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A Model for the Low-Spin, Non-Heme, Thiolate-Ligated Iron Site of Nitrile Hydratase

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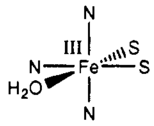
Nitrile hydratase is the first and only current example of a metalloenzyme containing a single non-heme Fe in a mixed N/S-ligated coordination site.[1-3] Its function is to hydrolyze nitriles to the corresponding amides in organisms which can live on R−CN as their sole C and N source. On the basis of EPR,[4] MCD,[5] resonance Raman, EXAFS,[2] and ENDOR[1] studies, the active site of nitrile hydratase is proposed to contain a six-coordinate, low-spin ($S = {}^1/_2$) Fe^{3+} ion ligated by two *cis*-cysteinates, three N's, and a water:



The spectroscopic properties of nitrile hydratase are pH-dependent, and two distinct forms (pH ∼ 7 and pH = 9) have been characterized.[6,7] The high-pH form[6] appears to be identical to the "substrate-bound" form on the basis of its spectroscopic properties.[4] Two impediments to the synthetic modeling of this enzyme have been the ease with which ferric ions oxidize thiolates to disulfides and the propensity of metal thiolates to oligomerize.[8,9] The small number of reported monomeric Fe(III) thiolate complexes attests to this.[10-13] Mixed N-/SR^--ligated Fe(III) systems are even rarer.[14-16] Low-spin complexes of this type are extremely rare; most Fe(III) compounds are $S = {}^5/_2$ high-spin[17,18] or, at best, exist in a $S = {}^5/_2 \leftrightarrow {}^1/_2$ spin equilibrium.[19] Previously reported model compounds approximate the coordination geometry and metric parameters of the nitrile hydratase active site but do not reproduce the spin state and electronic spectral properties.[14] Herein we report the synthesis, structure, and properties of a stable low-spin Fe(III) thiolate complex which is remarkably similar to nitrile hydratase in terms of its electronic and geometric structure.

Thiolate-ligated $[Fe^{III}(AMIT)_2]Cl$ (**1**) was synthesized[20] via the Schiff-base condensation of ethylenediamine with α-mercaptoacetone at an Fe^{3+} template. This approach has provided access to a variety of mixed amine/thiolate S_xN_y-ligated transition-metal complexes by our group.[21-23] Although we have not yet obtained X-ray-quality crystals of **1**,

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(20) Under anaerobic conditions, the α-mercaptoacetone dimer 2,5-dimethyl-2,5-dihydroxy-1,4-dithiane (0.54 g, 3 mmol) was combined with 1 equiv of NaOMe (0.32 g, 6 mmol) in 30 mL of MeOH, followed by 1 equiv of ethylenediamine (0.36 g, 6 mmol). This mixture was then added to a MeOH (30 mL) solution of $FeCl_3$ (0.49 g, 3 mmol) at −35 °C. The solution was reduced in volume to ∼30 mL under vacuum, filtered, and placed in a freezer overnight to afford 0.5 g (40% yield) of bis(5-amino-2-methyl-3-azapent-2-ene-1-thiolato)iron-(III) chloride, $[Fe^{III}(AMIT)_2]Cl\cdot 2CH_3OH$ (**1**), as dark green needles. Anal. Calcd for $C_{12}H_{30}N_4O_2S_2ClFe$: C, 34.50; H, 7.24; N, 13.41. Found: C, 34.12; H, 7.16; N, 13.63.

