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Highly Fluorinated Metal Complexes as Dual ^{19}F and PARACEST Imaging Agents

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Abstract

We reported a set of water-soluble transition metal complexes that can serve as both ^{19}F and PARACEST magnetic resonance imaging agents. The high number of equivalent fluorine atoms and the paramagnetic effect of metals offer these complexes high ^{19}F sensitivity as demonstrated by *in vitro* ^{19}F MRI experiments. The complexes contain carboxamide groups appended onto a cyclen macrocycle, which provide ^1H CEST peaks well differentiated from bulk water. The Co(II) agent displays two CEST peaks that can be utilized for ratiometric pH determination and the concept of combining ^{19}F MR and PARACEST as complementary imaging techniques was demonstrated with the Fe(II) complex.

Graphical Abstract

Introduction

Magnetic resonance imaging (MRI) is a powerful and noninvasive diagnostic tool that uses long wavelength radio frequency to excite nuclei of interest and generate high resolution images. Various imaging techniques have been developed based upon MRI. Proton (^1H) MRI is most frequently employed, and due to the high abundance of ^1H in the body, ^1H MRI provides excellent images of soft tissues. ^{19}F MRI has had growing interest as the ^{19}F nucleus has a similar sensitivity to ^1H and the lack of endogenous ^{19}F *in vivo* can allow for quantitative analysis and more accurate localization of imaging agent-derived signal.¹

† Footnotes relating to the title and/or authors should appear here.

Conflicts of interest

There are no conflicts to declare.

Ideally, ^{19}F imaging agents contain high concentration of a single ^{19}F signal to maximize signal intensity and agent sensitivity. Paramagnetic metals can further enhance ^{19}F signal by reducing longitudinal relaxation times (T_1), enabling more efficient image acquisition.² Another MR imaging modality relies on the chemical exchange saturation transfer (CEST) mechanism. CEST functions through frequency-selective irradiation of protons which are in exchange with bulk water, resulting in a decrease in the bulk H_2O signal due to magnetization transfer.^{3,4} The inherent sensitivity of CEST signal towards the local pH environment makes it an ideal candidate for accurate pH mapping within the body.^{5,6}

Currently, there is a growing interest in combining multiple imaging modalities in order to offer complimentary diagnostic information.^{7–10} Nevertheless, the execution of multi-modal imaging is usually complicated and requires the administration of a cocktail of different MR imaging agents assuming similar biodistribution or colocalization.^{11,12} To solve these problems, we have developed new, water-soluble MR imaging agents capable of acquiring ^{19}F /CEST MRI simultaneously using a single molecule without compromising the sensitivity. Thus, the combination of these two modalities into a single molecule can enable the control of CEST signal at will by turning on/off of the saturation pulse as well as facilitate the quantification of signal using ^{19}F MRI.

Our aim was to design an agent that chelates paramagnetic metal ions, exhibits well-shifted water exchangeable proton frequency with reasonable exchange rate, possesses high fluorine density for maximum ^{19}F MR signal, and good water solubility for biological applications. To meet these criteria, we synthesized a class of paramagnetic MRI contrast agent consists of three primary components: the carboxyamide appended macrocycle, a bifunctional linker incorporated with high fluorine density, and a glucose moiety for solubility (Scheme 1). The paramagnetic metal center plays two pivotal roles: as a relaxation enhancer to shorten the relaxation times of the proximal fluorine moiety to improve the ^{19}F MRI sensitivity and as a chemical shift agent to generate CEST signal isolated from bulk water. The fluorine bearing moiety is designed in such a way that a robust, single fluorine resonance can be observed which is one of the key prerequisites for effective ^{19}F MR imaging. Due to the high hydrophobicity of the fluorine moiety, a glucose fragment was introduced to improve hydrophilicity and water solubility. Three different metal complexes were synthesized through direct complexation of the final ligand with Fe^{2+} , Co^{2+} , and Ni^{2+} metal salts in water and their properties were further studied. Our agents contain excellent water solubility, high equivalent ^{19}F density, and relatively large separation between the ^1H CEST signal and water, giving them several advantages over previously reported agents.^{13,14}

