An Activatable Manganese-Based MR Contrast Agent for the Imaging of Redox Environment

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Introduction

Redox reactions are ubiquitous in biochemical activities. Disruption of redox homeostasis has been associated with a large number of pathological conditions such as atherosclerosis, stroke, Alzheimer's disease, Parkinson's disease and cancer. However, the lack of suitable redox responsive probes has hindered the non-invasive observation of intracellular redox activities through molecular imaging. Some reports have described redox chemistry of the ligand being utilized to develop gadolinium-based redox probes (T_1 agents)[1]. We reasoned that taking advantage of metal-based redox chemistry would result in large changes in relaxivity. Unfortunately other oxidation states of Gd or Eu are both energetically unfavorable and unstable. We hypothesized that a redox-activated MR probe based on the Mn^{2+}/Mn^{3+} couple would be stable with respect to metal dissociation in both oxidation states, sensitive to biological reductants, and sufficiently reactive to act as an in vivo sensor. Ligand field strengths are known to affect the potential of a redox couple. For example EDTA forms stable Mn(II) complexes, whereas replacing two carboxylate donors in EDTA with phenolates to give the HBED ligand favors the Mn(III) state. We hypothesized that a ligand structure (HBET) that is intermediate between EDTA and HBED – containing only one phenolate donor – could potentially exhibit metastability toward either oxidation state. Here we report the design and characterization of a potential redox probe.

Methods

HBET (hydroxybenzylethylenediamine triacetic acid) was synthesized from reductive amination of mono BOC-protected ethylene diammine with salicaldehyde, followed by alkylation with t-butyl bromoacetic acid, and then acid deprotection to give the free ligand in overall 45% yield. The reaction of one equivalent of ligand with $MnCl_2$ led to Mn^{II}-HBET in 84% isolated yield. The Mn^{III}-HBET complex was synthesized in 38% yield by aerial oxidation of $MnCl_2$ in the presence of one equivalent of the ligand followed by RP-HPLC purification. Relaxivity measurements were performed on a Bruker mq60 Minispec at 1.4 T and 37°C. Manganese concentrations were determined using an Agilent 7500a ICP-MS system. UV-Vis spectra were recorded on a SpectraMax M2 spectrophotometer using quartz cuvette with a 1 cm path length. Cyclic Voltammetry (CV) was recorded in TRIS buffer (pH = 7.4) containing 0.5 M KNO_3 as supporting electrolyte, at 100 mVs^{-1} scan rate. The $K_4Fe(CN)_6$/ $K_3Fe(CN)_6$ couple was used as the internal standard. Glassy carbon was used as the working electrode; Ag/AgCl as the reference electrode and Pt wire was used as the auxiliary electrode.

Results

The T_1 relaxivity of the Mn^{II}-HBET complex in TRIS buffer (pH 7.4, 37° C) is three to four fold higher than the Mn^{III}-HBET complex when measured at 1.4 and 4.7 T (2.76,3.95 $mM^{-1}s^{-1}$ vs 1.04,1.20 $mM^{-1}s^{-1}$). This difference is large considering that the Mn^{III} state still has four unpaired electrons. The CV scan for Mn(II)HBET shows a reversible oxidation peak at 0.356 V, indicating the potential for using this molecule as a redox probe. The feasibility of this probe to act as redox responsive MR agents was tested under physiologically relevant reducing conditions. The Mn^{III}-HBET complex is easily reduced to the Mn^{II} state in the presence of glutathione. Figure 1a shows the increase in relaxation rate ($1/T_1$, symbols) over 15 minutes as Mn^{III}-HBET (solid line) is reduced to the Mn^{II} complex. Figure 1b shows that this conversion proceeds directly to the product without the accumulation of any long-lived reaction intermediates or the formation of byproducts. Detailed kinetic experiments for this redox reaction indicate that the reaction is first order in both glutathione and [Mn^{III}-HBET] with an overall second-order rate constant of $(3.8\pm0.3)\times10^{-1}$ M^{-1} s^{-1} (95% C.I.).

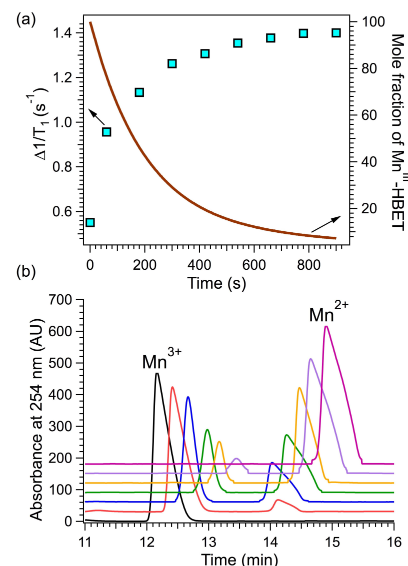

Figure 1. Reduction of Mn^{III}-HBET in the presence of 10 mM glutathione monitored by (a) increase in relaxivity (left axis) and decrease of UV absorbance at 375 nm (right axis) over time, and (b) an analytical LC-MS technique at 22 minute intervals.

Conclusion

For redox-activated probes, the conversion to the activated state must occur efficiently within a physiologically relevant redox range and should also be rapid with respect to probe washout. The Mn^{III}-HBET complex has a half-life of 3-30 minutes in the presence of 1-10 mM glutathione concentration (similar to the conditions within cells). By comparison the half-life of Mn^{III}-HBET in blood plasma, where the glutathione concentration is three orders of magnitude lower, would be greater than a week. Therefore, we anticipate that the probe activation would be limited primarily to regions where the normal mechanisms that regulate extracellular redox have been severely impaired.

Reference: (1) (a) Tu, C.; Nagao, R.; Louie, A. Y. *Angew. Chem.-Int. Edit.* **2009**, *48*, 6547; (b) Raghunand, N.; Jagadish, B.; Trouard, T. P.; Galons, J. P.; Gillies, R. J.; Mash, E. A. *Magn. Reson. Med.* **2006**, *55*, 1272.