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A PARACEST Agent Responsive to Inner- And Outer-Sphere Phosphate Ester Interactions for MRI Applications

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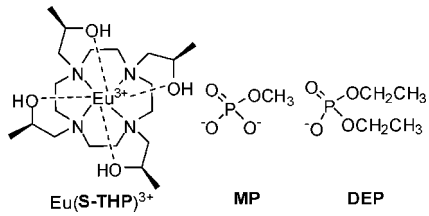
The development of magnetic resonance imaging (MRI) contrast agents that report on their environment through specific molecular recognition events is an active area of research.[1,2] MRI contrast agents have shown the potential to sense pH,[3] temperature,[4] metabolite concentration,[5−7] metal ions,[8−10] proteins,[11] or enzymes.[12,13] A major goal in these studies is to prepare MRI contrast agents that respond to molecules that serve as early biomarkers of disease.[1] Ln(III) complexes that function as contrast agents through paramagnetic chemical exchange saturation transfer (PARACEST) are especially promising for development as responsive MRI contrast agents. PARACEST agents have paramagnetically shifted mobile protons that are in slow exchange with bulk water protons. Application of a presaturation pulse to these mobile protons leads to a decrease in the intensity of the water signal.[14] The environment of the mobile protons is influenced by the interactions of Ln(III) PARACEST agents with macromolecules or small molecule metabolites.[7,11,15,16] The development of responsive PARACEST agents, however, is restricted by the limited number of ligand types with exchangeable protons.

To address this, we reported on Ln(III) macrocyclic complexes with exchangeable alcohol protons, but these complexes functioned as PARACEST agents only in water/acetonitrile mixtures.[17] The alcohol proton exchange rate constant was predicted to be too large to observe a CEST effect. Here we show for the first time that a Eu(III) macrocyclic complex with alcohol groups, Eu(**S-THP**)$^{3+}$ (Chart 1) acts as a PARACEST agent in pure water at controlled pH. In addition, the CEST spectrum of this complex is selectively responsive to two biologically important classes of phosphate esters. The modulation of the CEST effect is unexpectedly mediated by an outer-sphere phosphate diester or an inner-sphere phosphate monoester complex, as shown by direct-excitation Eu(III) luminescence spectroscopy. These differences may provide a basis for designing selectively responsive PARACEST agents.

Chart 1



The CEST spectrum of Eu(**S-THP**)$^{3+}$ shown in Figure 1 was recorded by applying a presaturation pulse in 1 ppm increments. There is a CEST feature at ∼6 ppm downfield of bulk water that arises from the alcohol group, as shown by the corresponding alcohol proton resonance (Figure S1 in the Supporting Information). A pronounced pH dependence is observed for the CEST spectrum of Eu(**S-THP**)$^{3+}$ over the pH range from 4.5 to 7.3, with an optimum pH of 5.9 (Figure S2). This pH dependence is characteristic of base-catalyzed exchange with a low-pH optimum due to the acidic alcohol protons.[18] In addition, anionic ligands such as phosphate esters modulate the pH dependence of the CEST effect.

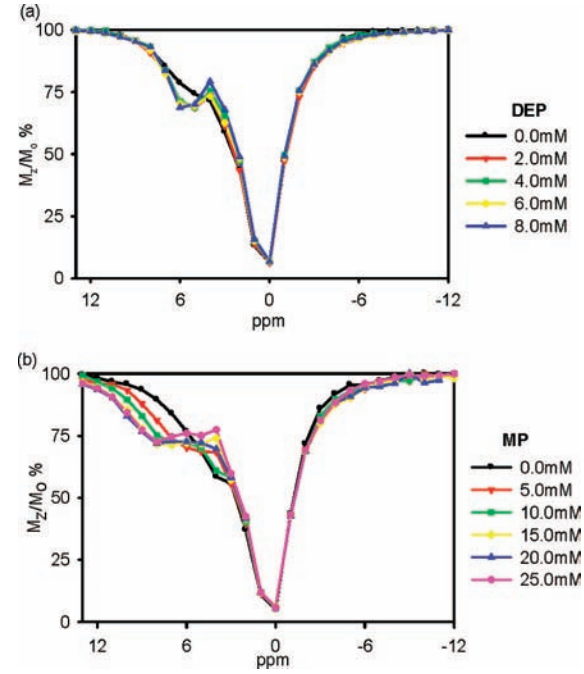


Figure 1. CEST spectra of 5.00 mM Eu(**S-THP**)$^{3+}$ with 20.0 mM MES, 100 mM NaCl and the addition of (a) diethyl phosphate at pH 6.6 and (b) methyl phosphate at pH 6.7. M_z is the water resonance intensity with saturation at the frequency shown, and M_o is the water resonance intensity without selective saturation.

Titration of Eu(**S-THP**)$^{3+}$ with diethyl phosphate (**DEP**) in a solution buffered at pH 6.6 and containing 100 mM NaCl increases the intensity of the existing CEST alcohol peak (Figure 1a). A plot of the Eu(**S-THP**)$^{3+}$ CEST response as a function of **DEP** concentration (Figure S3a) shows that even 1 equiv of **DEP** changes the CEST effect. In contrast, addition of methyl phosphate (**MP**) to Eu(**S-THP**)$^{3+}$ changes the CEST spectrum in two ways (Figure 1b): the alcohol CEST peak of Eu(**S-THP**)$^{3+}$ decreases, and a new CEST peak at ∼8 ppm grows in, corresponding to a new alcohol proton resonance (Figure S4). A plot of the intensity of the new CEST peak as a function of **MP** concentration was fit to a 1:1 binding curve with a dissociation constant of 10 mM (Figure S3b). These phosphate ester complexes of Eu(**S-THP**)$^{3+}$ have distinct pH-dependent CEST spectra over the pH range from 5.5 to 7.0. In the presence of 1 equiv of **DEP**, Eu(**S-THP**)$^{3+}$ has an optimal CEST effect at pH 7.0, and with **MP** there is a less pronounced pH dependence, with the strongest CEST effect from pH 5.5 to 6.5 (Figures S5−S8). In addition, **MP** and **DEP** modulate the CEST

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spectrum of Eu(**S-THP**)$^{3+}$ in an interdependent way when both esters (1−2 equiv each) are added (Figure S9).

