Oxidation-Responsive, Eu$^{II/III}$-Based, Multimodal Contrast Agent for Magnetic Resonance and Photoacoustic Imaging

Lina A. Basal, Yan Yan, Yimin Shen, E. Mark Haacke, Mohammad Mehrmohammadi, and Matthew J. Allen

INTRODUCTION

Perturbation of redox homeostasis has been correlated with pancreatic, colorectal, prostate, and lung cancers; neurodegenerative diseases; colitis and inflammatory bowel disease; and liver diseases. Because changes in redox environments are associated with many diseases, there is value in detecting these changes. Multimodal imaging agents that combine magnetic resonance imaging (MRI) and photoacoustic imaging are capable of producing images that provide molecular information, which is of potential utility in the imaging of oxidative stress. MRI and photoacoustic imaging have complementary properties: MRI enables scans over large volumes with practically unlimited depth penetration, and photoacoustic imaging can produce detailed, real-time images of areas that are accessible by sources of light. Orthogonal imaging techniques that use multimodal imaging agents are potentially useful with respect to validation and the avoidance of misinterpretation of data from a single imaging technique. However, reported multimodal MRI and photoacoustic contrast agents do not report changes in redox environments. Eu$^{II}$-containing complexes have been reported as oxidation-responsive contrast agents for MRI that shorten the $T_1$ relaxation times of protons on exchanging water, and we hypothesized that a subset of these complexes that change absorbance in the visible region of the electromagnetic spectrum as a function of oxidation state could serve as oxidation-responsive, multimodal contrast agents for magnetic resonance imaging and photoacoustic imaging. Here, we report the photoacoustic properties of Eu-containing species 1 and 2 (Figure 1) that are contrast agents for $T_1$-weighted and chemical exchange saturation transfer MRI, respectively.

Results and Discussion

The pair of complexes 1 and 2 has been previously reported as an oxidation-responsive contrast agent for MRI. Complex 1...
has a relaxivity of $3.73 \pm 0.04 \text{ mM}^{-1} \text{s}^{-1}$, and complex 2 has a relaxivity of zero but acts as a contrast agent for chemical exchange saturation transfer MRI. This difference in relaxivity is amenable to the visualization of oxidation from EuII to EuIII. Neither complex has been characterized with respect to photoacoustic imaging.

To study the optical absorption properties relevant to photoacoustic imaging, molar absorptivity values were calculated. The molar absorptivity at 410 nm was calculated because this wavelength is near the wavelength where the EuII-containing complexes in this study has the highest absorption on the photoacoustic imaging system used (Figure 1). To determine the molar absorptivity at 410 nm, UV–visible spectra were measured before and after oxidation, and molar absorptivity values were calculated from the spectra. The molar absorptivity of EuII-containing complex 1 was $3.0 \times 10^2 \text{ mM}^{-1} \text{ cm}^{-1}$, and there were no absorptions above the noise for EuIII-containing species 2 at 410 nm (molar absorptivity of 0 mM$^{-1}$ cm$^{-1}$); therefore, the difference in molar absorptivity between the oxidation states of Eu in complexes 1 and 2 was stark.

Because photoacoustic imaging agents rely on the absorption of visible light, we expected that complex 1 but not complex 2 would act as a contrast agent for photoacoustic imaging.

To determine whether measurable photoacoustic responses could be observed that changed with the oxidation state of Eu, we measured the photoacoustic response (25 °C, 14.5 mJ cm$^{-2}$ at 410 nm) of 1 and 2 from 410 to 700 nm. Glass tubes with thin walls (0.35 mm) were filled with solutions of complex 1 under an inert atmosphere, and the same samples were exposed to air to yield 2. The samples were placed in a bath of distilled water and irradiated with light, and the photoacoustic signal amplitude was recorded (Figure 2). The data revealed that complex 1 had a photoacoustic response at 410 nm, which was 4.3X higher than oxidized complex 2. To verify that the signal observed was different from the blank (phosphate-buffered saline), we calculated the minimum detectable concentration of 1. First, the photoacoustic signal amplitude was plotted as a function of concentration for 1 (Figure 2), and then the photoacoustic intensities of the lowest point of 2 (30 mM) and

Figure 1. (Top) EuII-containing complex 1 and EuIII-containing complex 2. Counterions are not shown for clarity. (Middle) Normalized absorbance spectra of 1 (●) and 2 (—). (Bottom) Absorbance at 410 nm vs concentrations of 1 and 2. The slopes of the lines of best fit are molar absorptivities (M$^{-1}$ cm$^{-1}$).

