA = 1,4,7,-10-tetraazacyclododecane tetrakis(methylacetamide)) at low temperatures in acetonitrile-d_3. We extended this observation by showing that the bound water ^{1}H signal of the Eu^{3+} complex of a similar DOTA-tetra(amide) derivative, **1** (1,4,7,10-tetraazacyclododecane tetrakis (ethyl-acetamidoacetate)), can be detected at 50 ppm in pure water at ambient temperatures.[6] The large difference in chemical shift between bound and bulk water suggested to us that [Eu(**1**)(H$_2$O)]$^{3+}$ might serve as an efficient MT contrast agent.

The solid-state structure of [Eu(**1**)(H$_2$O)](triflate)$_3$[6] shows that the Eu^{3+} is bound to four macrocyclic nitrogen atoms and four amide oxygen atoms in a square antiprismatic geometry with a twist angle of 38.5° between the N4 and O4 planes. A single bound water molecule occupies a typical mono-capped position on the O4 surface. A comparison of hyperfine shifts shows that the complex has this same structure in both acetonitrile and water.

The 500 MHz ^{1}H NMR spectrum of [Eu(**1**)(H$_2$O)]Cl$_3$ shows a bound water resonance at 49.7 ppm at 25 °C. This resonance broadens with increasing temperature but is observable even at 40 °C. Presaturation of this bound water resonance for variable time periods resulted in an incremental decrease in the bulk water signal (data not shown). These data were fit to eq 1 to give a bound water lifetime (τ_M^{295}) of 351 ± 12 μs and a bulk water relaxation time (T_{1sat}) of 537 ± 23 ms. These values indicated that significant magnetization transfer would be expected in water, even with low concentrations of Eu(**1**)$^{3+}$ (2.5% at 1 mM, 20% at 10 mM, 50% at 39 mM, and 61% at 63 mM). In comparison, it has been estimated that a diamagnetic agent with a 5 ppm chemical shift difference between exchanging sites would yield only a 10% decrease in bulk water signal at 39 mM.[2]

Figure 1 shows a plot M_S/M_0 versus saturation frequency offset for 63 mM [Eu(**1**)(H$_2$O)]Cl$_3$ at 25 °C. The peak at 0 ppm represents direct saturation of bulk water, while the peak centered near 50 ppm reflects chemical exchange. A 57% decrease in bulk water signal intensity was observed when a 1 s presaturation pulse was applied at +9800 Hz. No change in water intensity was observed when the presaturation pulse was at −9800 Hz. It is significant that a MT effect was observed even when the frequency of the presaturation pulse was set to 25−30 ppm, even though the change in bulk water intensity was only ∼20% at this frequency. In comparison to the data in the figure collected at 4.7 T, only a 6% decrease was observed when this same experiment was performed at 11.75 T. This illustrates that the frequency selectivity of such MT agents will depend on magnetic field strength because chemical exchange is closer to the slow exchange limit at the higher frequency. This also indicates that the frequency selectivity of MT agents such as this will be much broader at 1.5 T, the most common clinical imaging field.

T_1-weighted spin−echo images of a phantom containing 63 mM [Eu(**1**)(H$_2$O)]Cl$_3$ in an inner vial and pure water in a larger outer vial are shown in Figure 2. The intensity of the inner vial containing the MT agent in the two control images (no presaturation pulse, left image; presaturation pulse at −9800 Hz, right image) is higher than the surrounding water vial due to the bulk paramagnetism of the Eu^{3+} complex. Addition of a 1s presaturation pulse at +9800 Hz to both vials prior to the imaging sequence resulted in a 79% decrease in intensity of the inner vial

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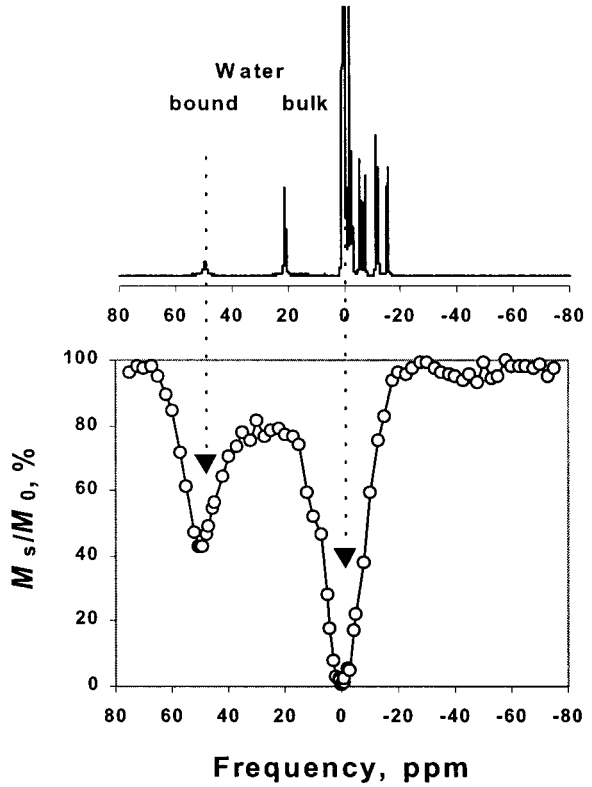

