Development of Hyperpolarizable $^{13}$C-PROBES for the Quantification of METALS In Vivo
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Introduction

- Disruption of the homeostasis of metals in the body has been correlated to a variety of diseases (cancer, Parkinson’s Disease, heavy metal poisoning).
- Current blood-based methods of metal quantification don’t give accurate concentrations of metals in tissue samples.
- $^{13}$C-tagged EDTA and EDTA probes have experienced a shift in carbonyl peak (singlet in the range of 170 to 180 ppm) unique to the type of metal it bound to (Figure 1) via $^{13}$C NMR.
- The area under the curve of these shifted peaks increased linearly with an increase in concentration of the metal.
- Sensitivity of signal was increased through hyperpolarization using dynamic nuclear polarization (DNP).
- Macrocycles tend to have a higher binding affinity than linear molecules due to a higher entropy associated with the chelation process.
- Increased binding to metals could give a stronger signal, which could be better for lower concentrations.
- Goal: Evaluate metal ion-binding properties of $^{13}$C-tagged EDTA, and other macrocycles

Materials and Methods

Synthesis of $^{13}$C-EDTA

- Synthesized according to Scheme 1.
- Ethylenediamine was tetraalkylated with $^{13}$C-ethyl bromoacetate.
- The resulting intermediate (1) was purified via extractions with DCM and deprotected with sodium hydroxide to yield the final product (2).
- Final product was adjusted to pH 7.5 and lyophilized.
- Identified via $^{1}H$ and $^{13}$C NMR spectroscopy.

Results and Discussion

Figure 1. Chemical shifts of $^{13}$C-EDTA when bound to different divalent metals via $^{13}$C NMR.

Figure 2. Overlay $^{13}$C NMR spectra from titration of (2) with Ca$^{2+}$ in 0.3 M HEPES buffer at pH 7.40 in D$_2$O acquired at 125 MHz.

- Appearance of a second carbonyl peak at 180.0 ppm was observed (Figure 2) upon addition of Ca$^{2+}$, which matched literature values
- Throughout titration, peaks in the range of 45 to 60 ppm experienced no observable change, these peaks correspond to non-$^{13}$C-tagged carbons on proton and carbons in HEPES buffer
- As the concentration of Ca$^{2+}$ in the sample increased, the “bound” peak at 175.0 ppm was observed to decrease in intensity and the “bound” peak increased in intensity

Figure 3. Binding curve of (2) with Ca$^{2+}$. AUC was normalized to total AUC, and ratios of bound and unbound AUC were plotted.

- Carbonyl peaks for bound and unbound $^{13}$C-EDTA were integrated and normalized to the total area under the curve (AUC).
- A function of the AUC and moles of Ca$^{2+}$ added was plotted (Figure 3).
- Moles Ca$^{2+}$ to fully complex ligand = moles $^{13}$C-EDTA, calculated using lines of best fit, used in order to find the percentage of $^{13}$C-EDTA in (2).
- The synthesized sample (2) was calculated to be 46.42% wt $^{13}$C-EDTA

Conclusions

- Behavior of $^{13}$C-EDTA observed in previous studies was confirmed upon binding Ca$^{2+}$.
- Area under the curve was utilized to determine the concentration of $^{13}$C-EDTA in the synthesized sample.

Future Work

- Titrate $^{13}$C-EDTA with other metal ions to verify chemical shifting when bound to different metals
- Synthesize $^{13}$C-tagged macroyclic ligands
- Investigate specific chemical shifts of $^{13}$C-tagged macrocycles upon binding to different metal ions
- Verify ability of $^{13}$C-tagged macrocycles to quantify metal concentrations
- Collaborate externally for hyperpolarization studies

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References


