Novel hydroxytryptophan-based Gd chelating substrate for imaging myeloperoxidase activity.

A. A. Bogdanov[1], Y. Xie[2], and M. S. Shazeeb[2]
[1]Radiology, UMASS Medical School, Worcester, MA, United States, [2]UMASS Medical School

Introduction: Myeloperoxidase (MPO) is one of the crucial imaging targets that has clear outcome- predictive value in several diseases, including myocardial infarction and stroke [1]. MPO has also been implicated in the progression of cancer, several CNS pathologies, and in the development of unstable atheroma. Because the catalytic activity of MPO is preserved in live tissue, the enzymatic activity of MPO can be used as a marker of inflammation sites *in vivo*. We previously synthesized and tested several paramagnetic complexes of mono- and bis- amides of macrocyclic and linear chelates that "sense" MPO activity [2-4]. As a result of MPO-mediated catalysis, the above sensors oligomerize and/or form covalent bonds with proteins resulting in increased molar relaxivity (r_1 and r_2). This enables MR imaging of MPO activity *in vivo*. Although the existing paramagnetic MPO sensors have very high kinetic stability [5], the thermodynamic stability of bisamides is usually compromised and the solubility of substituted tryptamides in water is limited. The goal of the current study was to synthesize and characterize a novel bifunctional MPO sensor with the same number of donor atoms as in DTPA (i.e. N3O5) in anticipation of improved thermodynamic stability and better solubility**.**

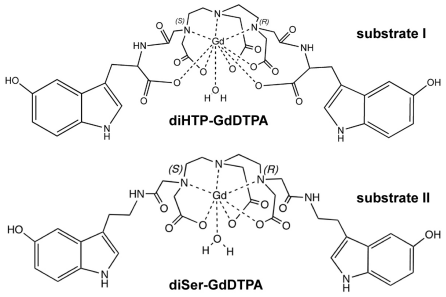

Fig. 1. Structures of MPO substrates I and II

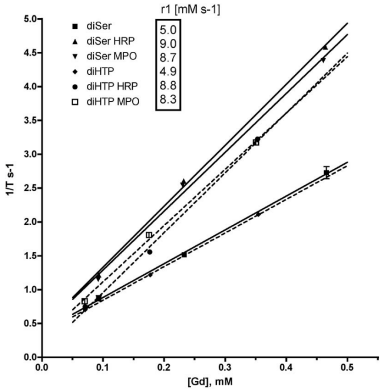

Fig. 2. Relaxivities of chelated Gd in substrate I and substrate II and relaxivities of reaction mixtures containing MPO/GOX or HRP/GOX enzyme pairs.

Methods: An octadentate ligand (bis-amide of DTPA) was synthesized by reacting hydroxytryptophan, a naturally occurring amino acid, with DTPA dianhydride in the presence of pyridine. Gadolinium was trans-chelated from Gd citrate. The resultant chelate was purified by acetone precipitation and purified by C18-HPLC using a gradient of acetonitrile. The obtained product (bis-HTrp-DTPA(Gd), substrate **I**) was characterized by using ^{1}H, ^{13}C NMR and TI-MS. The control substrate (bis-5-hydroxytryptamide) of Gd DTPA (substrate **II**) was synthesized as described in [4]. The comparative substrate activation studies were performed using glucose oxidase GOX/MPO or GOX/horseradish peroxidase (HRP, positive control) as complementing activity-coupled enzymatic pairs (3 iU GOX : 1.5 iU MPO or HRP) in the presence of 0.1-5 mM of paramagnetic MPO substrate and 5 mM glucose. The reaction was terminated by sodium azide (5 mg/ml) after 0.5 h (HRP) or 2 h (MPO). Gradient SDS-PAGE (5-15%) was performed using samples incubated in the presence or the absence of the substrates to account for potential protein cross-linking using silver staining.

