Iron complexes with an N/S chromophore relevant to the active site of the hydrolytic metalloenzyme nitrile hydratase†

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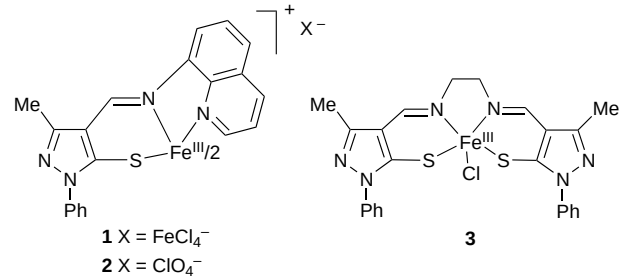
Iron(III) complexes with a 3N/3S chromophore reproduce a mixed N/S heterocyclic ligand environment, metal–ligand bond lengths, and low-spin state (S = 1/2) characteristic of the hydrolytic metalloenzyme nitrile hydratase; $Fe^{III}N_2S_2Cl$ species possesses a quantum mechanically admixed S = 3/2, 5/2 state and able to form low-spin solvent adducts, possible analogues of the substrate-bound form of the enzyme.

Iron–thiolate metal centers have until recently been considered mainly in the context of Fe–S cluster assemblies of metalloproteins and in relation to their role in electron-transfer processes. One of the emerging new non-redox functions of biological iron–thiolate centers is that associated with their Lewis-base activity in catalyzing hydration–dehydration reactions of organic substrates.[1,2] Even though most of these transformations are catalyzed by the metalloenzymes containing Fe–S clusters in their active site, *e.g.*, aconitase, certain others apparently lack cluster units. Another example of a metalloenzyme with an iron–thiolate active site that is not redox active and does not belong to the heme or Fe–S cluster families is bacterial nitrile hydratase, which catalyzes the conversion of nitriles to corresponding amides. Combined physicochemical results[3] provide solid evidence for the presence of a low-spin (S = 1/2) $Fe^{III}N_3S_2$ chromophore with a sixth coordination site occupied by a water molecule or hydroxide ion, which is probably replaced by acetonitrile in the catalytic cycle. Synthetic iron(III) complexes with mixed N/S donor sets, common in coordination chemistry, can be useful models of the protein active site. However, there are only few examples[4] of such species owing to the often facile oxidation of thiolate ligand by Fe^{III}. Studying synthetic models of blue copper proteins, tetracoordinate bis-chelate copper(II)–thiolato complexes of Schiff-base ligands notorious for the same type of redox instability, we found[5] that the presence of the potentially supplementary coordinating group in the ligand structure leads to the stabilization of the higher metal oxidation states.

Following this strategy, we report novel stable iron(III) complexes **1** and **2** with a 3N/3S chromophore simulating the N/S ligand environment of the nitrile hydratase active site and some of its spectroscopic properties. Complex **3** contains a potentially labile terminal chloride ligand which may be substituted by solvent molecules involving acetonitrile. Possibilities for the ligand modifications in **1**–**3** provide an entry to an extended series of Fe^{III}–N/S complexes which might provide insight into intimate structural and electronic features of general interest and those relevant to the active site of metalloenzyme.

Reaction of the ligands H_2L [*N*,*N*′-bis(5-mercapto-3-methyl-1-phenylpyrazol-4-ylmethylene)ethylenediamine] and HL [5-mercapto-1-phenyl-3-methyl-4-ylmethylene(8-amino)-quinoline] with $Fe(ClO_4)_3nH_2O$ or $FeCl_3{\cdot}6H_2O$ in ethanol affords in good yields complexes **1**–**3**.[6] The X-ray molecular structure§ of **1** is shown in Fig. 1 The iron center is octahedrally



1 X = $FeCl_4^-$
2 X = ClO_4^-
3

coordinated by the two azomethine, and two quinoline nitrogens and two thiolate sulfur atoms in *cis*-positions [S(1)–Fe–S(2) 95.15(8)°]. Three metal–ligand angles [S(1)–Fe–N(2) 175.1(2), S(2)–Fe–N(6) 173.2(2), N(1)–Fe–N(5) 176.5(2)°] are close to 180°, thus indicating a tendency for a cubic symmetry ligand arrangement. Iron–sulfur bond lengths, Fe(1)–S(1) 2.227(2) and Fe(1)–S(2) 2.230(2) Å, are similar to that reported for nitrile hydratase (2.21 Å from EXAFS data[3e] for a pH 7.3 form) and *ca*. 0.07 Å shorter than for a high-spin FeN_2S_3 complex.[4e] Iron–nitrogen bond lengths in **1**, Fe–N_{av} 1.99 Å, are also identical to those in the enzyme.

