Amino Acid-Derived Sensors For Specific Zn^{2+} Detection Using Hyperpolarized ^{13}C Magnetic Resonance Spectroscopy

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Alterations in Zn^{2+} 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 ^{13}C MRS have been developed. Here, we explored the use of HP ^{13}C MRS for Zn^{2+}-specific imaging.

We began our study by screening a variety of candidate Zn^{2+} ligands for their chemical shift response upon Zn^{2+} binding. Among the 34 tested compounds, cysteine and iminodiacetic acid were selected for further evaluation because of their large chemical shift response to Zn^{2+} binding (+4.6 and +7.3 ppm in the presence of equimolar Zn^{2+}, respectively), excellent water solubility, low molecular weight, predicting a long ^{13}C T_1, and convenience for ^{13}C labeling. Both probes showed excellent specificity for Zn^{2+} over other main physiologic cations Na^+, K^+, Ca^{2+} and Mg^{2+}. A quantitative method for determining Zn^{2+} concentration was developed using NMR. A titration curve was plotted to show the chemical shift difference between [1-^{13}C]Cys and internal standard urea as a function of the Zn^{2+} to [1-^{13}C]Cys ratio. The peak moved linearly with increasing Zn^{2+} up to 0.25 equivalents of Zn^{2+} to [1-^{13}C]Cys. The rate of chemical shift change decreased at higher Zn^{2+} concentrations.

Next, we developed and optimized a hyperpolarization method for ^{13}C labeled [1-^{13}C]Cys. For [1-^{13}C]Cys, the optimized preparation was obtained by a mixture of 1.0 eq of [1-^{13}C]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 T_1 relaxation time was 36.0 ± 1.8 seconds at a magnetic field of 3T. The ability of the probes to image Zn^{2+} concentration was tested using phantoms on a 3T MRI system. By using the best-fit linear model of chemical shift as a function of Zn^{2+} concentration, hyperpolarized [1-^{13}C]Cys was able to accurately determine the concentration over the physiologically relevant range of 0.2 – 20 mM Zn^{2+}. Finally, we verified that HP [1-^{13}C]Cys could accurately determine Zn^{2+} concentration in biological samples by comparing imaging results against a commercially available fluorescence based Zn^{2+} quantification kit.

Taken together, these data demonstrate that [1-^{13}C]Cys represent promising probes for imaging Zn^{2+} using hyperpolarized ^{13}C MRI. The probes demonstrate large changes in signal and chemical shift in response to Zn^{2+}, favorable T_1 and polarization parameters, and can be imaged in phantom experiments. [1-^{13}C]Cys accurately quantified Zn^{2+} concentration in biological samples at physiologically relevant concentrations. For this reason, [1-^{13}C]Cys is a particularly promising probe for future *in vivo* hyperpolarized magnetic resonance imaging of pathologies with alterations in Zn^{2+} homeostasis such as prostate cancer, neurodegenerative disease, and diabetes.