pH-DEPENDENT SPIN STATE POPULATION AND 19F NMR CHEMICAL SHIFT VIA REMOTE LIGAND PROTONATION IN AN IRON(II) COMPLEX (POSTPRINT)

Alexandra I. Gaudette, Agnes E. Thorarinsdottir and T. David Harris
Northwestern University

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**6. AUTHOR(S)**
Alexandra I. Gaudette, Agnes E. Thorarinsdottir, and T. David Harris - Northwestern University

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
Northwestern University
2145 Sheridan Road
Evanston, IL 60208

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An Fe(II) complex that features a pH-dependent spin state population, by virtue of a variable ligand protonation state, is described. This behavior leads to a highly pH-dependent 19F NMR chemical shift with a sensitivity of 13.9(5) ppm per pH unit at 37 °C, thereby demonstrating the potential utility of the complex as a 19F chemical shift-based pH sensor.

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An Fe$^{II}$ complex that features a pH-dependent spin state population, by virtue of a variable ligand protonation state, is described. This behavior leads to a highly pH-dependent $^{19}$F NMR chemical shift with a sensitivity of 13.9(5) ppm per pH unit at 37°C, thereby demonstrating the potential utility of the complex as a $^{19}$F chemical shift-based pH sensor.

The development of transition metal-based molecules and materials that can be switched between low-spin and high-spin electronic states has constituted a highly active area of research over the past several decades. Indeed, the magnetic bistability of such spin-crossover compounds makes them potential candidates for molecular switches and chemical sensors, as the spin transition can be controlled by a number of external stimuli, such as temperature, pressure, and light. Recently, we and others have begun to explore the potential for spin-switchable molecules as bioresponsive probes for temperature, anions, and enzyme activity. In addition, given the relationship between tissue acidosis and diseases, including cancer and ischemia, a compound that undergoes a spin state transition as a function of pH could serve as a valuable tool for pH sensing. Nevertheless, pH-induced spin state switching is rare, and compounds that exhibit such behavior are unsuitable for most biological sensing applications due to $pK_a$ values far from biological pH or poor stability in water.

One approach toward biological pH sensing is to employ pH-induced changes in the chemical shift of $^{19}$F resonances, typically caused by an interconversion between species of different protonation states. Here, the use of $^{19}$F magnetic resonance spectroscopy (MRS) offers key advantages over the more commonly employed $^1$H MRS, most notably the absence of endogenous fluorine in living systems. Furthermore, the chemical shift of the $^{19}$F nucleus is highly sensitive to its local environment, such that small chemical changes can lead to drastic changes in chemical shift. Indeed, diamagnetic $^{19}$F MRS pH probes with $pK_a$ values near 7 have been developed for in vivo applications, where a variation in $^{19}$F chemical shift of 12 ppm was observed between the protonated and deprotonated forms. In addition, the sensitivity of $^{19}$F MRS probes can be further improved by incorporating paramagnetic metal ions, as the difference in chemical shift between the protonated and deprotonated forms is amplified by the presence of contact (through-bond) and dipolar (through-space) contributions to the chemical shift.

We previously demonstrated that spin-crossover Fe$^{III}$ complexes can facilitate chemical shift-based MR thermometry. Here, since both the contact and dipolar contributions to the paramagnetic chemical shift scale with $S(S + 1)$, where $S$ is the electronic spin state, thermally-induced spin-crossover from an $S = 0$ ground state to an $S = 2$ excited state afforded a dramatic increase in chemical shift with temperature. Building on these results, we sought to develop a spin-crossover Fe$^{II}$ complex that undergoes a deprotonation-induced spin state change near biological pH for $^{19}$F chemical shift-based pH sensing. Herein, we report the synthesis and characterization of an Fe$^{II}$ complex that features a new asymmetric 1,4,7-triazacyclononane (TACN) ligand appended with two 4-hydroxypyridine donors, and demonstrate that pH-induced spin state switching can engender highly sensitive $^{19}$F MRS pH probes.

