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The Effect of Zn^{2+} Binding on the Chemistry of Tm^{3+} and Eu^{3+} Chelates

By

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Abstract

Current contrast agents for MRI are Gd^{3+} -based, which successfully enhance image contrast. These agents, however, are limited in detecting certain analytes associated with disease compared to new agents that operate through a CEST mechanism. In this study, both Eu^{3+} and Tm^{3+} chelates were examined as CEST agents for MRI imaging as a function of Zn^{2+} concentration. T_1 values were obtained using NMR spectrometry; these values were then used to fit the CEST data obtained for the compounds. The CEST spectra of the Tm^{3+} chelate were useful for examining CEST arising from the amide protons. A shift in the amide protons was observed as Zn^{2+} concentrations increased indicating a presence of multiple species and a change in coordination chemistry. The change of the amide peak at -52 ppm showed its potential to be used as a marker for Zn^{2+} concentration. The Eu^{3+} chelate was useful for examining CEST arising from the coordinated water. A distinct change in the water peak shape, intensity, and shift was observed as Zn^{2+} was added, showing increased water exchange kinetics.

INTRODUCTION

Over time, magnetic resonance imaging has become an indispensable tool both in diagnostic medicine as well as biomedical research. The mechanism by which MRI obtains images is through the principle of Nuclear Magnetic Resonance (NMR). If a sample is placed in a magnet the spins of the nuclei either orient along or against the magnetic field with the majority of the spins aligned along the magnetic field.¹⁸ The bulk magnetization is therefore aligned along the z axis as seen in Figure 1.¹⁸ The net magnetization has a torque and precesses at a specific frequency called the Larmor frequency.¹² This precessing magnetic field causes a current in a nearby magnetic coil, which generates a signal. A radiofrequency pulse is then applied which results in more spins being oriented against the magnetic field and reduces the bulk magnetization.¹⁸ The intensity of the signals produced in experiments with NMR and MRI are proportional to the net magnetization. Radiofrequency energy can be applied to the net magnetization causing two effects depending on the duration of the pulse. If a short duration, high power, pulse is applied excitation of the nuclei is observed. If a long duration, low power, pulse is applied saturation will occur. When energy is no longer added, the spins will relax back to their original, low energy- α , states.

Figure 1. The vector on the z axis represents the bulk magnetization of a sample (left). A radiofrequency pulse results in more high energy- β spins and a lower magnitude of the bulk magnetization (right).¹⁸

Often times in MRI differentiating between different tissue types is difficult and an exogenous agent must be used. These exogenous agents, or contrast agents, will enhance the signal in specific regions as the agent gets distributed throughout the body by altering T_1 , T_2 or both.¹⁶ Most commonly used contrast agents are gadolinium(III) complexes, small paramagnetic metal complexes, which shorten the relaxation times of the water protons by increasing the rate of longitudinal water proton relaxation and thereby result in an increase of signal intensity.^{18,19} The relaxivity of an agent is known as the water proton relaxation rate per unit concentration of contrast agent and an increase in relaxivity results in a more effective reduction in T_1 .¹⁹ However, Gd^{3+} -based agents have their drawbacks as well. Current Gd^{3+} contrast agents flow throughout all extracellular space, have comparatively low relaxivities of 4-5 $mM^{-1}s^{-1}$ [2] and must be provided in comparatively high doses of about 0.1 $mmol\ kg^{-1}$.¹⁹ A new group of contrast agents have been found by Balaban *et al*¹⁶, which may be used for MRI. These agents possess slowly exchanging $-NH$ or $-OH$ protons and operate through a Chemical Exchange Saturation Transfer, or CEST, of pre-saturated spins to bulk water.^{16,17} In a CEST mechanism a nucleus is exchanged from one site to a chemically different site.¹⁸ This process is

Figure 2. When a presaturation pulse is applied to Pool B the number of high energy spins increases. Given that the chemical exchange rate is large enough to overcome the effects of relaxation, chemical exchange will occur. If not, the high energy spins will relax back to equilibrium.¹⁸

