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Iron(II) Complexes Containing Octadentate Tetraazamacrocycles as ParaCEST Magnetic Resonance Imaging Contrast Agents

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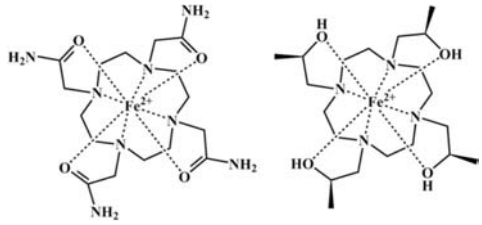
Supporting Information

ABSTRACT: Iron(II) complexes of the macrocyclic ligands 1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane (TCMC) and (1*S*,4*S*,7*S*,10*S*)-1,4,7,10-tetrakis(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane (STHP) contain a highly stabilized Fe^{II} center in the high-spin state, which is encapsulated by an octadentate macrocycle. The complexes are resistant to acid, metal cations, phosphate, carbonate, and oxygen in aqueous solution. $[Fe(TCMC)]^{2+}$ contains exchangeable amide protons, and $[Fe(STHP)]^{2+}$ contains exchangeable protons attributed to alcohol OH donors, which give chemical exchange saturation transfer (CEST) peaks at physiological pH and 37 °C at 50 and 54 ppm from bulk water, respectively. The distinct pH dependence of the CEST peak of the two complexes over the range of pH 6−8 shows that these two groups may be useful in the development of ratiometric pH sensors based on iron(II).

Magnetic resonance imaging (MRI) uses nonionizing radiation to image soft tissues deep in the body but may suffer from insufficient contrast when differentiating between malignant tumors and benign growths.[1] The development of MRI contrast agents that can distinguish between tumors and other tissue is a topic of much current interest. A relatively new type of MRI contrast agent that shows promise in this area enhances images through paramagnetic chemical exchange saturation transfer (paraCEST). ParaCEST agents are inherently sensitive to changes in pH and temperature, making them good candidates toward the development of responsive MRI contrast agents. Here we present new paraCEST contrast agents based on iron(II) that have two distinctly different responses to pH.

In a field dominated by trivalent lanthanide ion (Ln^{III}) paraCEST complexes, we recently reported on the first iron(II) paraCEST agents based on the macrocyclic ligand 1,4,7-triazacyclononane (TACN).[2] Here, we report on the first predominantly high-spin iron(II) complexes based on the 1,4,7,10-tetraazacyclododecane (CYCLEN) macrocycle, the amide-appended ligand (TCMC), and chiral-alcohol-appended ligand (STHP) (Chart 1). These macrocycles, which were developed originally for large metal ions such as Ln^{III}, function surprisingly well with Fe^{II}, a relatively large transition-metal ion in the high-spin state (r = 0.92 Å for eight-coordinate).[3] It is of interest to develop these complexes because the larger number of exchangeable protons of iron(II) macrocyclic complexes of CYCLEN compared to their TACN analogues is expected to produce more effective contrast.[4] In addition, iron(II) complexes of tetrasubstituted CYCLEN may be more inert toward dissociation than iron(II) complexes of trisubstituted TACN.[1]

Chart 1. Iron(II) Complexes for ParaCEST



ParaCEST agents induce hyperfine shifts of labile protons, which chemically exchange with bulk water. Upon application of selective radio-frequency (RF) irradiation, magnetization of the labile protons becomes saturated, and the process of chemical exchange causes the detected water signal to decrease. For paraCEST images, data are collected on-resonance, at the frequency of the exchangeable proton, and at an equivalent off-resonance frequency, which is subtracted to determine the difference in the signal and, hence, the contrast image. Selective RF irradiation thus acts as an on−off switch.

