Towards the Development of Redox-Responsive Eu(III) Complexes for Cancer Imaging

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Introduction and Project Goal

- Redox conditions within the cell are the result of homeostatic maintenance of numerous redox couples including GSSG/2GSH, NAD^+/NADH, and $NADP^+$/NADPH, all together to sustain a redox potential around -200 mV.[1]
- Cancer cells display redox dysregulation and lowered cytosolic pH as a means to maintain their over-proliferation.[2]
- The development of an approach to probe tissue redox state could potentially allow enhanced discrimination between healthy and cancerous tissues.
- MRI yields high-quality images without the use of ionizing radiation making it an attractive option for the diagnosis of numerous cancers.
- The quality of an MR image can be enhanced with the aid of contrast agents, the majority of which are Gd(III) complexes that provide a brightened image via T_1 weighted imaging.
- The reduction potential ($E_{1/2}$) of the Eu(II)/Eu(III) redox couple (-600 mV) has drawn attention for its relative proximity to biological redox conditions (-200 mV) and applications in MRI contrast agent design.
- The Eu(II) oxidation state has an identical electron configuration to Gd(III) and provides similar image brightening T_1 MRI properties.
- Eu(III) complexes provide darkened images or negative MRI contrast through a different MR imaging mechanism called PARACEST.
- However, most ligands used for Eu(III)-based PARACEST agents strongly stabilize the Eu(III) and as a result Eu(II) isn't present in substantial concentrations when these complexes are in an aqueous environment.

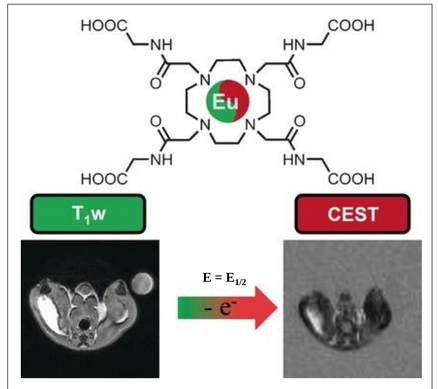

Figure 1. An example of the difference in contrast between Eu(II) and Eu(III)-based contrast agents.[3]

Project Goals

- The goal of this project is to synthesize a library of Eu(III) complexes with ligands containing various amino acid R groups and investigate the effects of amino acid charge, electron density, and pK_a on Eu(II)/Eu(III) redox potentials and stabilities by cyclic voltammetry.
- We will also investigate the response to pH of these properties over a pH range of 5.5-8.5.

Methods and Materials

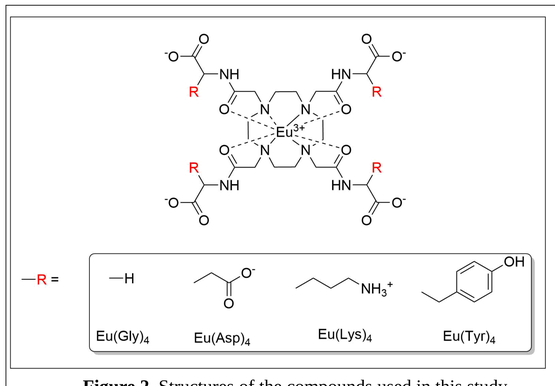

Figure 2. Structures of the compounds used in this study (glycine, aspartate, lysine and tyrosine).

Ligand Synthesis: All reagents and solvents were purchased from commercial vendors. Cyclen was tetra-alkylated with the corresponding protected chlorinated amino acid in the presence of base. The protected ligands were purified by flash chromatography before being deprotected by acid or base hydrolysis. All ligand identities were verified by ^{1}H NMR.

Metal Complexation: All ligands were complexed with an equimolar amount of $EuCl_3$ solution at pH 5.5–6.0. Excess metal presence was determined by Xylenol Orange test.