The EPR spectra of **I** recorded in frozen $CHCl_3$ and dimethylformamide solutions display a very large signal at $g \approx$

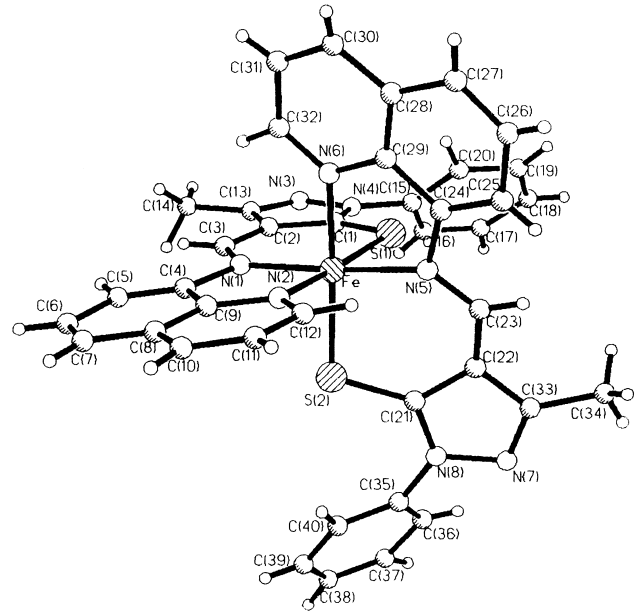


Fig. 1 Molecular structure of **1**. Selected bond lengths (Å) and angles (°): Fe–S(1) 2.227(2), Fe–S(2) 2.230(2), Fe–N(1) 1.969(5), Fe–N(2) 2.005(6), Fe–N(5) 1.980(5), Fe–N(6) 2.004(6); S(1)–Fe–S(2) 95.15(8), S(1)–Fe–N(2) 175.1(2), S(1)–Fe–N(1) 99.7(2), S(1)–Fe–N(5) 81.5(2), S(2)–Fe–N(6) 173.2(2), N(1)–Fe–N(5) 176.5(2).

2, superimposed on which is a rhombic spectrum with the components g = 2.19, 2.12 and 1.98, typical of a low-spin (S = 1/2) iron(III) complex. Given a high-spin (S = 5/2) nature of the anionic residue $FeCl_4^-$, low-spin features can be attributed to the $[FeL_2]^+$ species. Consistent with this assignment, complex **2** displays no broad component at g = 2 but only a well resolved low-spin spectrum with g = 2.193, 2.130 and 1.986.

Magnetic susceptibility measurements in the solid state reveal a low-spin (S = 1/2) iron species **2** with μ_{eff} = 1.95 μ_B, independent of temperature (77–300 K). Magnetic moments of **1**, containing $FeCl_4^-$ as a counter anion, are also temperature independent, 6.17 μ_B (per 2Fe) (4.44 μ_B per 1 Fe), which agrees well with presence of the contribution from the high-spin (S = 5/2) center (μ_{eff} = 5.95 μ_B).

The Mössbauer spectrum of **2** shows a symmetrical doublet with the following isomer shift and quadrupole splitting parameters at 77(300) K: δ = 0.15 (0.11) mm s^{-1} (relative to Fe metal), ΔE_Q = 2.01 (1.99) mm s^{-1}. This situation corresponds to the S = 1/2 iron complexes with an unpaired electron localized on the d_{xy} orbital. Low values of the isomer shift indicate strong metal–ligand π interactions.[7a] For **1**, in addition to a similar doublet, δ = 0.25 (0.15) mm s^{-1}, ΔE_Q = 1.98 (2.08) mm s^{-1} at 77(300) K there is another doublet with fairly small quadrupole splitting and isomer shift, δ = 0.29 (0.23) mm s^{-1}, ΔE_Q = 0.33(0.26) mm s^{-1} which is typical for four-coordinate high-spin (S = 5/2) iron(III) complexes and can be unequivocally assigned to the $FeCl_4^-$ anion.[7b]

Electronic spectra of **1** and **2** in $CHCl_3$ contain two bands in the IR region: λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) 750 (600), 1005 (1410) (**1**) and 750 (535), 995 (1250) (**2**). Compared to nitrile hydratase [λ_{max} = 715 nm (ε = 1100 dm^3 mol^{-1} cm^{-1}) at pH 7.3 and λ_{max} = 690 (ε = 1300) at pH 9], the 750 nm band may correspond to that observed in the enzyme and attributed to the Fe–S LMCT absorptions (with some contribution from an Fe–N chromophore) on the basis of a recent resonance-Raman study.[3d]