Iron(II) paraCEST agents must contain a high-spin Fe center, which is strongly stabilized in the divalent state so that the iron(II) complex is stable toward oxygen. Characterization of the spin state and oxidation state of the iron complexes was carried out in solution. The magnetic moments of $[Fe(STHP)]^{2+}$ and $[Fe(TCMC)]^{2+}$ as determined by Evans' method are 5.0 and 5.3 μ_B, respectively, consistent with high-spin Fe^{II} (S = 2).[6] This, in conjunction with the relatively narrow line widths and highly dispersed paramagnetic ^{1}H NMR spectra, shows that the complexes are predominantly high-spin Fe^{II} in aqueous solution.[7] Cyclic voltammograms showed reduction potentials of 800 mV versus normal hydrogen electrode (NHE)[5] for $[Fe(TCMC)]^{2+}$ and 1085 mV versus NHE for $[Fe(STHP)]^{2+}$. These very positive reduction potentials are consistent with a highly stabilized 2+ oxidation state and correspond to the inertness of the complexes toward reaction with oxygen over several days in aqueous solution.

Paramagnetic ^{1}H NMR spectroscopy was used to further characterize the complexes in solution. $[Fe(STHP)]^{2+}$ exhibits

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relatively sharp ^{1}H peaks in both CD_3CN and D_2O. There are eight nonexchangeable resonances, consistent with C_4 symmetry and coordination of all four pendent groups (Figure S1 in the Supporting Information, SI). The rigidity of the $[Fe(STHP)]^{2+}$ complex may be attributed to the chiral pendent groups on the ligand, which have been shown to impart a single helical conformation of alcohol arms and to produce a single diastereomer in solution for lanthanide(III) complexes.[8] $[Fe(TCMC)]^{2+}$, on the other hand, exhibits broad peaks for the CH protons of the macrocycle backbone and pendent groups, yet two very distinct, sharp peaks for the inequivalent NH amide protons observed at 57 and 3 ppm in CD_3CN (Figure S2 in the SI), arising from restricted rotation about the C−N bond.[9] This large chemical shift difference in amide proton resonances is similar to that of the TACN analogue reported previously.[2] Metal-ion complexes of TCMC frequently exist as two diastereomeric forms in solution that contain different arrangements of pendent arms and the macrocyclic backbone.[10] The six broad peaks are consistent with a dynamic structure due to interconverting diastereomers. Thus, although the spectra are distinctly different, both complexes yield ^{1}H NMR spectra that are consistent with octadentate coordination in solution. In support of an eight-coordinate complex, the Mn^{II} ion, which is similar in size to the Fe^{II} ion, forms an eight-coordinate complex with TCMC.[11]

CEST spectra were obtained by applying a presaturation pulse in 0.5 or 1 ppm increments and were plotted as the normalized water signal intensity (M_z/M_0 %) versus the frequency offset (ppm). Spectra were collected at 37 °C in the presence of 20 mM buffer and 100 mM NaCl, at pH 7.3. The CEST peak of $[Fe(TCMC)]^{2+}$ clearly arises from the amide NH protons, which are identified by their signature appearance as two highly separated resonances for the two chemically inequivalent protons.[9] The CEST peak of $[Fe(STHP)]^{2+}$ is most likely due to the hydroxyl protons, although we cannot rigorously rule out an exchangeable proton of a water ligand. However, it is unlikely that the $[Fe(STHP)]^{2+}$ complex, which contains an octadentate ligand, would have a bound water molecule. Notably, the CEST signal is much sharper for $[Fe(TCMC)]^{2+}$ than for $[Fe(STHP)]^{2+}$ (Figure 1). This corresponds to the narrow line width of the exchangeable proton resonance of this complex in CD_3CN compared to $[Fe(STHP)]^{2+}$, which has a very broad exchangeable proton resonance at 39 ppm in CD_3CN (Figures S1 and S2 in the SI). The very broad exchangeable OH proton resonance and corresponding CEST peak may be due to the formation of extensive hydrogen bonds with anions and solvent, as observed for analogous $[Ln(STHP)]^{3+}$ complexes.[8b] These interactions might give rise to a distribution of different hydroxyl environments. The T_1 values for the water protons are similar in the presence of 10 mM complex (380 ms, $[Fe(TCMC)]^{2+}$; 430 ms, $[Fe(STHP)]^{2+}$). However, the rate constant at 37 °C for alcohol proton exchange (3000 s^{-1} at pH 7.3) is sufficiently large to produce exchange broadening.[12] In comparison, the rate constant for the NH protons of the amide groups in $[Fe(TCMC)]^{2+}$ (400 s^{-1} at pH 7.4) is 7-fold lower. The larger rate constant for $[Fe(STHP)]^{2+}$ in comparison to $[Fe(TCMC)]^{2+}$ corresponds to the more acidic values for the OH protons (pK_a = 9.2; Table S2 in the SI) compared to the NH protons (pK_a > 11).[5]