These studies show that binding of the two phosphate esters to Eu(**S-THP**)$^{3+}$ gives rise to different CEST spectra and that **DEP** influences the CEST spectrum at lower concentrations than does **MP**, a surprising observation in view of previous work on the relative binding strengths of phosphate esters to Ln(III) macrocyclic complexes.[19] In order to study the nature of the phosphate ester interactions, direct-excitation Eu(III) luminescence spectroscopy was used. Figure 2 shows the $^7F_0 \rightarrow {}^5D_0$ excitation spectra for Eu(**S-THP**)$^{3+}$ as **DEP** or **MP** is added. The $^7F_0 \rightarrow {}^5D_0$ transition is especially useful because both the ground and excited states are nondegenerate, which means that the number of observed peaks corresponds to the number of different Eu(III) species in solution.[20] At pH 6.6, the Eu(**S-THP**)$^{3+}$ complex has one excitation peak for the aqua complex Eu(**S-THP**)$(OH_2)^{3+}$ (579.32 nm, Figure 2a).[21] Addition of 10 equiv of **DEP** to Eu(**S-THP**)$^{3+}$ at pH 6.6 does not change the intensity of the excitation peak at 579.32 nm. Luminescence lifetime data in H_2O and D_2O show that the number of bound water ligands does not change upon addition of excess **DEP** (Table S1). Taken together, these data suggest that the interaction of Eu(**S-THP**)$^{3+}$ with **DEP** under these conditions does not involve direct interaction with Eu(III) through water ligand displacement. In contrast, addition of **MP** to Eu(**S-THP**)$^{3+}$ leads to a decrease in the major excitation peak at 579.32 nm for Eu(**S-THP**)$(OH_2)^{3+}$ (Figure 2b). Binding occurs through displacement of a water ligand to give Eu(**S-THP**)(**MP**)$^+$, as confirmed by luminescence lifetime data (Table S1). Data for luminescence intensity and lifetime versus **MP** concentration were plotted and fit to a 1:1 binding isotherm to give K_d values of 22 and 7.0 mM, respectively (Figures S10 and S11), which are close to that measured in the CEST titration under similar conditions. Luminescence data show that the complex is stable over several days even in the presence of physiologically relevant concentrations of phosphate and carbonate (Figures S12 and S13).

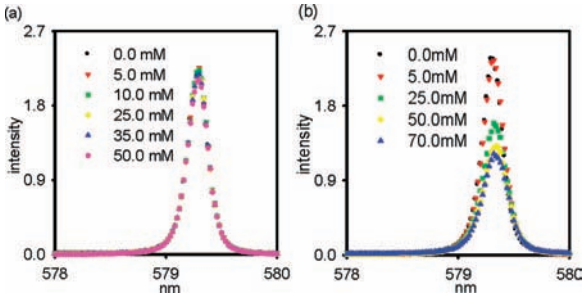

Figure 2. Luminescence excitation spectra ($^7F_0 \rightarrow {}^5D_0$, excitation at 579.32 nm, emission at 628 ± 27 nm) of 5.00 mM Eu(**S-THP**)$^{3+}$ in 20.0 mM MES and 100 mM NaCl at pH 6.6 with addition of (a) **DEP** and (b) **MP**.

These results highlight the different binding modes of the two phosphate esters that lead to distinct CEST responses for Eu(**S-THP**)$^{3+}$. The CEST spectrum modulation by outer-sphere **DEP** is mediated through a change in the rate constant for alcohol proton exchange, as suggested by the sharpening of both the CEST alcohol peak (Figure 1a) and the ^{1}H NMR alcohol resonance (Figure S1). This effect likely involves the interaction of **DEP** with the alcohol protons of Eu(**S-THP**)$^{3+}$ through an outer-sphere binding pocket.[22] In contrast, the inner-sphere **MP** complex gives rise to a new CEST peak corresponding to a new alcohol resonance. The distinct pH dependence of the CEST effect of the **MP** complex is attributed to a change in the acidity of the hydroxyl groups.

In summary, Ln(III) macrocyclic complexes with pendant alcohol groups are a promising new class of responsive PARACEST agents. The inner-sphere/outer-sphere binding dichotomy for two different ligands suggests a mechanism for developing selectively responsive CEST agents. Good inner-sphere ligands such as **MP** replace a water ligand, giving rise to a new pH-dependent CEST peak that is characteristic of the new complex. Poor ligands such as **DEP** do not replace the water ligand under similar conditions and thus promote a distinct CEST response. Nonetheless, low concentrations of **DEP** modulate the CEST spectrum of Eu(**S-THP**)$(OH_2)^{3+}$ in high concentrations of NaCl and buffer, supporting a specific interaction that may be further tuned. The binding constants reported here suggest that it may be feasible to develop PARACEST agents for the detection of phosphate-containing metabolites that are present in low millimolar concentrations.[23]

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Supporting Information Available: Luminescence excitation spectra, CEST and ^{1}H NMR spectra, binding curves, and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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