With respect to a specific low-spin nature of both substrate-free and bound nitrile hydratase, a significance of the particular ligand field strength may be to allow ligand substitution reactions to occur appropriately (at the early steps of nitrile binding) and also to activate a nitrile bond. To follow this line, we synthesized **3**, the complex with a potentially labile terminal ligand. Hydrolytic properties of the $Fe^{III}N_2S_2Cl$ species are implicated by the early results of Murray *et al.*,[4b] who failed to isolate a sulfur analogue of [Fe^{III}(salen)Cl] but only the complex with a partly hydrolyzed Schiff-base ligand, *N*-[(2-aminoethyl)thio]salicylideneamine. In contrast to the low-spin species **1** and high-spin [Fe^{III}(salen)Cl],[8] the magnetic moment of **3** in the solid state, μ_{eff} = 4.45 μ_B (300 K), indicates the less common intermediate-spin (S = 3/2) state. This value drops to 3.84 μ_B at 77 K. The temperature dependence of the magnetic susceptibility for **3** suggests a contribution from the quantum mechanically admixtured S = 5/2 spin state.[9] Consistently, the Mössbauer spectra of **3** display a single symmetrical doublet with similar parameters at 77(300) K: δ = 0.31(0.23) mm s^{-1}, ΔE_Q = 2.68 mm s^{-1}, typical of the mixed S = 3/2, 5/2 state iron porphyrin complexes and also detected for S = 3/2 iron species.[9b,10] In frozen solutions of **3**, we observed a low-spin species with spectral patterns similar to that for **2** in dmf (g = 2.191, 2.159, 1.981), pyridine–DMF (1 : 1) (g = 2.169, 1.979) and $CHCl_3$ (g = 2.193, 2.162, 1.988) whereas in MeCN there is a low-spin spectrum (g = 2.190, 2.161, 1.985) superimposed on the very broad signal. This result may be explained by formation of either a six-coordinate solvent adduct **3**·solv or a chloride-dissociated species with two axial solvent molecules.¶ The terminal ligand substitution reaction with a concomitant ground spin-state alteration are well established in porphyrin systems, although they usually occur with N-donor type ligands or strongly coordinating solvents.[9c] Complex **3** may be a suitable starting point to pursue an ultimate goal of nitrile hydrolysis[11] given the opportunities of ligand modifications, *e.g.*, conversion to corresponding aminothiol, variation of the bridging group and incorporation of the nitrile functionality into the ligand framework.

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Footnotes and References

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§ *Crystal data* for **1**: $C_{40}H_{30}Cl_4Fe_2N_8S_2$, M = 940.4, monoclinic, space group $P2_1/c$, a = 11.485(2), b = 17.489(30), c = 20.574(5) Å, β = 103.51(3)°, U = 4018.2(5) Å^3, Z = 4, D_c = 1.55 g cm^{-3}, Mo-Kα radiation (0.710 73 Å^3), SYNTEX P2$_1$ diffractometer, T = 295 K. The structure was solved and the data reduced using the SHELX-93 program package. Hydrogen atoms were included at calculated positions with fixed isotropic thermal parameters (U_H = 0.049 Å^2); 2θ < 56°; 5100 unique reflections; 3732 observed reflections with $I > 3\sigma(I)$; number of the refined parameters 596; R = 0.059 (R_w = 0.081). The chlorine atoms of the $FeCl_4^-$ anion are disordered as can be judged from their large thermal displaced parameters (U_{Cl} = 0.115 Å^2). CCDC 182/556.

¶ **3**: UV–VIS [$CHCl_3$, λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})]: 420 (2960), 470(2715), 770(205)(sh). ^{1}H NMR (δ, 300 K): 10.2 (*m*-H, 4 H), 5.7 (*p*-H, 2 H) 2.7 (*o*-H 4 H), 1.4 (CH=N, 2 H), −0.55 (CH_3, 6 H) in $CDCl_3$; 9.8 (*m*-H), 6.4 (*p*-H), 3.4 (br), 2.7 (br), −0.5 (CH_3) in CD_3CN; 10.2 (*m*-H), 5.8 (*p*-H), 2.4 (br, CH=N, *o*-H) in $(CD_3)_2SO$; 8.65 (*m*-H), 6.25 (*p*-H) and 4.0 (br) in $(CD_3)_2SO$–[$^2H_5$]pyridine (1 : 1). Owing to poor solubility, it was possible to determine μ_{eff} for **3** by the Evans method only in $(CD_3)_2SO$ (2.04 μ_B).

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