Results and Discussion: The structure of the purified chelate was proven by 1H NMR spectrometry and MALDI-ITMS (m/z 796.4 ($C_{36}H_{43}N_7O_{14}$ - H+)). The gadolinium complex of the above chelate (m/z 951.3, Fig. 1), i.e. substrate **I** , was further tested in the presence of peroxide-generating GOX and either MPO, or HRP. The additional substrate *in vitro* efficacy testing involved comparing the MPO and HRP-mediated relaxivity increase in the presence of a hydrogen peroxide source (glucose/glucose oxidase) to that of a previously described and *in vivo* tested substrate **II** [2,5].

We observed an MPO-dependent increase of molar relaxivity of Gd in the reaction mixture from 4.9 to 8.3 $[mM.s]^{-1}$ (1.7 fold at 0.47T) at 2 h (Fig. 2). HRP, which has a higher effective kinetic constant, showed a similar 1.8-fold increase of relaxivity under identical conditions. In the case of bis-5HT-DTPA(Gd), we observed very similar changes of relaxivity (1.7 vs. 1.8 times, respectively), suggesting that both Gd chelates **I** and **II** were used by peroxidases as reducing substrates. However, there were notable differences in terms of the type of reaction products: substrate **II** and HRP gave insoluble cross-linked final products if the reaction proceeded for several hours while the products of substrate **I** were soluble. Gradient SDS-PAGE analysis of enzymatic activation of substrate **I** showed the formation of 10-20 kDa oligomerized products (Fig. 3, arrow) in the case of HRP, while in the reaction mixture containing MPO we observed the formation of a new band of cross-linked proteins. This result suggested covalent binding to proteins as a potential mechanism of relaxivity increase as a consequence of an MPO-catalyzed reaction. No apparent cross-linking of proteins was observed in the case of substrate **II.** Imaging of phantoms at 3T (Fig. 3, spin-echo sequence, TR/TE=200/30ms, NEX=1), as expected, showed smaller increase of r1 (20%) at this field strength than at 0.47T. HRP resulted in a strong 1.9-fold increase of r2 in the case of substrate **II** but no such changes were observed with substrate **I** proving a lack of product aggregation.

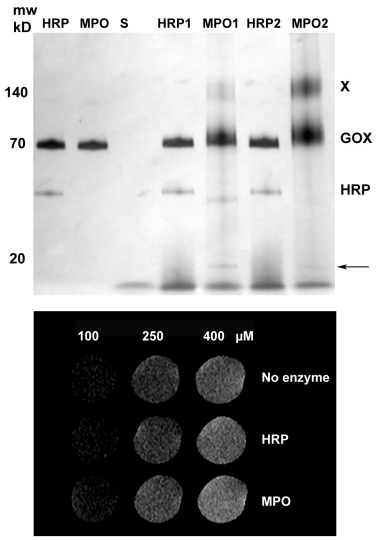

Fig. 3. Top: Gel electrophoresis of MPO and HRP-containing reaction mixtures. Cross-linked product is marked with X. **Bottom:** T1W SE images of reaction mixtures containing MPO/GOX or HRP/GOX enzyme pairs at 3T.

Conclusions: Bis-HTrp-DTPA(Gd) is a novel paramagnetic reducing substrate of MPO. It results in MPO-mediated increase of Gd relaxivity similar to that of bis-5HT-DTPA(Gd). However, Bis-HTrp-DTPA(Gd) has high water solubility and undergoes MPO-specific conversion into protein cross-linking reactive intermediates. We anticipate it to be an efficient sensor for *in vivo* imaging of MPO activity due to potential binding to tissue proteins in inflammatory lesions.

References: 1. Brennan ML *et al.* NE J Med 349: 1595, 2003; 2. Ronald JA *et al.* Circulation 120:592, 2009; 3. Chen JW *et al.* MRM 52:1021, 2004; 4. Querol M *et al.*, Org Lett. 17, 1719, 2005; 5. Rodriguez E *et al.* JACS 132:168, 2009,