in competition with relaxation and can be seen in Figure 2.¹⁸ The CEST process would occur in a system that has two pools, A and B, of exchanging protons as long as the pools have relaxation times that are longer than the exchange rates.¹⁸ The exchange rates should also be as fast as possible to reduce relaxation effects.¹⁸ Following a presaturation pulse, Pool A would have an increase in high energy spins, against the magnetic field, and a decrease in low energy spins, along the magnetic field.¹⁸ This moves Pool A closer to saturation, reducing the bulk magnetization and the signal intensity. Pool B would have a signal intensity of almost 0.¹⁸ Using this mechanism, signal intensity of one resonance can therefore be altered by applying a presaturation pulse to a second resonance that is in exchange with the first resonance and ultimately use this effect to change signal intensity to produce contrast in MRI images.¹⁸ Paramagnetic compounds that have exchangeable proton sites (-NH, -OH or H₂O) frequency shifted away from the bulk water NMR frequency may increase the effectiveness of a CEST agent by maximizing the frequency difference between the two pools, $\Delta\omega$, and consequently also the rate of chemical exchange.¹⁸ These compounds are called PARACEST agents. Another advantage of PARACEST agents is their ability to act as “smart” agents and respond to changes in molecular concentrations within the body.¹⁸ So far, systems that are capable of reporting a number of biologically relevant variables have been constructed, such as: pH, temperature, lactate, glucose and zinc.¹

Divalent zinc is the second most abundant transition-metal ion in the human body^{4,9,11,14} and deficiency in the ion affects digestive, immune and neuronal systems.^{10,14,15} It has also been found to be important for regulation of cell death and synaptic transmission,³ but perhaps most importantly Zn²⁺ is bound up with insulin in pancreatic β -cells. Because Zn²⁺ is co-released with insulin in response to elevated glucose levels imaging the presence of Zn²⁺ could be beneficial to

the medical field and to understanding diabetes in particular. In the human body, Zn^{2+} exists both as bound Zn^{2+} and free Zn^{2+} . Bound Zn^{2+} can be found on membrane receptors, transporters and channels regulating their activity.⁷ Bound Zn^{2+} on loosely bound metal-protein complexes in certain cell types and organelles are released to form free Zn^{2+} ion concentrations ranging from 10^{-12} - 10^{-5} M to about 0.3 mM.^{1,5,8} To be able to study how and why it is released inside the body a mode of detection will be necessary. In the past there has been success in sensing Zn^{2+} ions using Gd^{3+} complexes, where the first one designed decreased water relaxivity by about 30% when Zn^{2+} was present.⁶ A CEST agent with Eu^{3+} has also been designed and was shown to be promising in sensing Zn^{2+} concentrations that were within range for free Zn^{2+} ions in human tissues.¹³

Methods

Figure 3. The two contrast agents studied were the thulium complex (left) and europium complex (right).

Two contrast agents were studied, including a thulium complex and a europium complex. The structures of these contrast agents can be seen in Figure 3. Each complex was studied in four separate samples of varying Zn^{2+} concentration: 0, 0.5, 1, and 2 Eq Zn^{2+} . They were then analyzed on a Bruker 600 MHz NMR spectrometer to obtain T_1 values of bulk water. All T_1 values were measured through saturation recovery except for the europium with zero equivalents

of Zn^{2+} , which was measured through inversion recovery. The T_1 values were then used to fit the data on MATLAB software.

Results

The T_1 values for the Tm^{3+} samples with 0, 0.5, 1 and 2 equivalents of Zn^{2+} were 0.377 s, 0.370 s, 0.365 s, and 0.396 s respectively. The T_1 values for the Eu^{3+} samples with the same Zn^{2+} equivalents were 3.384 s, 2.489 s, 2.559 s, and 2.587 s respectively.

Eqs of Zn^{2+}	0	0.5	1	2
[EuL] (mM)	12.778	8.333	6.945	5.833
τ_M (us)	293.3	105.1	76	67.2
τ_{NH} (ms)	5	0.9588	0.84	0.5009
$\delta \text{H}_2\text{O}$	0.3357	0.5913	0.4711	0.4644
δ bound	50.8941	50.8218	50.6135	49.3208
δNH	-4.5	-5.0554	-4.8617	-5.1599
T_1 bulk	2.48	2.25	2.55	2.58
T_2 bulk	0.6748	0.8082	0.8576	0.5707
T_1 bound	0.045	0.03	0.03	0.03
T_2 bound	0.01	0.01	0.01	0.01
$T_1 \text{NH}$	1.5	1.5	1.5	1.5
$T_2 \text{NH}$	1.5	0.3	0.3001	1.5

Table 1. Fitting parameters for the Eu^{3+} chelate.

[Zn] =	0	0.5	1	2
τ_M (μs)	2711.3	4094	972.1	1465.5
τ_{NH} (ms)	2.169	1.2081	2.9585	0.7353
$\delta \text{H}_2\text{O}$	-0.1747	-0.2936	-0.4485	-0.7757
δ bound	-48.2598	-47.5	-51.5	-50.7996
δNH	-51.8	-52	-59.5	-59.6928
T_1 bulk	0.3806	0.3719	0.3669	0.3999
T_2 bulk	0.0045	0.0045	0.0053	0.0091
T_1 bound	0.0005	0.0006	0.1396	0.0018
T_2 bound	0.001	0.001	0.0004	0.0003
$T_1 \text{NH}$	0.0005	0.0012	0.2	0.0019
$T_2 \text{NH}$	0.0006	0.0003	0.0045	0.0003

Table 2. Fitting parameters for the Tm^{3+} chelate.

