Evaluation of a TGase-responsive PARACEST MRI contrast agent: the influence of conformations and non-covalent adducts

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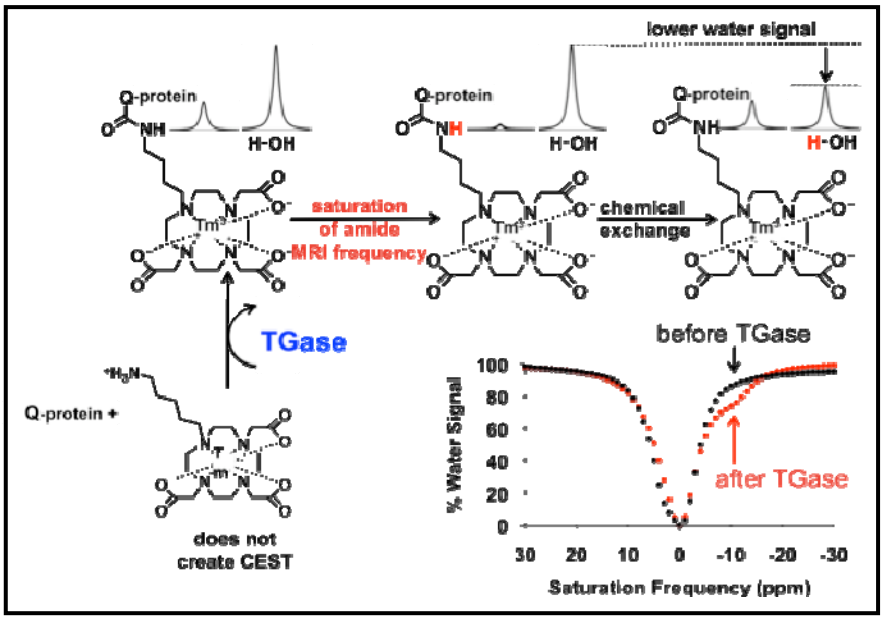
INTRODUCTION: A PARACEST MRI contrast agent, tm-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid-10-cadaverine (Tm-DO3A-cadaverine), has been shown to conjugate to the side chain of glutamine via catalysis with transglutaminase (TGase) [1]. This demonstration showed the appearance of CEST after the creation of a covalent bond by TGase. However, this demonstration was only performed with a hydrophobic peptide, ZQG. We sought to improve the utility of this agent by investigating the conjugation of this agent to a protein and to hydrophilic peptides that contain arginine. Furthermore, we investigated the chemical exchange rates before and after the TGase-mediated conjugation reactions to further characterize the behavior of this PARACEST agent.

METHODS: **Sample Preparation:** Tm-DO3A-caddaverine was synthesized by alkylating DO3A with bromopentylamine and using the product to chelate Tm(III). A total of 0.5 units of recombinant microbial TGase (Zedira GmbH) was incubated with 20 mM of Tm-DO3A-cadaverine, 0.75 mM of the albumin, and 8 mM of glutathione in tris-HCl buffer at pH 7.0 and 37°C for 24 hours. We also carried out the reaction by replacing albumin with 20mM of QR or GQR peptides. CEST Spectra were acquired for each reactant, glutathione, and the reaction mixture before and after adding TGase. **CEST Spectrum:** A series of 1D NMR spectra were acquired using a 4 second saturation pulse applied at 20 μT power and with saturation frequencies spanning +35 to -35 ppm in 0.25 ppm increments (NEX=4, 37.3°C, 11.7T). Water peak heights were used to construct CEST spectra of a sample before and after adding the enzyme. Lorentzian line fitting was performed to measure the magnitude of CEST from the reactants and the Tm-DO3A-cadaverine-albumin product (Fig. 1) [2]. Bloch fitting was performed to measure chemical exchange rates [3,4].

RESULTS: The synthesis intermediates and product prior to chelation, and the reactants and products of the enzyme reactions, were confirmed with Mass Spectrometry and NMR spectroscopy. The TGase reaction with albumin, QR or GQR caused CEST to appear at -11 and +4 ppm (Fig. 1). Chemical exchange rate analyses were used to validate that CEST arising from proximity to Tm(III) was from a paramagnetic agent. Comparisons of CEST spectra of reactants and products were also used to validate the identify of each CEST effect. These results confirmed that Tm-DO3A-cadaverine can detect TGase enzyme activity



The CEST spectrum of Tm-DO3A-cadaverine and the arginine-containing peptides prior to adding TGase showed a weak CEST effect at -11 ppm. This CEST effect was assigned to a non-covalent, supramolecular adduct of Tm-DO3A-cadaverine and the arginine side chain of the peptide. Similar results have been reported for a solution of polyarginine and a Tm(III) chelate [5].

DISCUSSION: The appearance of CEST at -11 ppm differs from the previous report of this agent, which listed the appearance of CEST at +4, +11, and +22 ppm after conjugation of Tm-DO3A-cadaverine to a hydrophobic ZQR peptide. This result demonstrates the outstanding specificity of CEST for different molecular conformations, which may be further exploited to design responsive CEST agents. The appearance of a weak CEST effect at -11 ppm before TGase-catalyzed conjugation suggests the formation of a non-covalent supramolecular adduct, which may be further exploited to design responsive CEST agents.

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