A self-calibrating PARACEST MRI contrast agent that detects esterase enzyme activity

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Abstract: We have developed a PARACEST MRI contrast agent, Yb-DO3A-oAA-TML-ester, that detects esterase enzyme activity. Esterase caused the agent to spontaneously disassemble to create a PARACEST agent with two detectable CEST effects. The ratio of the two CEST effects was independent of concentration and T_{1sat} relaxation, so that this agent was self-calibrating with respect to these factors. This ratiometric method was dependent on temperature, although a more detailed analysis could account for this effect. Therefore, a self-calibrating PARACEST MRI contrast agent can more accurately detect a molecular biomarker such as esterase enzyme activity.

Introduction: Many MRI contrast agents change in response to a molecular biomarker, but other molecular biomarkers or environmental factors can influence the agent, so that a MRI signal change is not necessarily conclusive proof for detecting a biomarker.[1,2] To overcome this problem, a second control CEST effect may be included in the same PARACEST agent, which is responsive to all factors that alter the first CEST effect except for the biomarker to be measured. To investigate this approach, a PARACEST MRI contrast agent was developed with one CEST effect that is responsive to esterase enzyme activity and a second control CEST effect (Figure 1A).

Methods: CEST MR studies were conducted using a 600 MHz Varian Inova NMR spectrometer by prepending a standard pulse-acquire protocol with 3.0 s of continuous-wave radio frequency saturation at a power of 14.8 μT, with saturation frequencies ranging from +30 to -30 ppm in 1 ppm increments.[3] A single function that consisted of a sum of three Lorentzian lineshapes was fit to each CEST spectrum using custom routines written for Matlab R2009B. An inversion-recovery NMR protocol with 14.8 μT selective saturation was used to measure T_{1sat} relaxation times. To study the in vitro enzyme reaction, a CEST spectrum was acquired before and 24 hours after combining 25 mM of Yb-DO3A-oAA-TML-ester and 617 nM of porcine liver esterase. To perform the cell study, a CEST spectrum was acquired after creating a 25 mM solution of Yb-DO3A-oAA-TML-ester with media used to culture 10^9 cells/mL of PA10145-U P. aeruginosa bacterial cells. Additional CEST spectra were acquired to test the effects of concentration (3-20 mM), T_{1sat} relaxation time (0.74-1.42 sec), temperature (17.2-53.2 °C), and saturation time (0-6 sec).

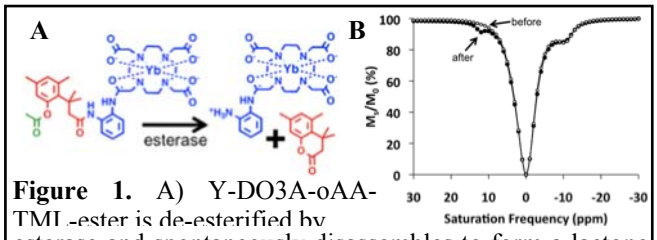

Figure 1. A) Y-DO3A-oAA-TML-ester is de-esterified by esterase and spontaneously disassembles to form a lactone (red) and Yb-DO3A-oAA (blue). B) Y-DO3A-oAA-TML-ester showed a CEST effect at -10 ppm (open circles). Adding esterase to create Yb-DO3A-oAA caused a second CEST effect to appear at +12 ppm (filled circles).

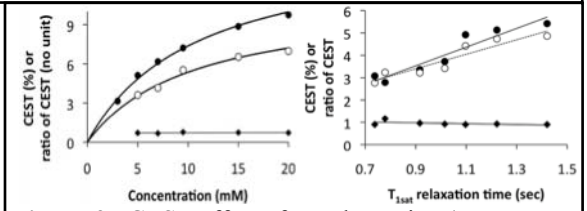

Figure 2. CEST effects from the amine (open circles) and amide (filled circles) of Yb-DO3A-oAA were dependent on concentration and T_{1sat} relaxation, but the ratio of the CEST effects (diamonds) was independent of these factors.

Results: The PARACEST agent, Yb-DO3A-oAA-TML-ester, showed a single CEST effect from the amide that is near the lanthanide ion. After adding esterase to a solution of the agent, a second CEST effect was detected from an amine, indicating that the esterase had catalyzed de-esterification that led to spontaneous disassembly of the agent (Figure 1B). A similar result was obtained by adding the PARACEST agent to media used to culture *P. aeruginosa*, a bacterium known to secrete esterases.[4] As further proof that the agent detected esterase activity, no reaction was detected when the media was autoclaved before adding the agent.

The ratio of the two CEST effects of the product after the enzyme reaction, Yb-DO3A-oAA, was independent of concentration and T_{1sat} and therefore the agent is self-calibrating with respect to these factors (Figure 2). Although the ratio of the CEST effects was dependent on temperature, this dependence followed the Arrhenius Equation so that this self-calibrating, ratiometric approach is still feasable as long as temperature is stable during the CEST MR study. The ratio of the CEST effects was dependent on pH, so that knowing the pH is a prerequisite for this method. A concentration-dependent Hanes method and the T_{1sat}-dependent Hanes method were used to measure the chemical exchange rates of the amide and amine.[5] These Hanes methods showed that the agent was incompletely saturated, although the incomplete saturation did not affect the comparison of CEST effects of the single agent.

Discussion: These results demonstrated that Yb-DO3A-oAA-TML-ester was responsive to esterase activity. Furthermore, the ratio of the CEST effects can more conclusively report on enzyme activity because the ratio is independent of concentration and T_{1sat} relaxation times and can also account for the effects of temperature.

References

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