A New MRI PARACEST Agent for Sensing Glucose

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Introduction

The importance of glucose as a key fuel in mammalian cells and the known clinical complications associated with abnormal glucose levels have resulted in the development of numerous analytical methods to detect and quantify glucose. A recent report demonstrated that a PARACEST-based glucose sensor could potentially allow non-invasive mapping of physiological levels of glucose with the sensitivity of water ([water]/[glucose] ratio is ~ 10,000). PARACEST agents have the added advantage in that they can be activated (switched on-and-off) by applying a frequency-selective RF pulse at a mobile proton pool far away from bulk water, thereby altering the water signal intensity at will. Preliminary investigations of this previous PARACEST agent *in vivo* indicated modest toxicity likely due to the net positive charge on the agent, with or without glucose bound. Thus, a new glucose sensor reported here, EuDOTAM-bis(phenylboronate)-bis(glycinylamide) (Eu**L1**) was designed to increase the negative charge on the complex and thereby lower its toxicity. Like the previous agent, Eu**L1** can be activated by applying a saturation pulse at ~ 44 ppm at 37°C. We have found that Eu**L1** has other advantages over the previous system including higher water solubility and lower *in vivo* toxicity while maintaining a high sensitivity for glucose at physiological pH and temperature. Preliminary MRI data reported here show that this new glucose sensor is quite promising for *in vivo* imaging of glucose.

Methods

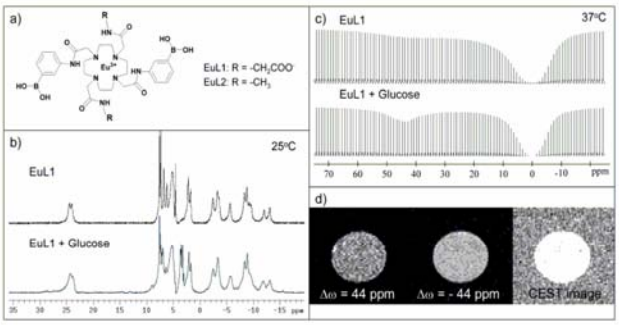
The new glucose sensor Eu**L1** (Figure 1a) was prepared by mixing stoichiometric amounts of $EuCl_3$ and the ligand and heating the mixture for 5 hrs at 90°C while maintaining the pH between 7~8. Filtration and evaporation of this solution resulted in a powder that was redissolved in DI water (50 mM) for use as a stock solution. Diluted samples of this stock (20 mM) were characterized by NMR (Figure 1b), CEST (Figure 1c) and MRI (Figure 1d). To test the *in vivo* toxicity of this sample, 400 μl of 50 mM Eu**L1** at pH7.4 and osmolality of ~ 300 mM was slowly injected through jugular vein catheter into the body of a female C57BL6 mouse (22 g). CEST was measured at 9.4T (Varian Inova) at 25°C and 37°C by a CW frequency-selective pulse at multiple B_1 values (200 – 2 kHz) in a duration range of 500 ms – 2 s. MRI spin echo (SE) microimages (data maxtrix 64x64, FOV 30x30 mm^2, thickness: 3 mm; TR/TE 2 s/10 ms) were obtained at 9.4T, pH 7.4, with and without glucose. The sample was mixed with hydrogel to mimic the viscosity of tissue and loaded into 5 mm tubes and pre-saturated at ± 44 ppm by a Gaussian-shaped pulse with B_1 power of 500 Hz and duration of 1s in the MRI experiments.

Results and Discussion

Eu**L1** was found to be highly water soluble (~100 mM) at pH 7 and 37°C and stable for prolonged storage (no visable precipitation for at least 2 months). There was no apparent toxicity or side effects as the animal appeared to be healthy and breathing normally after injection of 400 μl of 50 mM Eu**L1**, a reasonable dosage for *in vivo* CEST imaging. The ^{1}H NMR spectrum of Eu**L1** at 25°C showed a typical shift pattern for a square antiprism (SAP) coordination geometry similar to other well-characterized EuDOTA and EuDOTAM-like complexes. The CEST spectrum of Eu**L1** at 25°C showed a "dip" at 52 ppm with a maximum of 25% water attenuation (data not shown) but this "dip" was not apparent at 37°C (Figure 1c top). In contrast, the addition of 1:1 glucose to Eu**L1** resulted in an ~30% decrease in the bulk water intensity after application of presaturation pulse at 44 ppm (Figure 1c bottom). This is characteristic of CEST from a Eu^{3+}-bound water molecule in exchange with bulk water. A sharpening of bulk water resonance in the CEST spectrum (Figure 1c) of the Eu**L1**-glucose sample compared to Eu**L1** alone suggests that the exchange between these two water pools slowed upon binding of glucose. Interestingly, the high resolution ^{1}H NMR spectrum of Eu**L1**-glucose (Fiure 1b bottom) showed extra small broadened peaks at low field (2 peaks between 27 – 30 ppm and two more peaks between 12-16 ppm), which might belong to another structurally different species or from the binding glucose. The 9.4T images of EuL-glucose images with saturation at 44 ppm ("on-resonance") and -44 ppm ("off-resonance") showed visible image contrast, which generated a clear CEST image by pixel-by-pixel subtraction of "on-" from "off-resonance" images (Figure 1d). Application of this new glucose sensor for *in vivo* imaging of glucose is currently under way.

Conclusion

A new glucose sensor has been characterized by NMR and MRI and tested for *in vivo* toxicity in mice. Preliminary data showed that Eu**L1** has low *in vivo* toxicity and high CEST performance in responding to glucose binding at biological conditions, and therefore could be useful for *in vivo* imaging of tissue glucose.



References

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