The T_1 of bulk water was constrained to these T_1 values and CEST spectra were fitted to the Bloch equations using a three-pool model of bulk, bound and NH. The other T_1 and T_2 values

were constrained to reasonable values. The fitting parameters are shown in Table 1 and 2 for the Eu^{3+} and Tm^{3+} chelates respectively.

Figure 4. Full CEST spectra of the Tm^{3+} chelate with 0, 0.5, 1 and 2 equivalents of Zn^{2+} (left) and just the two amide peaks (right).

The full CEST spectra of the Tm^{3+} chelate (Figure 4) was visualized for the four samples. The two peaks corresponding to the amide protons, which were expected for a chelate with C_2 symmetry, can be seen closer on the right of Figure 4. Prior to any addition of Zn^{2+} the two CEST peaks were at -48 ppm and -52 ppm, with the downfield, -48 ppm, peak having greater intensity. At half of an equivalent of Zn^{2+} the peaks were at the same location, however the upfield peak, -52 ppm, became more intense. After the addition of one equivalent of Zn^{2+} the two amide peaks shifted to -52 ppm and -60 ppm with the downfield peak, -52 ppm, being more

Figure 5. CEST for the two amide protons at -52 ppm and -60 ppm on the Tm^{3+} chelate with increasing Zn^{2+} concentration. Ratio of the two is represented by the curve above.

intense. At two equivalents of Zn^{2+} the peaks experienced no shift, however the upfield peak, -60 ppm, was the most intense. Overall, with increasing concentrations of Zn^{2+} , CEST at -60 ppm decreased from 0.934 to 0.844 and CEST at -52 ppm increased from 0.868 to 0.884 (Figure 5). The ratio of -52 to -60 increased from 0.93 to 1.05 as the concentration of Zn^{2+} increased.

Figure 6. Full CEST spectra of the Eu^{3+} chelate with 0, 0.5, 1 and 2 equivalents of Zn^{2+} (left) and just the bound water peak (right).

CEST spectra of the Eu^{3+} chelate (Figure 6) showed the bound water peak at 50 ppm and the bulk water peak centered at 0 ppm. A closer view of the bound water peak shows that as the concentration of Zn^{2+} increases, the peak broadens, reduces in intensity and shifts toward the bulk water peak upfield. Titration of the Eu^{3+} chelate with 0 to 2.25 equivalents of Zn^{2+} resulted

Figure 7. ^1H NMR Spectrum from titrating the Eu^{3+} chelate with varying concentrations, 0 to 2.25 equivalents, of Zn^{2+} .

in a noticeable change in the ^1H NMR spectrum with increasing Zn^{2+} concentration, especially exceeding one equivalent (Figure 7).

Discussion

The shift of the amide protons in the CEST spectra of the Tm^{3+} chelate makes it likely that there is a different chelate structure in the presence of Zn^{2+} than when Zn^{2+} is absent. This was supported by the change in the ^1H NMR spectrum following titration of Zn^{2+} into a solution of the Eu^{3+} chelate. When examining the CEST spectra of the two amide protons it can be seen that the upfield CEST peak without Zn^{2+} and the downfield CEST peak with Zn^{2+} both occur at -52 ppm, but vary in their intensity. This change in intensity in the presence of Zn^{2+} could make it usable as a marker for Zn^{2+} concentration. The peak at -60 ppm can be used as a reporter of the Zn^{2+} concentration in a ratiometric method, but the peak at -48 ppm may be too weak. Figure 4 further indicates the presence of multiple species as Zn^{2+} is added to solution. The line broadening is the result of individual species having different shifts as well as the changing exchange rate. This causes the appearance of deceptively faster exchange rates. It may also explain why the concentration of chelate appears to decrease with increasing Zn^{2+} concentrations.

The broadening, shifting, and reduction in intensity of the bound water peak of the Eu^{3+} CEST spectra indicates that water exchange kinetics are increasing with the presence of Zn^{2+} . The fit was also not adequate on the bound water peaks when concentration of the CEST agent was kept constant and consistent with the known initial concentration. Only when the concentration was progressively decreased for each sample did the data fit better. The concentration values in Table 1 also show that with added Zn^{2+} a reduction in the hydration state or exchange capacity of the CEST agent is observed.

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