Figure 1. 500 MHz ^{1}H NMR spectrum of 250 mM [Eu(**1**)(H_2O)]Cl_3 aqueous solution at neutral pH and 25 °C (upper; the huge bulk water peak was truncated to make the small bound water peak more visible). A curve of MT effect (M_S/M_0) vs saturation frequency offset for 63 mM [Eu(**1**)(H_2O)]Cl_3 aqueous solution at neutral pH and ~22 °C (lower). These data were obtained using a Brüker 4.7 T Omega imaging system, a 2.5 cm surface coil, and 16.4 db of presaturation power over 1s.

and no change in the outer vial. This demonstrates the potential utility of such MT complexes as a novel way to alter image contrast.

Biomolecules intrinsic to tissues with proton exchangeable groups such as OH and NH have been also been proposed as CEST agents.[2,7] One disadvantage of such diamagnetic agents is that the chemical shifts of exchangeable groups in these diamagnetic systems is only ~1−5 ppm away from bulk water. As many tissues show an inherent magnetization transfer effect from a broadened water resonance associated with proteins and other large biomolecules, it may be difficult to distinguish the effects of inherent tissue MT versus those of a diamagnetic CEST agent. The advantage of a paramagnetic system such as Eu(**1**)$^{3+}$ for this application is the highly shifted water resonance should allow easy differentiation of the CEST agent from MT effects originating in tissue. Eu(**1**)$^{3+}$ may show an additional advantage in this regard at 1.5 T because the CEST peak will be naturally broadened by chemical exchange at this field so it may prove possible to move the presaturation frequency 50−70 ppm away from bulk water and still see a significant CEST effect.

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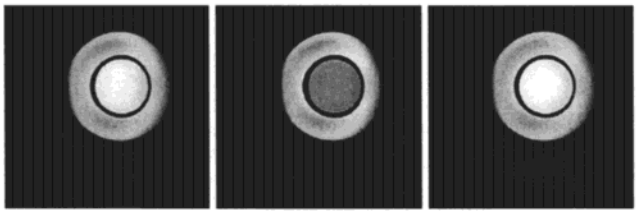

Figure 2. 4.7 T (~22 °C) magnetization transfer (MT) T_1-weighted spin−echo images for a phantom with no saturation (left), saturation at +9800 Hz (middle, Eu^{3+}-bound water position), and saturation at −9800 Hz (right), using a 2.5 cm diameter surface coil with a saturation duration time of 1s and power of 0, 16.4, 16.4 db, respectively. The outer vial contained deionized water, while inner vial contained 63 mM [Eu(**1**)-(H_2O)]Cl_3 dissolved in pure water.

In summary, [Eu(**1**)(H_2O)]Cl_3 shows a novel CEST effect in pure water that may useful for certain imaging applications. One significant advantage of a CEST agent over typical relaxation or broadening agents based upon gadolinium or dysprosium is that the CEST effect can be switched on/off at will. It may ultimately prove possible to design other Ln^{3+}-based systems with differing water or proton exchange rates that are altered by the biological environment.

Experimental Section. The preparation of **1** and the [Eu(**1**)-(H_2O)](triflate)$_3$ complex had been reported.[6] [Eu(**1**)(H_2O)]Cl_3 was prepared by mixing a 1:1 molar ratio of ligand **1** and $EuCl_3$ in aqueous solution at room temperature and allowing the mixture to reach equilibrium overnight. No free Eu^{3+} could be detected after a few hours (xylenol orange in 0.5 M NaAc/HAc buffer, pH 5.3, was used to test for free Eu^{3+}). The pale yellowish freeze-dried complex was used without further purification. High-resolution ^{1}H NMR confirmed that the complex was fully formed.

Variable temperature ^{1}H-, ^{13}C-, and ^{17}O NMR spectra were recorded on a Varian INOVA-500 NMR spectrometer at 500, 125.6, and 67.8 MHz, respectively. NMR samples were allowed to stand in the probe for at least 10 min at each the temperature before data acquisition. The temperature was constant to within 0.5 °C. Proton T_1-weighted spin−echo images were collected using a Brüker 4.7 T Omega imaging system. The decoupler channel was used to presaturate the bound water signal. A 2.5 cm surface coil was used in all imaging experiments. Typical imaging parameters included a 1 s presaturation pulse with a power level of 0−50% (The maximum saturation power could be as high as 82 db for this imaging system), a TE of 18 ms and a TR of 500 ms.

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