Session Title: Educational Course in Molecular imaging

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Highlights:

- Transition metal ion (Fe^{II}, Co^{II} or Ni^{II}) based paraCEST MRI contrast agents (paraCEST = paramagnetic chemical exchange saturation transfer)
- Design of ligands and complexes for effective contrast
- Characterization of complexes and assessment of their stability
- CEST MR imaging experiments in biological media

Title: paraCEST MRI contrast agents containing iron, cobalt or nickel

Target Audience: Radiologists, chemists or biologists with an interest in the design and testing of transition metal ion based MRI contrast agents for mapping pH or redox potential.

Outcome/objectives: Attendees will understand the basics behind tuning contrast agent stability, redox potential, spin state, molecular dynamics and contrast signal optimization for paraCEST agents.

Purpose: To gain an understanding of the basics behind the design of effective MRI contrast agents based on Fe^{II}, Co^{II} or Ni^{II}.

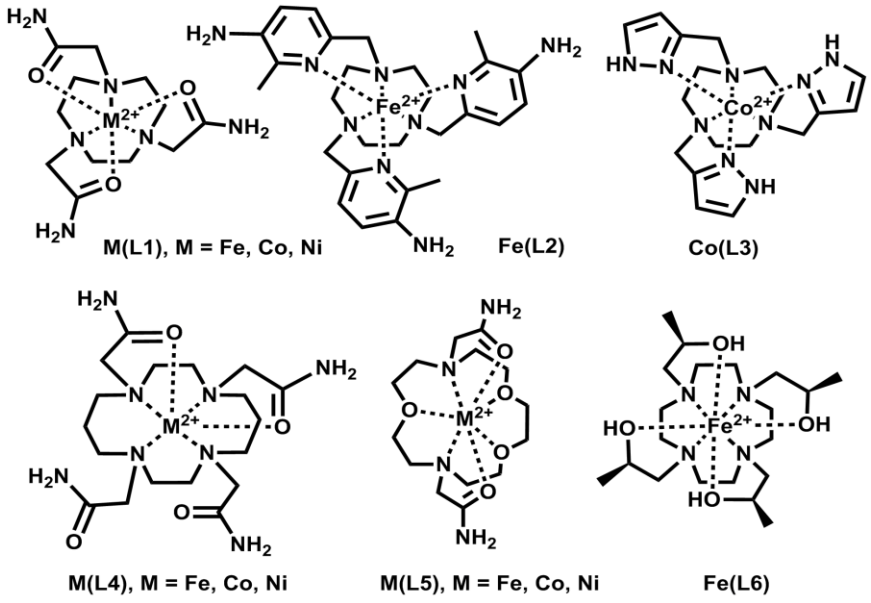
The development of MRI contrast agents that are activated by changes in biological environment such as pH, temperature or redox status is of current interest. One relatively new class of inherently "smart" MRI contrast agents contains metal ion complexes that produce contrast through chemical exchange saturation transfer (CEST).[1] These agents have ligands that give proton NMR resonances which are distinct from those of bulk water, but are in chemical exchange with water protons. A presaturation pulse is introduced at the radiofrequency of the ligand proton resonance to saturate the magnetization of the proton. The saturated ligand proton exchanges with protons of water to produce a decrease in the bulk water signal. CEST MRI contrast agents frequently contain metal ions with unpaired electrons. These paramagnetic complexes (paraCEST agents) shift the exchangeable proton resonance far away from the bulk water signal to reduce interference from magnetization transfer effects in tissue.

Lanthanide ion based paraCEST agents are quite promising and are under development for several applications including stem cell tracking[2] and pH mapping.[3] Recent work shows that transition metal ion complexes containing iron, cobalt or nickel are also promising as paraCEST agents.[4-12] These are biologically relevant metal ions and thus may have certain advantages for development as contrast agents. The development of Fe^{II} contrast agents is of special interest because the body has mechanisms for handling excess iron, the most abundant transition metal ion in the body. Other advantages include the multiple accessible oxidation states of these metal ions that can be used for contrast agents that register redox status. Furthermore, the rich coordination chemistry of the first row transition metal elements may facilitate the development of new types of paraCEST contrast agents that are responsive to biological environment.

Choice of transition metal ion complexes

The paramagnetic metal ion in paraCEST agents produces a large shift in the exchangeable proton resonance without producing extensive line broadening.[1] In other words, the metal ion must have poor relaxivity properties. Certain paramagnetic transition metal ions have these favorable magnetic properties[13] for development as paraCEST MRI contrast agents including Fe^{II}, Co^{II} and Ni^{II}.[5-10,12] However there are complicating features in the design of transition metal ion agents that make development more challenging in comparison to lanthanide(III) complexes. In particular, there must be careful control of the metal ion spin state and oxidation state. Fe^{II} and Ni^{II} complexes may be either paramagnetic or diamagnetic depending on ligand environment. Fe^{II} and Co^{II} may be oxidized to Fe^{III} or Co^{III}, respectively, to give complexes that are not paraCEST agents. There are also considerations that are common to all contrast agents such as stability of the complexes and their interaction with biologically common molecules that might influence contrast.