Results and Discussion

Synthesis and solution characterization

The synthesis of ligand L and its M^{2+} (Fe , Co , Ni) complexes is described in the Supporting Information. Owing to the good hydrophilicity of the DOTA-tetraamide core and glucose, all complexes show excellent water solubility despite the presence of highly hydrophobic fluorine moiety: up to at least 30 mM concentrations could be achieved in 100% aqueous solution. Importantly, excellent air and aqueous stability were observed for all three complexes as monitored by ^{19}F NMR. The magnetic moments were determined to be 4.9,

4.5, and 3.5 μB for FeL, CoL, and NiL, respectively, using Evans' method¹⁵, indicative of high-spin metal complexes in all cases. The ^{19}F NMR spectra of FeL, CoL, and NiL in buffer revealed a sharp, single ^{19}F signal for all three complexes which is crucial for optimal imaging sensitivity (Figure S1). The ^{19}F chemical shifts are very close to that of the ligand (Table 1)—the small ^{19}F pseudo contact shift (PCS) is consistent with weak through-space interactions between the paramagnetic metals and ^{19}F nuclei, which is likely due to relatively long distances between them.¹⁶ The ^{19}F T_1 and T_2 relaxation times were determined for all complexes as well as the ligand and the results are summarized in Table 1. The relaxation times of the complexes are significantly shortened compared to the free ligand, demonstrating the relaxation enhancement offered by paramagnetic transition metals. Intriguingly, a considerable field dependence of ^{19}F relaxation times was observed for NiL in particular. This phenomenon is attributed to the relative longer electronic relaxation time of Ni^{2+} , the effect of which dwindles at stronger magnetic field.¹⁷

***In vitro* ^{19}F MRI experiments**

Given the promising NMR properties of FeL, CoL, and NiL, we evaluated their capability as ^{19}F MR image agents *in vitro* using a 7T preclinical MRI scanner. Phantom images were acquired at different concentrations using a fast-spin echo pulse sequence with parameters optimized to the relaxation properties of each complex. The limit of detection (LOD) was measured by linearly fitting the signal-to-noise ratios (SNRs) against concentrations and assuming a minimum detectable threshold of 3.5 (Figure 1, S4). Impressively, the LOD was determined to be 50, 60 and 40 μM for FeL, CoL, and NiL, respectively following a 12-minute scan. The ligand itself, on the other hand, shows a lower sensitivity corresponding to a LOD of 160 μM within same amount of acquisition time, indicating the imperative role of the paramagnetic metal ion in enhancing imaging sensitivity. Alternatively, this can be directly visualized by comparing the SNRs at the same probe concentration of 1 mM where the signal intensities of metal complexes are roughly 3–4 fold of that of ligand itself (Figure 1). These values are lower than similar reported paramagnetic ^{19}F MR probes, demonstrating the superior sensitivity of these complexes.^{18–20} This result can be attributed to two main factors: (1) High ^{19}F density; as our probes possess by far the highest number of equivalent fluorine atoms amongst small molecule, water-soluble paramagnetic ^{19}F MR probes^{18,20–23}; (2) Relaxation enhancement provided by Fe(II), Co(II), and Ni(II) reduces the ^{19}F relaxation times so that a shorter repetition time ($\text{TR} = 100 \text{ ms}$) can be employed to allow for more acquisitions within a set time period which further increases sensitivity.

CEST properties

Given the presence of water exchangeable amide protons, we investigated the CEST properties of these complexes. ^1H NMR spectra were acquired to identify the presence of exchangeable protons and their resonance frequencies. By comparing the ^1H NMR spectra in buffered solutions of H_2O and D_2O , we were able to locate exchangeable protons at 68 and 62 ppm, 62 and 52 ppm, and 77 ppm for FeL, CoL, and NiL, respectively (Figure S5, S6, S7). These paramagnetically shifted exchangeable protons were assigned to the $-\text{NH}_2$ protons of metal-bound amide groups.^{24,25} The additional amide proton feature with FeL and CoL indicates a different coordination environment in solution compared to NiL. Though we were unable to obtain a crystal structure, the Ni(II) center within the current

