

Amino Acid-Derived Sensors For Specific Zn²⁺ Detection Using Hyperpolarized ¹³C Magnetic Resonance Spectroscopy

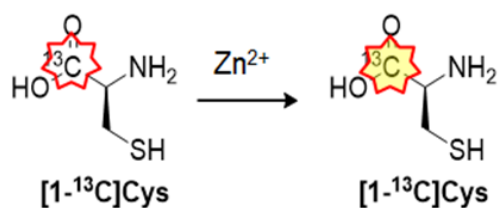
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Alterations in Zn²⁺ homeostasis have been targeted for diagnosis and treatment of human diseases including cancer, diabetes, and neurodegenerative illness. Enabled by hyperpolarization techniques, hyperpolarized (HP) probes for *in vivo* detection of a variety of analytes using HP ¹³C MRS have been developed. Here, we explored the use of HP ¹³C MRS for Zn²⁺-specific imaging.

We began our study by screening a variety of candidate Zn²⁺ ligands for their chemical shift response upon Zn²⁺ binding. Among the 34 tested compounds, cysteine and iminodiacetic acid were selected for further evaluation because of their large chemical shift response to Zn²⁺ binding (+4.6 and +7.3 ppm in the presence of equimolar Zn²⁺, respectively), excellent water solubility, low molecular weight, predicting a long ¹³C T₁, and convenience for ¹³C labeling. Both probes showed excellent specificity for Zn²⁺ over other main physiologic cations Na⁺, K⁺, Ca²⁺ and Mg²⁺. A quantitative method for determining Zn²⁺ concentration was developed using NMR. A titration curve was plotted to show the chemical shift difference between [1-¹³C]Cys and internal standard urea as a function of the Zn²⁺ to [1-¹³C]Cys ratio. The peak moved linearly with increasing Zn²⁺ up to 0.25 equivalents of Zn²⁺ to [1-¹³C]Cys. The rate of chemical shift change decreased at higher Zn²⁺ concentrations.

Next, we developed and optimized a hyperpolarization method for ¹³C labeled [1-¹³C]Cys. For [1-¹³C]Cys, the optimized preparation was obtained by a mixture of 1.0 eq of [1-¹³C]Cys, 0.5 eq of 4N HCl and 2.65 eq of glycerol, with 20 mM OX063 radical. Using this method, 13.4 ± 0.6% back-calculated polarization was obtained with a polarization time constant of 1227 ± 30 (n = 3). The T₁ relaxation time was 36.0 ± 1.8 seconds at a magnetic field of 3T. The ability of the probes to image Zn²⁺ concentration was tested using phantoms on a 3T MRI system. By using the best-fit linear model of chemical shift as a function of Zn²⁺ concentration, hyperpolarized [1-¹³C]Cys was able to accurately determine the concentration over the physiologically relevant range of 0.2 – 20 mM Zn²⁺. Finally, we verified that HP [1-¹³C]Cys could accurately determine Zn²⁺ concentration in biological samples by comparing imaging results against a commercially available fluorescence based Zn²⁺ quantification kit.

Taken together, these data demonstrate that [1-¹³C]Cys represent promising probes for imaging Zn²⁺ using hyperpolarized ¹³C MRI. The probes demonstrate large changes in signal and chemical shift in response to Zn²⁺, favorable T₁ and polarization parameters, and can be imaged in phantom experiments. [1-¹³C]Cys accurately quantified Zn²⁺ concentration in biological samples at physiologically relevant concentrations. For this reason, [1-¹³C]Cys is a particularly promising probe for future *in vivo* hyperpolarized magnetic resonance imaging of pathologies with alterations in Zn²⁺ homeostasis such as prostate cancer, neurodegenerative disease, and diabetes.



- Biocompatible Zn²⁺ probe
- Good selectivity
- Signal enhanced ~3400 fold
- High accuracy