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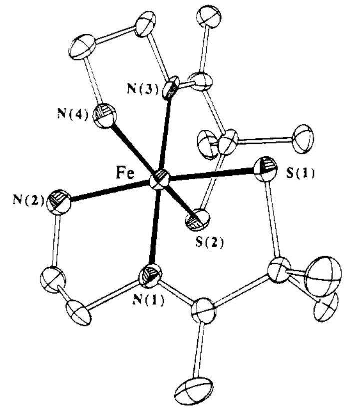

Figure 1. ORTEP diagram of $[Fe^{III}(ADIT)_2]^+$ (**2**). Selected distances (Å) and angles (deg): Fe−S(1), 2.200(1); Fe−S(2), 2.207(1); Fe−N(1), 1.938(2); Fe−N(2), 2.043(3); Fe−N(3), 1.938(2); Fe−N(4), 2.059(3); S(1)−Fe−S(2), 97.3(1); S(1)−Fe−N(2), 167.9(1); S(2)−Fe−N(4), 168.9(1); N(1)−Fe−N(3), 175.7(1). Hydrogens are omitted for clarity.

the structure of its methyl-substituted derivative[24] $[Fe^{III}(ADIT)_2]Cl{\cdot}MeCN$ (**2**) was determined[25] and is shown in Figure 1, minus the chloro anion and cocrystallized MeCN. Methyl protection at the carbon α to the sulfurs appears to stabilize **2** relative to **1**.

The structural and electronic properties of **1** and **2** closely match those of both the low-pH and high-pH forms of nitrile hydratase. For example, the crystallographically determined Fe−S distance (2.203(5) Å) in **2** is virtually identical to the EXAFS-determined Fe−S distance (2.21(3) Å) of the enzyme.[2] The Fe^{3+} ion in **2** sits in an S_2N_4 coordination sphere which consists of two *cis*-thiolates, two *trans*-imines, and two *cis*-amines. The structural feature which distinguishes **2** from most other Fe thiolates[26] is the short mean Fe−S distance. This distance provided the first clue that **2** exists in a low-spin state. The only other example of an Fe thiolate with short Fe−S distances is $[Fe^{III}(Haetsaln)_2]^+$.[16] The mean Fe−N distance in **2** (1.99(7) Å) is also short and approximately the same as that observed in nitrile hydratase (1.98(3) Å).

The ambient-temperature magnetic moment of **2** (μ = 1.85 μ_B)[27] supports the low-spin ($S = {}^1/_2$) state assignment suggested by the short Fe−S distances, and both the solution (μ = 1.95 μ_B) and solid state (μ = 1.69 μ_B) magnetic moments of **1** are characteristic of an $S = {}^1/_2$ ground state, indicating that its structure is probably similar to that of **2**. These spin-state assignments were confirmed by EPR at 77 K. In a MeOH/EtOH 9:1 glass, compounds **1** and **2** gave signals (**1**, g = 2.20, 2.16, 2.00; **2**, g = 2.19, 2.13, 2.01) which closely resemble those of the pH = 9 form (g = 2.20, 2.12, 1.99) of nitrile hydratase.[6] The low-spin state of nitrile hydratase has been somewhat of a puzzle, since the majority of six-coordinate Fe(III) compounds are high-spin[17,18] or exist in a spin equilibrium.[19] With sulfur in the coordination sphere, it is true that π-interactions favor a high-spin state. However, in opposition to this, electrons would be delocalized within the covalent Fe−S bonds (the nephelauxetic effect).[28] This favors a low-spin state. Perhaps there is a delicate balance which is dependent on the number of sulfurs in the coordination sphere. We are currently investigating this possibility.

