PARACEST agents based upon DOTA-like conjugates with poly-L-lysine

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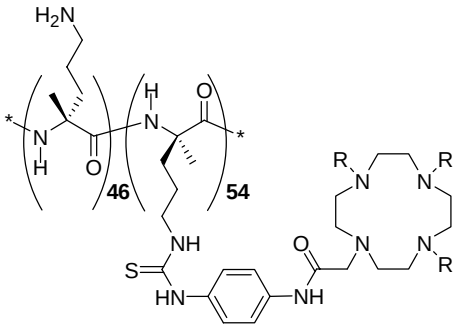
Introduction

Recently, a novel kind of MRI contrast agent has been described that takes advantage of both the paramagnetic shift properties of lanthanide ions and the widely diverse exchange dynamics of the bound water protons and/or the amide protons.[1] Such paramagnetic complexes are now referred to as PARACEST (paramagnetic chemical exchange saturation transfer) agents. Recently, an ion-pairing system has been reported to improve the PARACEST efficiency based upon a positively charged poly-L-arginine (to provide a large number of exchangable guanidine protons) and the negatively charged shift reagent ($TmDOTP^{5-}$).[2] However, systems such as this are not practical for *in vivo* use because 1) the ionic pairing interaction would likely be interfered with by endogenous cations or positively charged macromolecules and 2) the ionic partners may not coexist in the same compartment because poly-L-arginine is a macromolecule while TmDOTP is relatively small and easily diffusable. Here, a somewhat different approach is presented that uses two covalently conjugated systems to enhance the PARACEST effect.

Results and Discussion

As a demonstration of feasibility, two different DOTA-tetraamide ligands were covalently conjugated to poly-L-lysine (PLL) having an average of 150 lysine residues. Polymer **1** is based on DOTA-4AmC while Polymer **2** is based on the simple tetraamide of DOTA. The Eu^{3+} complex of DOTA-4AmC has been previously shown to have a slowly exchangeable bound water that can be activated to produce a PARACEST effect. Water exchange in the corresponding EuDOTAM (simple amide) system is too fast to be useful but the analogous YbDOTAM complex is useful *via* activation through the more highly shifted amide protons in this complex. NMR and elemental analysis indicated that ~54% of the lysine residues had been conjugated. High resolution ^1H NMR spectra of the Eu^{3+} and Yb^{3+} polymers had similar characteristics as the corresponding monomeric complexes.

To test the PARACEST efficiency of these systems, variable amounts of polymers were added to 100 mM HEPES buffer at pH 7 and Z-spectra were recorded at room temperature using a 4.7T animal imaging system and a 2 cm surface coil for RF transmission. A 2 s presaturation pulse (B_1 = 1020 Hz) was used and the saturation frequency was swept from 100 to -100 ppm in steps of 1 ppm (the bulk water frequency was set to 0 ppm). The Z-spectra of the polymers were similar to the corresponding monomer complexes. The Eu^{3+}-H_2O had a resonance at ~50 ppm while the Yb^{3+}-amide protons were shifted upfield near -17 ppm. Their PARACEST efficiencies *versus* concentrations were plotted in Figure 1. By fitting to the theory, the exchange lifetimes (τ_M) of 310 μs and 1.2 ms were obtained for Eu^{3+}-H_2O (polymer **1**) and Yb^{3+}-amide protons (polymer **2**), respectively. The most important feature is that these two conjugates show a significant CEST effect using polymer concentrations in the μM range.



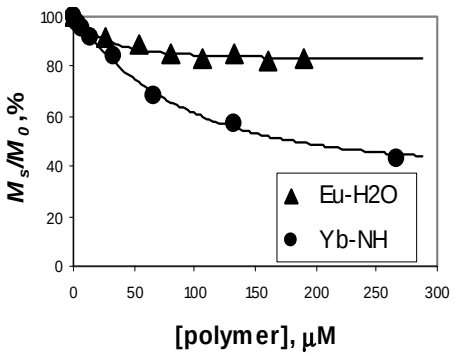


Figure 1. PARACEST efficiencies of polymer **1** (Eu complex) and **2** (Yb complex) at different concentration, and the best fit to the theory.

Conclusions

Two DOTA-tetraamide/PLL conjugates were synthesized and their lanthanide complexes were tested *in vitro* as prototype PARACEST agents. Since these systems are covalently conjugated and therefore should be suitable for blood pool imaging *in vivo*. Further modifications to introduce target-specific functional groups at the free lysine residues are possible.

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References

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