All ligands used to date for transition metal ion paraCEST agents are macrocyclic ligands.[7] Macrocyclic ligands are strong chelates that protect the metal ion from interaction with other biologically important molecules that might bind and prevent metal ion dissociation. There are four different types of macrocyclic backbones that have been used including those with tetraaza-macrocycles, triaza-macrocycles and a mixed diaza-trioxa-macrocycle. Pendent groups attached to the macrocycle backbone are chosen so that five-membered chelates are formed with the metal ion. A wide variety of pendent groups are possible with transition metal ions including amines, alcohols, amides and heterocyclic amino groups such as benzimidazole, pyridines and pyrazoles.



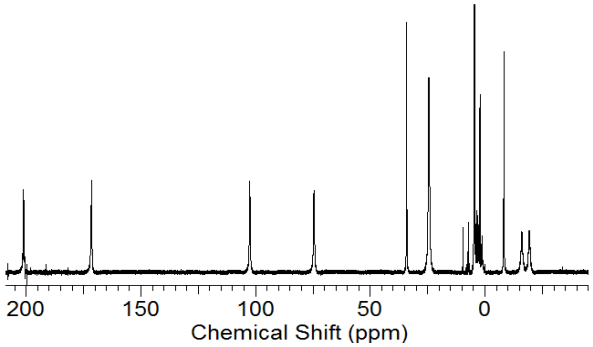
Transition metal ion paraCEST agents

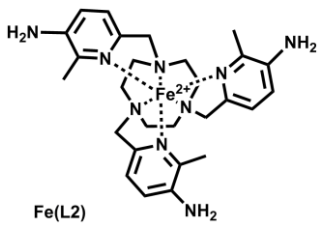
Maintaining a paramagnetic (high spin) Fe^{II} or Ni^{II} center requires optimization of the ligand donor groups (groups bound to the metal ion) and geometry through judicious choice of ligand. For Ni^{II} it is important to avoid ligands that would give a diamagnetic square planar Ni^{II} complex such as macrocyclic ligands that have four consecutive amine donors and no other donor groups. To produce high spin Fe^{II}, it is important to avoid coordination environments that give six strong bonds in an octahedral geometry, or the Fe^{II} may be diamagnetic. For example, the methyl substitutents on the pyridine pendent groups in **Fe(L2)** are necessary to favor high spin Fe^{II} by increasing the Fe-N bond length.[9]

To maintain the divalent state of the metal ion, it is useful to have neutral donor groups such as alcohols, amides, or amines. Amide and alcohol pendent groups that bind through oxygen donors favor the divalent state of the metal ion regardless of the macrocycle framework.[8] Similarly, 2-methylpyridines favor divalent iron and cobalt complexes. In contrast, pyrazole pendents on ligands such as L3 produce complexes of Co^{II} and Fe^{II} that are readily oxidized to the trivalent state. The potential of the Co^{II}/Co^{III} redox couple is tunable over a wide range based on choice of ligand. The Co(L3) complex is readily oxidized in air to the Co^{III} complex.[12] This complex has a diamagnetic/ paraCEST silent form and a paramagnetic/MRI active form that can be switched back and forth based on concentration of oxygen as an oxidant or cysteine as a reductant.

Characterization of the complexes

Characterization of the "solution chemistry" of the new complexes is an important step. The complex is examined to determine stability and kinetic inertness towards dissociation, dynamic processes, reactivity towards cations, anions and acidic conditions, oxidation and spin state (magnetic moment) and if possible, location of exchangeable protons. Paramagnetic proton NMR spectroscopy is the primary tool that is useful for defining all of these characteristics. For example, Fe(L2) shows nine highly shifted and relatively sharp proton resonances as shown below.[9] This is consistent with coordination of all six nitrogen donors in a highly symmetric and rigid complex containing the divalent iron (Fe^{II}) state. Magnetic moments (5.8 μ_B) as measured by Evan's method in solution are also consistent with high spin Fe^{II}. To identify exchangeable protons, the proton NMR spectrum of the complex is examined in non-protic solvents such as deuterated acetonitrile or DMSO. Addition of a few drops of D_2O results in the reduction of the exchangeable proton resonance.





^{1}H NMR spectrum of Fe(L2) in D_2O showing highly shifted proton resonances of the ligand in the complex.