scaffold is most likely 6-coordinate in solution, with four ligand donors coming from the cyclen macrocycle and 2 from coordinating amide arms.^{25–27} The Co(II) complex is more likely 7-coordinate in solution based on the integration of the two amide peaks (2:1) which suggests that all three -NH_2 amides are bound to the metal center. This is consistent with a recently published 7-coordinate Co(II) complex with a similar coordinating ligand.²⁶ The Fe(II) system could be either 7- or 8-coordinate but favors 8-coordinate based on reported structures.^{14,25} CEST spectra (Z-spectra) were subsequently obtained by selective presaturation of FeL, CoL, and NiL in HEPES buffer at 1 ppm increments. The normalized bulk water signal integrations were plotted against frequency offset to generate Z-spectra. Consistent with the ^1H NMR spectrum, NiL displays a single CEST peak centered at 73 ppm relative to bulk water (Figure 2). Unlike ^1H NMR, only one broad peak appears in the CEST spectrum of FeL at 63 ppm, which we attribute to overlap between the two CEST signals (Figure 2). Indeed, close scrutiny of the CEST spectrum at lower saturation powers reveals two distinct peaks centered at 63 ppm and 59 ppm, respectively, that match well with the peaks identified in the ^1H NMR spectrum (Figure S9). Aligned with what is observed in the ^1H NMR spectra, the two CEST peaks are more distinguishable in CoL (Figure 2).

The pH dependence of the CEST signal for FeL, CoL, and NiL was further investigated. Overall, stronger CEST intensity was observed at more basic conditions due to base-catalyzed proton exchange (Figure 3. S8, S11, S15). Little variation in magnetic susceptibility was seen between pH 6.5–8.0 for all three complexes (Figure S3). The pK_a of the amide protons in FeL and CoL are likely high since no signal attenuation was observed when the pH was approaching 8 where signal coalescence arising from fast proton exchange is normally expected.^{24,25,28} The CEST signal of the NiL, however, plateaued around pH 7.8 and declined afterwards (Figure S15), suggesting a lower pK_a value associated with NiL. This phenomenon is in accordance with the stronger Lewis acidity

of Ni(II) comparing to Fe(II) and Co(II). The proton exchange rates (k_{ex}) were determined using a concentration-independent method (Omega plot).²⁹ The proton exchange rates were measured to be $2.7 \times 10^3 \text{ s}^{-1}$, $2.7 \times 10^3 \text{ s}^{-1}$ (58 ppm), and $4.8 \times 10^3 \text{ s}^{-1}$ in pH 7.4 HEPES buffer for FeL, CoL, and NiL, respectively (Figure S10, S14, S16). Again, the higher k_{ex} of NiL agrees with its lower pK_a . Exchange rates at different pH values were also measured, showing a gradual increase of k_{ex} with increasing pH (Figure S10, S14, S16). The two CEST peaks of CoL possess different exchange rates. Specifically, the minor CEST feature at 52 ppm has a faster k_{ex} of $3.5 \times 10^3 \text{ s}^{-1}$ at pH 7.4. This phenomenon stimulated our interest in further exploring the potential of employing CoL in ratiometric pH measurement. As shown in Figure 3, the CEST ratio of the two peaks in CoL were plotted against pH, and a good linear correlation was observed between pH 7.2 and 8.0. The concentration of CoL does not alter the ratio significantly, demonstrating the possibility of using CoL for concentration-independent pH determination. In the future, electron withdrawing groups could be installed around the amide group to adjust the pH window to more acidic region which is of paramount interest in tumor pH mapping.^{14,30}