Figure 2. Photoacoustic signal amplitude as a function of wavelength for 1 (□), 2 (■), and phosphate-buffered saline (pH 7.4) (◇); (bottom) photoacoustic intensity vs concentration of 1.
the blank were each measured using seven independently prepared samples. The minimum detectable concentration was calculated to be $3\sigma/m$, where $\sigma$ is the standard deviation of the seven repeated measurements at 30 mM and $m$ is the slope of the best fit line in Figure 2. The minimum detectable concentration for I was determined to be 15 mM. To place this value in context, another discrete molecule has been imaged with photoacoustic imaging in mice with an injection concentration of 3.3 mM. The photoacoustic response data demonstrate that the Eu-containing species studied show detectable photoacoustic response in the reduced form (+2 oxidation state of Eu) and do not show photoacoustic response in the oxidized form (+3 oxidation state of Eu).

To visualize the photoacoustic response before and after oxidation, samples of 1 and 2 were imaged with photoacoustic imaging (410 nm, 25 °C, Figure 3). Images of 1 and 2 were collected at 30, 60, and 100 mM because these were the concentrations used in calculating the minimum detectable concentration (Figure 2). The photoacoustic images of 1 appeared bright relative to those of 2, as expected based on the photoacoustic signal amplitudes in Figure 2. Furthermore, $T_1$-weighted images showed positive contrast enhancement for complex 1 that contained Eu II, and no visible contrast enhancement in phosphate-buffered saline (pH 7.4); (J) 1 (30 mM in phosphate-buffered saline, pH 7.4); and (K) 2 (30 mM in phosphate-buffered saline, pH 7.4). The color map shows the normalized intensity for photoacoustic images; and $T_1$-weighted images of (I) phosphate-buffered saline (pH 7.4); (J) 1 (30 mM in phosphate-buffered saline, pH 7.4); and (K) 2 (30 mM in phosphate-buffered saline, pH 7.4). The samples for MRI and photoacoustic imaging were in tubes with diameters of 3 and 5 mm, respectively.

Figure 3. Photoacoustic images of (A) 1 (100 mM in phosphate-buffered saline, pH 7.4); (B) 1 (60 mM in phosphate-buffered saline, pH 7.4); (C) 1 (30 mM in phosphate-buffered saline, pH 7.4); (D) optical glass tube with a wall thickness of 0.35 mm in a bath of distilled water, demonstrating an absence of scattering due to the glass tube; (E) 2 (100 mM in phosphate-buffered saline, pH 7.4); (F) 2 (60 mM in phosphate-buffered saline, pH 7.4); (G) 2 (30 mM in phosphate-buffered saline, pH 7.4); and (H) phosphate-buffered saline (pH 7.4). The color map shows the normalized intensity for photoacoustic images; and $T_1$-weighted images of (I) phosphate-buffered saline (pH 7.4); (J) 1 (30 mM in phosphate-buffered saline, pH 7.4); and (K) 2 (30 mM in phosphate-buffered saline, pH 7.4). The samples for MRI and photoacoustic imaging were in tubes with diameters of 3 and 5 mm, respectively.

of-concept relative to the reported multimodal, photoacoustic imaging agents, which are capable of picomolar limits of detection, use near-IR light, or both, include the relatively high detection limit and use of relatively high energy visible light in our system. We are currently pursuing ways to decrease the limit of detection and red-shift the wavelength of Eu-based systems by forming aggregates of the Eu-containing complexes in solution and changing the ligand field to shift absorbance of the Eu-containing species. These findings are a step toward multimodal sensing of redox environments using photoacoustic imaging.