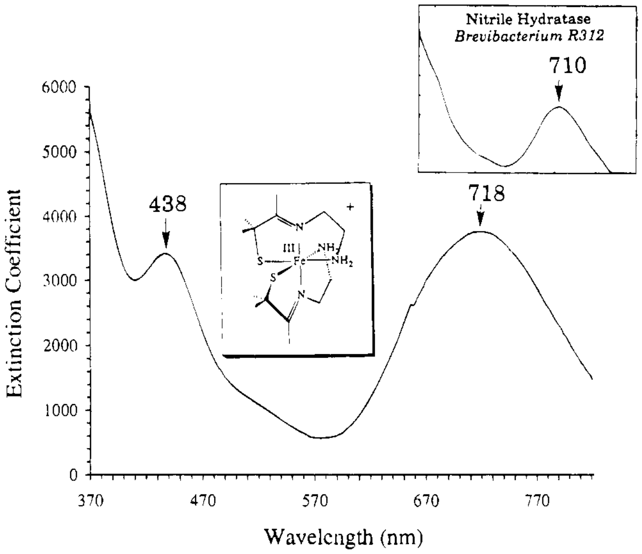

Figure 2. Comparison of the electronic spectra of $[Fe^{III}(ADIT)_2]Cl{\cdot}MeCN$ (**2**) (MeCN, 298 K) and nitrile hydratase from *Brevibacterium* sp. strain R312 at pH ∼ 7. Inset: Reprinted with permission from ref 3. Copyright 1986 Academic Press.

Perhaps the clearest demonstration of the striking similarity between model compound **2** and nitrile hydratase is the close match in their electronic spectral properties. This is illustrated in Figure 2. The low-energy band present in the spectra of both nitrile hydratase[3,6] and **2** at ca. 700 nm appears to be a sensitive probe of the local coordination environment. Increasing the pH by ca. 2 pH units shifts this band from 710 nm ($\epsilon \sim 1200$) to 690 nm ($\epsilon \sim 1200$) in the enzyme.[6] In model compounds **1** and **2**, replacement of H by Me at the carbon α to the sulfur shifts this band from 696 nm ($\epsilon \sim 2760$) to 718 nm ($\epsilon \sim 3754$). The MCD spectral properties of models **1** and **2** are also nearly identical to those of nitrile hydratase, at least in the lower energy region, which lacks interference from imine $\pi \rightarrow \pi^*$ transitions.[5]

In conclusion, the close match in properties between our structurally characterized *cis*-thiolate Fe(III) model complex and the enzyme nitrile hydratase supports the proposed *cis*-cysteinate-ligated Fe(III) structure of nitrile hydratase. We are continuing to work on the syntheses of more exact structural models which contain Fe(III) in an S_2N_3O environment and of more reactive, five-coordinate models.

Acknowledgment. We thank Mark Nelson (Dupont) for providing us with unpublished data and Jurek Krzystek and Jack Peisach (Albert Einstein College of Medicine) for collecting our EPR data. Financial support from the National Institutes of Health (Grant GM45881) is gratefully acknowledged.

Supporting Information Available: The EPR spectrum of **2** at 77 K (Figure S-1), text giving a summary of the structure determination, and tables of crystallographic data and details of data collection, atomic positional and thermal parameters, bond distances and angles, and hydrogen atom positional parameters for **2** (8 pages). Ordering information is given on any current masthead page.

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(24) Dark green blocks of bis(6-amino-2,3-dimethyl-4-azahex-3-ene-2-thiolato)iron(III) chloride, $[Fe^{III}(ADIT)_2]Cl{\cdot}MeCN$ (**2**) were obtained by using 3-methyl-3-mercapto-2-butanone in place of α-mercaptoacetone in the procedure outlined for **1** in footnote 20, followed by recrystallization from acetonitrile.

(25) Crystallographic data (Mo Kα (λ = 0.710 69 Å) radiation, Enraf-Nonius CAD4 diffractometer, 183 K) for **2**·MeCN: $C_{16}H_{33}N_5S_2ClFe$, monoclinic, space group $P2_1/c$, a = 11.200(3) Å, b = 10.164(2) Å, c = 19.954(4) Å, β = 98.22(3)°, V = 2248.1(11) $Å^3$, Z = 4, 2885 observed reflections ($F > 4.0\sigma(F)$), R = 0.0330, R_w = 0.0474. The structure was solved by direct methods (SHELXTL PLUS, pc version).

(26) Most reported Fe−S distances fall in the range 2.26−2.35 Å. See refs 10−14 and: Shoner, S. C.; Barnhart, D.; Kovacs, J. A. Unpublished results.

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