One major concern of applying PARACEST agents for *in vivo* pH measurement is that the signal change is dependent on probe concentration. The CEST signal intensity of FeL at pH 7.4 varies proportionally with complex concentration (Figure S17), and also depends on the

sample pH. The relationship between CEST intensity and pH is shown in Figure 4A and is fitted exponentially at representative concentrations, 5.1 mM and 10.5 mM. Therefore, exact probe concentration is required in order to obtain accurate pH information based on CEST. ^{19}F MRI can address this issue by serving as an “internal” reference for probe quantification. Given that ^{19}F signal intensity solely correlates with ^{19}F density, pH results in negligible effect as long as the magnetic properties of complexes remain unchanged. To demonstrate this, we obtained CEST data for samples (Sample A and Sample B) that display equal CEST % but are not equivalent in composition (Figure 4B). The $[\text{}^{19}\text{F}]$ of these samples were then determined by ^{19}F NMR, identifying Sample A as containing 5.1 mM FeL and Sample B containing 10.5 mM FeL (Figure 4C). Based on this information, the appropriate pH calibration curve can be selected and the pH of each sample can be determined. In this case, the pH of Sample A is 7.71 and the pH of Sample B is 7.91 (Figure 4D), which is consistent with the pH determined by independent measurements with a pH meter (7.69 for Sample A, 7.86 for Sample B). While this is a simple proof-of-concept experiment, this demonstrates that with the appropriate calibration curves, the combination of ^{19}F and CEST measurements can provide a quantitative assessment of pH changes that takes into account the concentration-dependent response of CEST agents.

Conclusion

In summary, we have synthesized a series of water-soluble, paramagnetic metal complexes that can serve as both ^{19}F MR imaging agents and PARACEST agents. The high fluorine density, combined with the paramagnetic relaxation effect offered by the metal ions, allows for great sensitivity as demonstrated by the ^{19}F MRI experiments. Meanwhile, the exchangeable amide protons provide these complexes with the additional capability to act as PARACEST agents. Though their *in vivo* applications remain to be examined, the study presents us with a valuable and novel class of complexes for the designing of water-soluble, biocompatible small molecular MR probes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

^{19}F MRI limit of detection study for FeL, CoL, and, NiL in 50 mM HEPES buffer (0.1 M NaCl, pH=7.4). Detailed scanning parameters are provided in the supporting information.

Figure 2.

CEST spectra recorded at 23 °C for 7–9 mM aqueous solutions of complexes in 50 mM HEPES and 100 mM NaCl buffered at pH 7.3–7.5. Power level $B_1 = 25 \mu\text{T}$, 4 s saturation pulse. Inset shows the zoomed-in area of CEST peaks.

Figure 3.

Left: CEST signal change of 10.9 mM buffer solution of CoL at pH 6.49–8.01. Recorded at 23 °C, power level $B_1 = 25 \mu\text{T}$, 4 s saturation pulse; Right: CEST ratio ($(M_z/M_0)_{58 \text{ ppm}}/(M_z/M_0)_{52 \text{ ppm}}$) versus pH with 9.7 mM, 10.9 mM and 15.1 mM CoL.

Figure 4.

A: CEST signal change of 5.1 mM and 10.5 mM buffer solution of FeL at pH 6.5–8.0.

Recorded at 23 °C, power level $B_1 = 25 \mu\text{T}$, 4 s saturation pulse (curves represent the nonlinear fit of the data); B: Overlaid CEST spectra of sample A and B; C: Determination of probe concentration of sample A and B using ^{19}F NMR (5-fluorocytosine was used as an internal reference); D: Calculation of pH by fitting the CEST signal intensities of sample A and B into the calibration curve.

Scheme 1.
Structure of metal complexes in this study.

Table 1.

Measured ^{19}F T_1 and T_2 relaxation times of 1 mM ligand and complexes in HEPES buffer (50 mM, 0.1 M NaCl, pH 7.4) at 1.4 T (37 °C) and 9.4 T (23 °C).

	1.4 T			9.4 T			
	T_1 (ms)	T_2 (ms)	R_2/R_1	T_1 (ms)	T_2 (ms)	R_2/R_1	δ_F (ppm)
FeL	56.1	50.6	1.11	38.4	18.0	2.13	69.8
CoL	45.3	41.2	1.10	41.6	29.6	1.40	70.8
NiL	63.1	55.2	1.14	14.5	12.2	1.19	70.5
L	1510	840	1.80	631	352	1.79	70.6