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A Paramagnetic CEST Agent for Imaging Glucose by MRI

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Balaban et al.[1,2] recently reported that low molecular weight compounds with slowly exchanging −NH or −OH protons may be used to alter tissue MRI contrast via *c*hemical *e*xchange *s*aturation *t*ransfer (CEST) of presaturated spins to bulk water. One important feature of this contrast mechanism is that it permits modulation of the contrast effect via gating the RF presaturation pulse. Van Zijl et al.[3−5] have shown that the effect can be amplified by using exogenous macromolecules with large numbers of exchanging sites or endogeneous proteins and peptides. This idea was extended to paramagnetic europium (III) DOTA−tetraamide complexes[6] (DOTA = 1,4,7,10-tetraazacyclododecane-*N*,*N*′, *N*″,*N*‴-tetraacetic acids) when it was shown that such complexes display a slowly exchanging ($\tau_M^{298} \approx 380\ \mu s$) bound water resonance near 50 ppm. Paramagnetic CEST agents offer several advantages over diamagnetic agents. The larger frequency shift of the exchanging site ($\Delta\omega$) means that agents with much faster exchange may be used without approaching the slow exchange limit ($\Delta\omega^*\tau_M \gg 1$), and less off-resonance irradiation of bulk water should occur. Importantly, paramagnetic CEST agents with a wide variety of chemical and physical properties can be designed by slight modification of the ligand structure (fine-tuning of τ_M) or choice paramagnetic ion (to vary both $\Delta\omega$ and τ_M).[7−9] Several paramagnetic CEST agents have now been reported that are capable of sensing indices such as pH[10−12] or lactate concentration.[13] The theoretical and experimental aspects of such paramagnetic CEST agents had been reviewed recently.[14] In short, it opens an entirely new avenue to those who are interested in MR cellular or molecular imaging. Here we report on the design of a paramagnetic CEST agent that can provide a metabolic map of glucose by MRI.

Boronic acids are known to bind selectively and reversibly with structures containing cis-diols, a property that has been widely exploited in the design of new saccharide sensors.[15,16] This property has also been used to target Gd^{3+} chelates to glycated proteins to improve the relaxivity.[17,18] With this background, we set out to build a prototype CEST agent (Chart 1) for detecting levels of tissue glucose.

The solubility of free ligand in water is low, but the corresponding lanthanide(III) complexes are quite soluble (up to 0.5 M). This feature makes it convenient to prepare the pure complexes in aqueous solution by adding excess ligand (5−10%) and filtering off the excess after complexation. The low-field portion of ^{1}H NMR spectra of Eu(**1**) in the absence (lower) and presence (upper) of glucose are given in Figure 1. The macrocyclic H_4 resonance (∼21 ppm) serves as a convenient internal reference. The appearance of a broad resonance near 50 ppm, easily assigned to a Eu^{3+}-bound water species,[6] suggests that binding of glucose to Eu(**1**) slows exchange of the Eu^{3+}-bound water molecule.

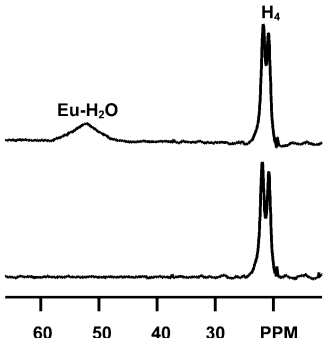

Figure 1. Low-field portion of the 500 MHz ^{1}H NMR spectra of 10 mM Eu(**1**) in the absence (lower) and presence (upper) of 100 mM glucose at pH 7.2 and 20 °C (the bulk water peak was set to 0 ppm).

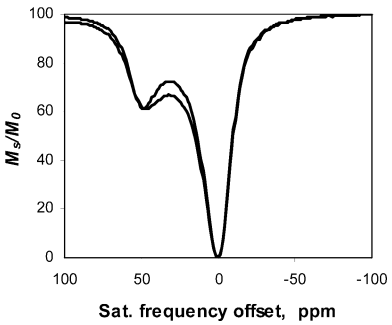

Figure 2. Z-spectra of 10 mM Eu(**1**) in the absence (lower) and presence (upper) of 20 mM glucose, pH 7.0, 25 °C.