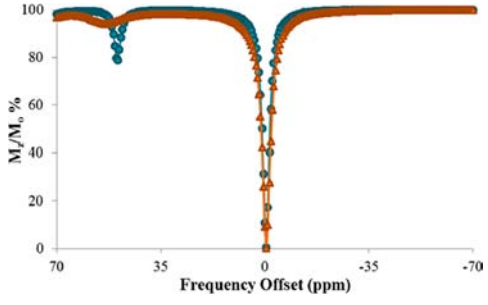

Figure 1. Overlaid CEST spectra collected on a Varian Inova 11.7 T spectrometer at 37 °C of 10 mM $[Fe(TCMC)]^{2+}$ (blue circles) and 10 mM $[Fe(STHP)]^{2+}$ (orange triangles). Samples contained 20 mM HEPES and 100 mM NaCl, pH 7.3. B_1 irradiation at 12 μT was applied for 2 s in increments of 0.5 ppm.

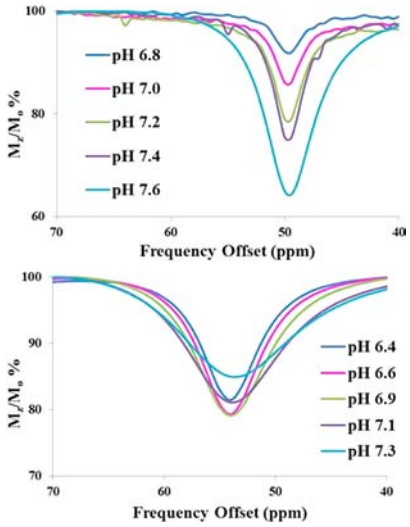

Figure 2. CEST spectra of the exchangeable proton region at 37 °C. Samples contained 10 mM $[Fe(TCMC)]^{2+}$ (top) or 10 mM $[Fe(STHP)]^{2+}$ (bottom), 20 mM buffer (HEPES or MES), and 100 mM NaCl. B_1 irradiation was applied for 2 s at 24 μT.

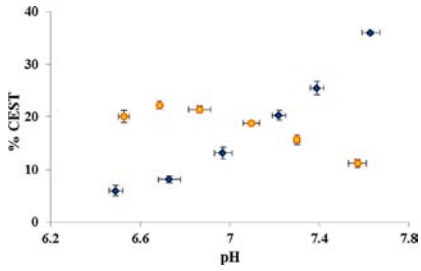

Figure 3. pH dependence of 10 mM $[Fe(TCMC)]^{2+}$ (blue diamonds) and $[Fe(STHP)]^{2+}$ (orange circles) at 37 °C in 20 mM HEPES or MES buffer and 100 mM NaCl. B_1 = 24 μT applied for 2 s in increments of 1 ppm (% CEST = 100 − M_z/M_0 %).

$[Fe(TCMC)]^{2+}$ shows a pronounced CEST signal at physiological pH, which increases in intensity in the range of pH 6.5−7.6 with no apparent exchange broadening (Figures 2 and 3). This is consistent with an increase in the rate constant with increasing pH due to base-catalyzed proton exchange.[13] By contrast, the change in the CEST signal for $[Fe(STHP)]^{2+}$ shows a pH optimum at an acidic pH of 6.7 with a decrease in the intensity at higher pH values as the CEST peak broadens further.[12] In contrast to $[Ln(STHP)]^{3+}$ with optimal CEST at pH 3−6,[8b] $[Fe(STHP)]^{2+}$ shows a more physiologically