The chemical shift of the exchangeable proton is quite important in the design of a paraCEST agent. The paramagnetic induced shift of a proton is produced by a combination of dipolar (through space) and contact (through bond) interactions. For transition metal ions, both contributions may be substantial, even several bonds away from the paramagnetic center.[13] The prediction of the chemical shift of the paramagnetically shifted exchangeable proton is quite difficult due to the combination of these two contributions. Chemical shifts of exchangeable protons in transition metal ion paraCEST agents vary from +170 ppm to -68 ppm versus water protons. A few complexes of low symmetry give multiple CEST peaks such as the Co^{II} complex of L4 which has four major CEST peaks, two of them highly shifted (95 and 112 ppm).[6] Given that each CEST peak has a unique pH dependence, the ratio of the peak intensity gives a measure of pH which is not dependent on the concentration of the agent. Such ratiometric paraCEST agents may ultimately be useful for mapping pH.

The reactivity of the paraCEST agents towards metal ion release, or interaction with biologically abundant anions, cations or proteins can be followed by monitoring the proton NMR spectrum of the complex. Release of the metal ion gives new proton resonances in the diamagnetic region of the NMR spectrum for the free ligand. Interaction of the metal ion complex with anions other ligands produces a shift in the paramagnetically shifted proton resonances. Such a shift in the proton resonances upon addition of a biologically relevant molecule may be useful for the development of responsive paraCEST agents. Alternatively, many transition metal ion complexes are highly colored so that UV-vis spectroscopy is useful for monitoring the dissociation or reactivity of the complex in solution.[8,12]

Whether the complex is reactive or not relies partly on a good match of ligand and coordination preferences of the metal ion. For example, the seven-coordinate Co^{II} and Fe^{II} complexes of L5 are inert towards phosphate and carbonate, whereas the Ni(L5) complex reacts with carbonate.[6,10] X-ray crystallographic studies show that the smaller Ni^{II} ion forms a complex of L5 which has a very long bond to one of the macrocyclic oxygens, consistent with a labile site and higher reactivity. Another example is found for paraCEST agents that have alcohol pendents such as **Fe(L6)**. The alcohol OH groups interact with carbonate and phosphate to produce a change in the alcohol proton chemical shift and modulation of the CEST signal.[5]

CEST NMR and CEST MR Imaging

For complexes of Fe^{II}, Co^{II} or Ni^{II} that have promising solution chemistry, CEST spectra or Z-spectra are recorded. This involves setting up a series of NMR experiments with variable presaturation pulse frequency at 0.5 ppm intervals and the effect of the presaturation pulse on the bulk water proton resonance is monitored. The CEST effect or CEST peak is produced by a decrease in the water peak intensity, showing up as a dip in the CEST spectrum. For these experiments, it is best to have located the approximate chemical shift of the exchangeable proton in order to set the range of the presaturation pulse values. CEST spectra are typically dependent on pH, so pH values must be carefully controlled in these experiments. Locating the position of the CEST peak and characterizing the temperature and pH dependence of the CEST effect is important information used in subsequent experiments on a MRI scanner.

The rate constant for the exchange of the proton with water is an important characteristic of a paraCEST agent.[1] An initial restriction is that the rate constant for proton exchange cannot be larger than the difference in frequency between water and contrast agent protons. This is necessary in order to selectively saturate the paraCEST agent protons with the radiofrequency pulse without saturating the bulk water protons. In general, larger rate constants correspond to a greater number of protons exchanged per unit time to produce a greater CEST effect. However, large rate constants require higher pulse power for an optimal CEST effect. The CEST effect is optimal for rate constants equal to $2\pi B_1$, where B_1 is the presaturation pulse power in Hz and is limited by allowable power deposition in vivo.[14] Thus, in addition to tuning the chemical shift of the exchangeable proton, it is advantageous to be able to modulate the rate constant for exchange by choice of different pendent groups. Proton exchange rate constants for transition metal ion paraCEST agents range from 12,000 s^{-1} to 300 s^{-1} and are obtained by constructing an Omega plot.[15]

Imaging of phantoms on a MRI scanner may use one of several different methods.[16] Our lab has used a pulse train comprised of five Gauss pulses at 12 μT for 1 s, interpulse delay of 200 μs at +/-135 ppm. CEST phantom images are collected by subtracting the ratio of "on" resonance and "off" resonance. Other imaging protocols may be used including CEST-FISP (FISP = fast imaging with steady-state free precession). CEST-FISP improves data precision by averaging multiple data points. The Co(L5) complex was imaged down to 250 μM by using this method.[6] It is useful to image transition metal ion paraCEST agents in serum or in agarose to access the effect of biological media on the CEST contrast. The CEST effect varies for some complexes in different media as does T_1 relaxivity, a competing process. The complex with the most highly shifted CEST peak typically shows the least interference from magnetization transfer interference in agarose as a tissue mimic. Some paraCEST agents such as Ni(L5) interact with components in serum to give a decrease in signal while others show enhanced contrast due to an increase in the proton exchange rate constant.

Conclusions

Fe^{II}, Co^{II} and Ni^{II} paraCEST agents show promise for further development. These transition metal ion complexes have a rich coordination chemistry that can be used to advantage to optimize MRI contrast and to design agents that are responsive to biological environment.

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