To determine whether this difference in water exchange could be detected in a CEST experiment, Z-spectra were recorded on samples of 10 mM Eu(**1**) and various amounts of glucose (0−100 mM).[6] On average, the bulk water signal intensity in the sample containing only Eu(**1**) decreased ∼38% after applying a 2 s saturation pulse ($B_1 = 1020$ Hz) at the bound water position (∼50 ppm at 25 °C). Although a similar CEST effect was observed at the peak maximum (∼50 ppm) after addition of excess glucose, the shape of the Z-spectra indicated that water exchange is indeed slower when glucose is bound to Eu(**1**). This was evidenced by an increase in intensity of the Z-spectrum at all frequencies between ∼25 and 35 ppm (Figure 2). Even though this difference is relatively small, a plot of the CEST ratio (bulk water intensity after applying a saturation pulse at 30 ppm/intensity after applying a saturation pulse at 50 ppm) versus glucose concentration is shown in Figure 3. This curve fits well to a 1:1 binding model with an association constant of 383 M^{-1}, a constant that agrees closely with a value obtained in an independent experiment using CD spectroscopy (K_a = 377 M^{-1}; see Figure S1). This association constant is ∼200-fold larger than that of phenylboronic acid[19] and larger than or similar to those of other diboronic fluorescent sensors.[20,21] Most

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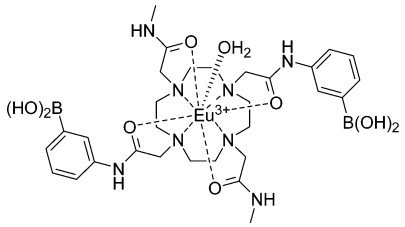
Chart 1



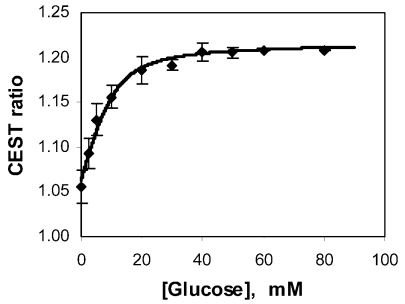


Figure 3. A plot of CEST ratio versus glucose concentration for 10 mM Eu(**1**) complex in 100 mM PIPES buffer at pH 7.0, 25 °C. The CEST ratios were calculated from the corresponding CEST spectra by applying a 2 s saturation pulse (B_1 = 1020 Hz) at frequency offsets of 50 and 30 ppm. The data points and the error bars are the average values and the standard deviations from three individual experiments, and the solid line is the best-fitted curve to a 1:1 binding model with a K_a of 383 M^{-1}. CEST subtraction also gave similar result (see Figure S2).

importantly, the curve of Figure 3 shows that the most responsive range for detection of glucose using the CEST properties of Eu(**1**) is 0−20 mM.

To test whether this method can be used to image glucose by MRI, a phantom consisting of four plastic tubes (ID 4 mm) each containing 10 mM Eu(**1**) and different amounts of glucose was prepared in 100 mM PIPES buffer at pH 7.0. Images were acquired after alternatively applying identical presaturation pulses at 50 ppm (bound water) versus 30 ppm (between the bound and bulk water peaks). Although the intensities of these individual images were visually similar (see Figure S3), the difference images show clear intensity gradations that parallel the glucose concentration (Figure 4). Using a gray scale of 0−255 (from the darkest to the brightest), we found that the image intensities were 208, 148, 112, and 96 for samples containing 0, 5, 10, and 20 mM glucose, respectively. The uncertainty in the measured image intensity in the physiological range of interest (∼5 mM) is about 10% (n = 4).

In summary, a EuDOTA−tetraamide ligand with bis(phenyl boronate) arms is shown to bind glucose reversibly with an association constant of 383 M^{-1}. Such binding alters the water exchange between a Eu^{3+}-bound water molecule and bulk water, and this is easily detected by CEST imaging. Importantly, this prototype CEST agent is sensitive to changes in glucose over a range of physiological interest (5−10 mM). Furthermore, it should be possible to optimize the water exchange properties of such complexes to enhance the CEST effect by minor alterations in the structure of the appended side chains.[9] This technology offers for the first time the possibility of mapping the distribution of glucose in tissue by MRI using the bulk water protons as antenna.

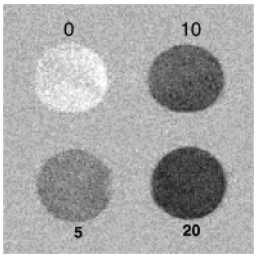


Figure 4. CEST images of phantoms containing 10 mM Eu(**1**) and either 0, 5, 10, or 20 mM glucose. PIPES (100 mM) was used to buffer the pH at 7.0. The image parameters were as follows: TR/TE = 3000/18 ms, FOV = 40 × 40 mm, thickness 2 mm, data matrix 256 × 256, saturation duration time of 2 s at a power of 1020 Hz at a frequency offset of 50 and 30 ppm. The CEST image was obtained by subtracting pixel by pixel the image at 50 ppm from that at 30 ppm.

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Supporting Information Available: CD spectra and a plot of their intensities versus glucose concentration for Eu(**1**) (Figure S1), a plot of CEST subtraction versus glucose concentration for Eu(**1**) (Figure S2), and the original MR images (Figure S3) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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