Cyclic Voltammetry Studies: Cyclic voltammograms were acquired with a Pine Research WaveNow Potentiostat using a glassy carbon working electrode, platinum counter electrode and Ag/AgCl reference electrode. The supporting electrolyte for all samples was 100 mM KCl. The pH was maintained using MES (5.5 & 6.5) and TRIS (7.5 & 8.5) buffers.

Results and Discussion

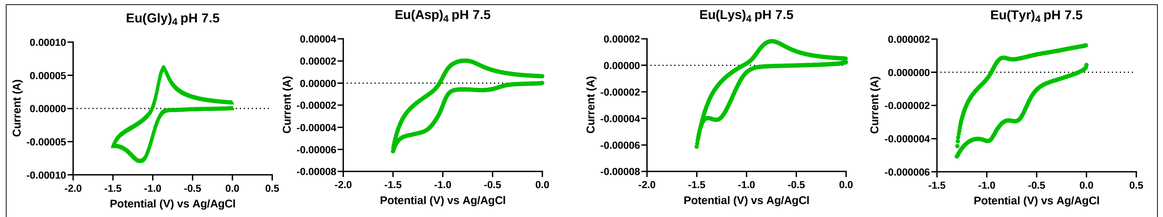

Figure 3. Cyclic voltammograms of the Eu(III) complexes with respective sidearms. Taken at a scan rate of 100 mv s^{-1}.

	$E_{1/2}$ (V) vs Ag/AgCl			
Complex	pH 5.5	pH 6.5	pH 7.5	pH 8.5
Eu-$(Gly)_4$	-1.007	-0.9904	-1.013	-0.9903
Eu-$(Asp)_4$	-0.7485	-0.9568	-0.9988	-1.037
Eu-$(Lys)_4$	-1.029	-1.036	-1.024	-1.024
Eu-$(Tyr)_4$	-0.9000	-0.9107	-0.9161	-0.9585
$EuCl_3$	-0.6028	-0.6070	-0.6327	N/A

Table 1. Electrochemical data over pH 5.5 – 8.5 range.

$E_{1/2}$ Data

- Complexes with the glycine and lysine sidearms demonstrated a constant $E_{1/2}$ over the pH range 5.5 – 8.5.
  - Eu-$(Gly)_4$ was found to have an average midpoint of -1.00 ± 0.01 V.
  - Eu-$(Lys)_4$ was found to have an average midpoint of -1.03 ± 0.005 V.
- The $E_{1/2}$ of the aspartate and tyrosine complexes demonstrated pH dependence.
  - Eu-$(Asp)_4$ demonstrated a 250 mV increase in Eu(II) stability at pH 5.5 relative to 8.5.
  - Eu-$(Tyr)_4$ demonstrated a 70 mV increase in Eu(II) stability at pH 5.5 relative to 8.5.

Discussion

- The pH dependent redox properties found in Eu-$(Asp)_4$ and Eu-$(Tyr)_4$ suggest that changes in the protonation state of their R-groups affect the ability of the europium ion to accept or donate electrons.
- Conversely, the pH independent redox properties found in Eu-$(Lys)_4$ and Eu-$(Gly)_4$ suggest these amino acid R-groups do not significantly affect the redox properties of the coordinated metal. In Eu-$(Gly)_4$ this is likely because there is no R group protonation occurring, and in Eu-$(Lys)_4$ we hypothesize there may be too large a distance between the amine and metal for any R-group protonation to affect the redox properties of the metal ion.

Conclusion

- R-groups in Eu-$(Asp)_4$ and Eu-$(Tyr)_4$ appear to induce a dependence of the redox potentials on pH. This is probably due to closer proximity of these groups to the metal, and their possession of pK_a values within the pH range studied.
- Eu-$(Asp)_4$ displayed the most redox sensitivity to pH over the pH 5.5 – 8.5 range.

Future Work

- Perform electrochemical investigations over an expanded pH range to encompass pK_as of all amino acids.
- Synthesize additional complexes with different amino acids and evaluate their redox properties.

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References

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