### EXPERIMENTAL PROCEDURES

Commercially available chemicals were of reagent-grade purity or better and were used without further purification unless otherwise noted. Water was purified using a PURELAB Ultra Mk2 water purification system (ELGA). Phosphate-buffered saline (10X) was purchased from Fisher BioReagents. Eu III–DOTA-4AmC [DOTA-4AmC = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraakis(acetamidoacetate)] (2) was purchased from Macrocyclics.

Samples of 1 were prepared in a wet glove box (water was allowed but not molecular oxygen) under an atmosphere of N2. Complex 1 was prepared following a published procedure.

UV–visible spectra were recorded using an Ocean Optics spectrophotometer (STS-UV-L-25-400-SMA) coupled to a high-power DH-MINI deuterium tungsten halogen source (Ocean Optics) or on a Shimadzu UVmini-1240 spectrophotometer. Extinction coefficients were calculated by plotting absorbance intensity at 410 nm as a function of concentration of 1 or 2. The UV–visible spectra of samples of 1 were measured under an atmosphere of N2 in a wet glove box or in airight cuvettes sealed with paraffin wax inside of the glove box and then removed from the glove box and measured within an hour of taking the samples out of the glove box.

Photoacoustic imaging was performed in the Department of Biomedical Engineering at Wayne State University with a photoacoustic imaging system consisting of a programmable ultrasound scanner (Verasonics Vantage) equipped with a linear array transducer (L114-v), a 30 Hz pulsed and tunable Nd:YAG pump/OPO laser (Spectra Physics PRO270/Verascan), and a field-programmable gate array (FPGA) control system. The images and amplitudes were acquired at 25 °C in a bath of distilled water. The samples of 1 (100, 60, and 30 mM) and 2 (100, 60, and 30 mM) were in 1X phosphate-buffered saline (pH 7.4). The solutions were imaged in optical glass tubes with a wall thickness of 0.35 mm. These tubes showed no observable signal in the absence of Eu-containing samples (see Figure 3). For samples of 1, the optical glass tubes were plugged with silica glue, waxed, and imaged within 1 h of preparation.

MRI was performed using a Bruker ClinScan small animal scanner (300.43 MHz, 7.06 T) equipped with a 30 cm bore magnet. The samples of 1 (30 mM) and 2 (30 mM) were in 1X phosphate-buffered saline (pH 7.4) under an atmosphere of N2, sealed with a cap and then paraffin wax, and measured within an hour of taking the samples out of the glove box. The $T_1$-weighted images were acquired at ambient temperature (21 °C) with an echo time of 3.26 ms, a repetition time of 21 ms, flip angles of 5°, 10°, 20°, 30°, 40°, 50°, and 60°, 16 slices at 2 mm thickness, a field of view of 35 × 70 mm², and a matrix size of 128 × 256.
Concentrations of Eu were determined using inductively coupled plasma mass spectrometry (ICP-MS) or energy-dispersive X-ray fluorescence (EDXF) spectroscopy. ICP-MS measurements were acquired on an Agilent Technologies 7700 series ICP-MS instrument in the Lumigen Instrument Center in the Department of Chemistry at Wayne State University. All dilutions were performed with 2% aqueous HNO₃ which was also used for blank samples during calibration. The calibration curves were created using the Eu isotope ion count for a 1–200 ppb concentration range (diluted from Fluka ICP standard solution, 100 mg of Eu L⁻¹). EDXF measurements were recorded using a Shimadzu EDX-7000 spectrometer (Shimadzu Scientific Instruments) in the Lumigen Instrument Center in the Department of Chemistry at Wayne State University. The calibration curves were created using the Eu fluorescence intensity at 5.845 keV for a 25–100 ppm concentration range (diluted from Fluka ICP standard solution, 100 mg of Eu L⁻¹).

**AUTHOR INFORMATION**

**Corresponding Authors**

*E-mail: mehr@wayne.edu. Phone: (313) 577-8833 (M.M.).

*E-mail: mallen@chem.wayne.edu. Phone: (313) 577-2070 (M.J.A.).

**ORCID**

Matthew J. Allen: 0000-0002-6868-8759

**Notes**

The authors declare no competing financial interest.

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