In order to develop an $^{19}$F MR probe that undergoes a pH-induced spin state transition, we set out to design a ligand that (1) forms a water-soluble complex with Fe$^{II}$, (2) features an $^{19}$F reporter group, and (3) affords a ligand field that changes dramatically with pH. Toward this end, we selected 4-hydroxy-3,5-dimethyl-2-pyrydyl groups as the pH sensing moieties, because the ability of 4-hydroxypyridines to engender significant changes in the electronic structure of transition metal compounds upon protonation or deprotonation has been demonstrated in V$^{III}$ Fe$^{II}$ Co$^{II}$ Ni$^{II}$ Ru$^{IV}$ Pt$^{IV}$ Re$^{IV}$ and Ir$^{IV}$ compounds. We thus hypothesized that incorporating hydroxypyridine groups into a spin-crossover complex could create a compound with a pH-sensitive spin state population (see Fig. 1). Along these lines, we targeted the macrocyclic TACN-based ligand H$_2$L (see Fig. 1, left).

Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208-3113, USA. E-mail: dbharris@northwestern.edu

† Electronic supplementary information (ESI) available: Experimental details, crystallographic data, additional magnetic and spectroscopic data, and crystallographic information file (CIF) for 1. CCDC 1574128. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c7cc08158h

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pH-Dependent spin state population and $^{19}$F NMR chemical shift via remote ligand protonation in an iron(II) complex†

Alexandra I. Gaudette, Agnes E. Thorarinsdottir and T. David Harris
The ligand H₂L was prepared following a four-step synthesis outlined in Scheme S1 (ESI†). Incorporation of two 4-methoxy-3,5-dimethyl-2-picolyl donors onto a tosylated TACN macrocycle via nucleophilic substitution reaction, followed by deprotection with H₂SO₄ and reductive amination with 3-fluoro-6-methyl-2-pyridinecarboxaldehyde, afforded H₂L as a yellow solid. Under anaerobic conditions, reaction of H₂L with one equivalent of [Fe(H₂O)₆](BF₄)²⁻ in MeCN, followed by aqueous workup, gave [([H₂L]Fe)BF₄]²⁺ (I) as a yellow-green solid in 52% yield. Subsequent slow cooling of an aqueous solution afforded yellow-green block-shaped single crystals of I.

Single-crystal X-ray diffraction analysis for I was carried out at 100 K (see Fig. 2 and Table S1, ESI†). The compound crystallized in the orthorhombic space group Pbcn, with one [H₂L]Fe²⁺ cation and two [BF₄]⁻ anions in the asymmetric unit. The two acidic protons on the hydroxypyridine moieties were located in the Fourier difference map and were freely refined. The coordination environment at the Fe²⁺ center in [([H₂L]Fe)BF₄]²⁻ is highly distorted from octahedral geometry. The Fe-N bond distances range from 2.109(5) to 2.223(5) Å with an average distance of 2.165(5) Å, and the cis N-Fe-N angles range from 75.1(2) to 111.3(2)° with an average angle of 90.8(2)°. These structural metrics indicate the presence of high-spin Fe²⁺ in the solid state at 100 K. Considering the observation of low-spin Fe²⁺ in related compounds at 100 K,† we surmise the high-spin Fe²⁺ center in I likely results from crystal packing effects.

We next examined the effects of pH on the electronic structure of I in aqueous solution. UV-Visible absorption spectra were collected for 100 mM phosphate buffer solutions containing 2% (v/v) DMSO between pH 5.39 and 7.94 at 25 °C (see Fig. S1, ESI†). At pH 7.94, the spectrum displays an intense absorption band at 271 nm (εmax = 27 000 M⁻¹ cm⁻¹) and three less intense bands at 334 (εmax = 2300 M⁻¹ cm⁻¹), 416 (εmax = 960 M⁻¹ cm⁻¹), and 453 nm (εmax = 1200 M⁻¹ cm⁻¹). We assign these features to π–π* and metal–ligand charge transfer (MLCT) transitions, respectively, as observed in similar compounds. As the pH is decreased to 5.39, the intensity of the π–π* absorption decreases (εmax = 12 000 M⁻¹ cm⁻¹), while the intensities of the MLCT bands increase significantly with concomitant shifts to 383 (εmax = 2800 M⁻¹ cm⁻¹) and 451 nm (εmax = 1600 M⁻¹ cm⁻¹). This increase in MLCT band intensity with decreasing pH suggests that the low-spin state becomes more populated when the ligand is fully protonated. Furthermore, the presence of a single set of three nearly isosbestic points at 330, 472, and 525 nm indicates an equilibrium between two species, likely (L²⁻)Fe and ([H₂L]Fe)²⁺, and therefore suggests highly similar values of pKₐ for the two hydroxyl protons. Such nearly simultaneous double deprotonation in aqueous solution has been reported for similar complexes, and is further supported by a single sigmoidal inflection point of ε vs. pH plots at 271 and 380 nm, providing pKₐ values of 5.95(7) and 5.90(2), respectively (see Fig. S2, S3 and Table S2, ESI†).

