

T1 modulation of CEST in a Eu(III)-DOTA-tetraamide-bis(nitroxyl) complex

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Introduction

Paramagnetic lanthanide-based DOTA-tetraamide complexes provide an alternative to traditional MRI relaxation agents by a chemical exchange saturation transfer (CEST) mechanism. Selective radiofrequency saturation of the protons on a Ln³⁺-bound water molecule followed by chemical exchange of that bound water with bulk water results in a reduction in total magnetization of bulk water.¹ Since the magnitude of PARACEST is quite sensitive to the water exchange rate in such complexes, modulation of the water exchange rate by steric and the electronic factors around the coordination cage has provided a platform by which responsive PARACEST agents have been designed.² The ¹H T₁ and T₂ relaxation times of the exchanging pools have also influence the magnitude of CEST contrast. Here, we report the first rationally designed PARACEST agent in which the CEST efficiency is modulated by the longitudinal relaxation time, T₁, of bulk water protons. The ligand design includes two covalently appended TEMPO (2,2,6,6-tetramethylpiperidin-1-yl)oxyl units to the pendant arms of a DOTA-tetraamide scaffold. It is anticipated that the CEST signal coming from the paramagnetic biradical complex would be small or negligible due to an reduced T₁ but the reduction of the nitroxyl groups to the diamagnetic hydroxylamine groups would result in a substantial increase in CEST contrast.

Experimental

The ligand was synthesized by alkylation of 4-bromoacetyl amino 2,2,6,6-(tetramethylpiperidin-1-yl)oxyl radical with the 1,4-bis-(N-tert-butylacetate)-1,4,7,10-tetraazacyclododecane-1,4,-tetraacetamide in the presence of a base followed by the acid hydrolysis of the tert-butyl ester groups. The Eu³⁺ complex was obtained by reaction of ligand (1) with EuCl₃ in water.

¹H and CEST spectroscopy

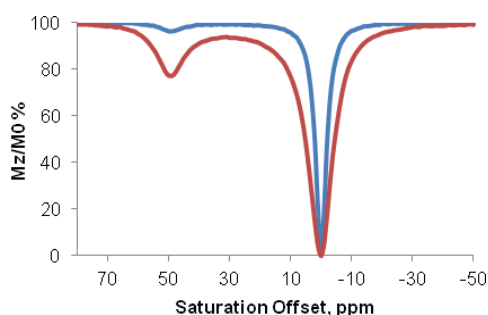


Figure 1. CEST spectra of Eu³⁺(1) (blue) and Eu³⁺(2) (red) collected using an applied B₁ of 10 μ T and 5s saturation.

The ¹H NMR spectrum of the Eu³⁺(1) complex in D₂O at 9.4 T showed highly shifted ligand resonances near +24 ppm, characteristic of the H4 axial protons of the macrocyclic ring. This indicates that Eu³⁺(1) exists largely as the square antiprismatic (SAP) coordination isomer. The CEST spectrum of a 20 mM sample of Eu³⁺(1) recorded at 9.4 T showed a distinct exchange peak for the metal bound water at +49 ppm, but its magnitude was less than 5% (Figure 2, blue line). The T₁ of water in this sample, determined by inversion recovery, was 200 ms. After addition of 2 equivalents of L-ascorbic acid, the T₁ of bulk water increased from 200 ms to 2500 ms and, consequently, the CEST signal from the exchanging water molecule increased to 25% as illustrated in Figure 1 (red line).

Discussion

Paramagnetic relaxation of bulk water by the TEMPO moieties in the ligand scaffold of the Eu³⁺(1) decreases the T₁ of bulk water protons to 200 ms. This reduced T₁ results in inefficient transfer of saturated spins from the Eu³⁺-bound water molecule into the bulk water proton pool. The Eu³⁺(1) complex, even though it has the preferred coordinating geometry (SAP) for slow water exchange, exhibits much less efficient CEST effect than many other Eu³⁺-DOTA-tetraamide complexes.¹ Chemical reduction of the TEMPO units to the diamagnetic hydroxylamine by reducing agents such as L-ascorbic acid increases the T₁ of the bulk water from 200 ms to 2500 ms and, as a result of the longer T₁, the transfer efficiency of saturated spins increases and the CEST properties of the system are turned on. CEST imaging of two phantoms shows a difference in contrast intensity between samples containing 20 mM Eu³⁺(1) and 20 mM Eu³⁺(2) both at pH 7 (Figure 2).

Summary

We have demonstrated that the CEST properties of a Eu³⁺-DOTA-tetraamide complex containing two covalently appended nitroxyl free radical moieties is quenched by T₁ relaxation of bulk water protons by the nitroxyl radicals. The CEST signal fully recovers after reduction of free radical moieties. Since nitroxyl radicals can be deactivated under certain biological conditions, this preliminary work implies that Eu³⁺-DOTA-tetraamide nitroxyl derivatives may be used as redox sensitive PARACEST probes.

References

- 1) Viswanathan, S., et.al., *Chem. Rev.* **2010**, *110*, 2960; 2) Ratnakar, S. J., et.al., *J. Am. Chem. Soc.* **2008**, *130*, 6; b) Viswanathan, S. et.al., *Angew. Chem., Int. Ed.* **2009**, *48*, 9330; c) Mani, T. et.al., *Contrast Media Mol. Imaging* **2009**, *4*, 183.

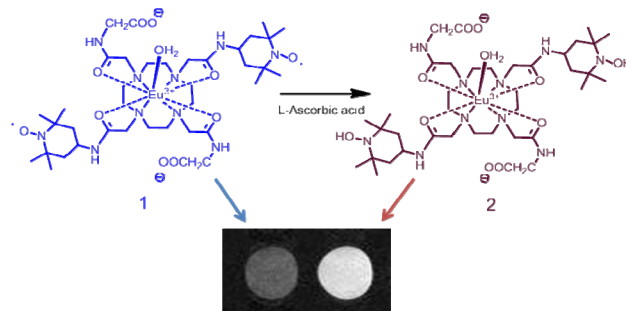


Figure 2. PARACEST phantom images at 9.4 T acquired by a fast spin echo sequence (TR/TE=5100/10ms), 128 x 128, with a 5 sec, 10 μ T presaturation pulse.