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relevant pH response, corresponding to the less Lewis acidic Fe^{II}, as indicated by the higher pK_a value of the Fe^{II}-bound alcohol pendent group. In summary, these data show that both types of exchangeable protons give complexes that demonstrate a CEST effect over a physiological pH range and may ultimately be useful in the development of iron(II) paraCEST agents that are ratiometric pH sensors. The incorporation of mixed amide/alcohol pendent groups into the complex is the next step toward producing ratiometric contrast agents.[14]

To examine biologically relevant conditions, CEST spectra were recorded in 20 mM HEPES and 100 mM NaCl, with 0.4 mM phosphate and 25 mM carbonate at slightly basic pH. The CEST spectrum of $[Fe(TCMC)]^{2+}$ does not show any significant change compared to spectra taken in the absence of carbonate and phosphate, while the $[Fe(STHP)]^{2+}$ CEST peak shifts 1 ppm, the shape of the peak is altered, and the CEST effect becomes less intense (Figures S3 and S4 in the SI). The response of $[Fe(STHP)]^{2+}$ to anions is reminiscent of the effect of anions on $[Ln(STHP)]^{3+}$ CEST spectra.[15] Given that the ^{1}H NMR spectra of the iron(II) complex changes very little in the presence of carbonate and phosphate, the change in the CEST peak is attributed to an outer-sphere anion interaction that disrupts the hydrogen-bonding network in place between the alcohol groups rather than direct binding of the anions to the Fe^{II} center.[15,16]

The complexes studied here are moderately thermodynamically stable, with formation constants of log K = 7.5[5] for $[Fe(TCMC)]^{2+}$ and 9.3 for $[Fe(STHP)]^{2+}$ in an aqueous solution containing 100 mM NaCl. Interestingly, these formation constants are lower than those for the TACN-derived complexes previously reported.[2] The speciation diagram shows that $[Fe(STHP)]^{2+}$ predominates in the pH range studied (Tables S1 and S2 and Figures S5 and S6 in the SI). However, kinetic inertness to dissociation is an important index of the viability of the complex for in vivo studies.[17] Despite the lower thermodynamic stability of the tetraazamacrocyclic complexes, the kinetic inertness of the complexes in the presence of acid, biologically relevant anions, and excess zinc(II) is quite high.

$[Fe(STHP)]^{2+}$ was monitored by ^{1}H NMR over 12 h of incubation at 37 °C to determine the degree of dissociation in the presence of biologically relevant anions, acid, and excess zinc(II) for 12 h. In all experiments, there is negligible change over 12 h with respect to changes in the proton chemical shifts or increased free ligand due to dissociation (Figures S7−S9 in the SI). Peaks observed in the diamagnetic region correspond to excess free ligand [or Zn^{II}-bound ligand] in the sample. The intensity and integration of the diamagnetic peaks in the presence of anions and Zn^{II} do not increase appreciably over time (0−2%) relative to the internal standard of 3-(trimethylsilyl)-1-propanesulfonic acid. Acidic conditions at pD 4 show the largest change of 15%. $[Fe(TCMC)]^{2+}$ shows no appreciable dissociation under any of these conditions.[5]

In summary, we have developed the first iron(II) complexes containing amide and alcohol pendant groups on a CYCLEN framework that give rise to a CEST effect in the physiologically relevant range between pH 6 and 8. NMR spectroscopic data are consistent with iron(II) complexes containing an octadentate macrocycle in solution. These complexes show surprising kinetic inertness toward dissociation of Fe^{II} in solution and are highly stabilized in the divalent state. The pH-dependent behavior of the CEST effect differs markedly for the alcohol and amide groups, making these systems of interest for the design of ratiometric responsive MRI contrast agent probes. In addition, the $[Fe(STHP)]^{2+}$ complex shows promise as an anion-responsive CEST agent. CYCLEN-based macrocycles may prove to be a highly versatile platform for the further development of iron(II) paraCEST MRI contrast agents for in vivo applications.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental methods, 1D ^{1}H NMR spectra, pH potentiometric data, and additional CEST spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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