To probe the magnetic properties of I and further examine the solution electronic structure as a function of pH, variable-temperature dc magnetic susceptibility data were collected using the Evans method in the temperature range 5–60 °C (see Fig. 3, upper). Samples were prepared in 100 mM phosphate buffer solutions with 2% (v/v) DMSO added as a reference. At pH 4.74, the value of χM/T increases with increasing temperature, from 1.78(1) cm³ K mol⁻¹ at 5 °C to 4.07(1) cm³ K mol⁻¹ at 60 °C. This temperature dependence indicates the presence of thermally-induced spin-crossover, with significant population of both an S = 0 ground state and S = 2 excited state in this temperature range. Assuming a value of g = 2 for the S = 2 state, insertion into the Curie law gives percentages of high-spin molecules (%HS) of 59% at 5 °C and 80% at 60 °C. In contrast, the variable-temperature magnetic susceptibility data for an analogous solution of I at pH 7.82 show much smaller temperature dependence. Here, χM/T increases only slightly with increasing temperature, from 2.62(1) cm³ K mol⁻¹ at 5 °C to 2.70(1) cm³ K mol⁻¹ at 60 °C, corresponding to %HS = 87% at 5 °C and 90% at 60 °C. We attribute this shift toward greater population of the S = 2 state with increasing pH to a decreased ligand field strength of L²⁻ vs. H₂L, owing to the weaker π acidity of the former, which is consistent with pyridyl substituent effects observed for related spin-crossover iron(II) complexes.

To further probe the pH dependence of the spin state of I, dc magnetic susceptibility data were collected on an aqueous solution as a function of pH at 25 and 37 °C (see Fig. 3, lower). At 25 °C, χM/T rapidly increases with increasing pH, from a value of χM/T = 2.07(1) cm³ K mol⁻¹ at pH 4.74 to χM/T = 2.64(1) cm³ K mol⁻¹ at pH 7.82, corresponding to %HS = 69% at pH 4.74 and 88% at pH 7.82. The plot of χM/T vs. pH at 37 °C exhibits similar behavior, albeit with less pronounced pH dependence, where χM/T increases...
from 2.20(1) cm$^3$ K mol$^{-1}$ at pH 4.74 (%HS = 73%) to 2.65(1) cm$^3$ K mol$^{-1}$ at pH 7.82 (%HS = 88%). These results are in accord with the variable-pH behavior observed in the UV-Visible analysis described above, and confirm the pH dependence of the spin state population in 1.

Finally, we aimed to determine the effect of the pH-dependent spin state population on the $^{19}$F NMR chemical shift of 1. Variable-pH $^{19}$F NMR spectra were collected on 2 mM solutions of 1 in 100 mM phosphate buffers with 2% (v/v) DMSO at 25 and 37 °C (see Fig. S4 and S5, ESI†). In the pH range 4.61–7.82, these spectra display a single $^{19}$F resonance from the metalated ligand. At pH 4.61, when the hydroxypyridine groups are predominantly protonated, the $^{19}$F resonance appears at 11.89 ppm vs. trifluoroacetic acid (TFA) at 25 °C. As the pH is increased, this resonance shifts downfield, following sigmoidal behavior, to a maximum value of 47.03 ppm at pH 7.74. The data at 37 °C shift is an increase in population of the $S = 2$ state, owing to deprotonation of the hydroxypyridine donors.

The foregoing results demonstrate the potential utility of a pH-induced spin state change for pH sensing using $^{19}$F MRS. We show that a change in spin state upon deprotonation of [(H$_2$L)Fe]$^{2+}$ dramatically affects its $^{19}$F chemical shift, which can be employed to quantitate pH. Indeed, this complex displays greater pH sensitivity than the current most-sensitive lanthanide-based $^{19}$F MRS probes$^{17b}$ and the only reported transition metal-based $^{1}$H MR pH probe.$^{32}$ To the best of our knowledge, [(H$_2$L)Fe]$^{2+}$ represents the first example of an $^{19}$F MRS pH probe that employs a pH-induced spin state change. While the oxygen sensitivity of this compound precludes it from in vivo experiments, the principles outlined here could inform the design of chemical shift-based MR pH sensors. Future efforts will focus on developing similar compounds with higher stability under biological conditions.

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There are no conflicts to declare.