Fe(III) and Co(III) Centers with Carboxamido Nitrogen and Modified Sulfur Coordination: Lessons Learned from Nitrile Hydratase

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ABSTRACT

Nitrile hydratase (NHase) is a non-heme Fe(III) or non-corrinoid Co(III) metalloenzyme with an unprecedented coordination sphere comprising deprotonated carboxamido nitrogens and modified Cys-S ($-SO^-$ and $-SO_2^-$) sulfurs. We have synthesized model complexes derived from designed ligands that contain these donor groups. The model complexes mimic almost all the intrinsic properties of the unique M(III) (M = Fe, Co) active site of NHase. Even a functional Co(III) model has been synthesized that hydrolyzes nitriles catalytically at pH close to the optimum pH of the enzyme. Our studies have provided insight into how the unusual donor atoms dictate the overall properties of the biological M(III) sites.

Introduction

The microbial enzyme nitrile hydratase (NHase) is a unique metalloenzyme that catalyzes the hydrolysis of a wide variety of nitrile substrates into their corresponding amides.[1−3] This enzyme contains at its active site a low-spin non-heme Fe(III) or a non-corrinoid Co(III) metal center depending on the bacterial source. Crystallographic studies on several NHases reveal that the M(III) center is ligated to two deprotonated carboxamido nitrogens from the peptide backbone and three cysteine sulfurs located in a highly conserved −C−S−L−C−S−C− motif.[4−6] In addition, the sixth site is occupied by a water/hydroxide molecule in the active form. Structural analysis on the inactive form of Fe-NHase, namely, $NHase_{dark}$,[6] reveals coordination of a photolabile NO molecule to the Fe(III) center (Figure 1), which is replaced by water/hydroxide upon exposure to visible light.[7,8] Such photoregulation via binding of NO at the metal site is not observed with the Co-NHase. Interestingly, the two Cys-S residues in the basal plane of all NHases are post-translationally modified to sulfenate ($-SO^-$) and sulfinate ($-SO_2^-$) moieties. For Fe-NHase, the post-translational oxidation of the Cys-S residues has been shown to be essential for catalytic activity of the enzyme.[9] Collectively, these observations have generated great interest in NHases and particularly in Fe-NHase, since it is the only known low-spin, mononuclear, non-heme Fe(III) site with such a novel and unprecedented coordination structure.

FIGURE 1. Structure of the NO-bound iron site of NHase from *Rhodococcus* sp. N-771.

Interest in NHase also stems from its potential use as an industrial biocatalyst. Indeed, NHase has found wide industrial application including the kiloton-scale production of acrylamide and nicotinamide[3,10,11] and as an environmental remediation catalyst for treatment of agricultural and industrial wastewater.[12,13] However, from the perspective of bioinorganic chemists, this enzyme possesses many interesting features and properties not observed with any other biological metal site. In fact, NHase could easily be the world record holder in the category of "first examples of never seen in other metalloenzymes". For example, NHase is the first example of a metalloenzyme that (i) contains deprotonated carboxamido nitrogens in combination with Cys-S centers bound to a metal center, (ii) exhibits asymmetric post-translational oxidation at Cys-S residues to sulfenic ($Cys\text{-}SO^-$) and sulfinic ($Cys\text{-}SO_2^-$) moieties shown to be a requirement for catalytic activity, (iii) is photoregulated by reversible binding of nitric oxide (NO) (Fe-NHase only), and (iv) is fully active despite the presence of a low-spin Co(III) (d^6) center, generally thought of as kinetically inert. These unusual characteristics automatically raise the questions as to what properties do the donor atoms in NHase impart on the metal center and what implications do they have on the catalytic transformation of nitriles to amides. These questions clearly fall in the realm of synthetic bioinorganic chemistry where researchers explore and elucidate the intrinsic chemistry of the active sites of metalloenzymes by pursuing studies on smaller model complexes, an approach known as the "synthetic analogue approach".[14] Our research along this line during the past few years has

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Pradip K. Mascharak was born in Jaipur, India, in 1953. He received his Ph.D. from the Indian Institute of Technology, Kanpur, in 1979. In the same year, he joined the research group of Professor Richard Holm at Stanford University and later moved to Harvard University. He later worked with Professor Steven J. Lippard at the Massachusetts Institute of Technology for two years before joining the University of California, Santa Cruz (UCSC), in late 1984. He is currently a Professor of Chemistry and Biochemistry at UCSC. Modeling the active sites of metalloenzymes, design of complexes that exhibit oxygenase activity in the presence of peroxides and dioxygen, and syntheses of photoactive NO-donors are the major focus of his research.

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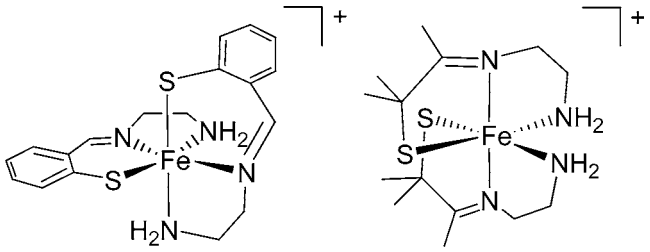


FIGURE 2. Structures of $[Fe(Ssal{:}N{\cdot}[CH_2]_2{\cdot}NH_2)_2]$ (cation of **1**) and $[Fe(ADIT)_2]^+$ (cation of **2**).

provided answers to these questions, and in this Account, we present the results along with results from two other groups working in this area.

When we embarked on our research, no examples of small Fe(III) or Co(III) model complexes with both carboxamido-N and thiolato/sulfe(i)nato-S coordination existed in the literature. This lack of precedence stems from several reasons. First, a metalloenzyme with such a coordination sphere did not exist, and hence, there was no interest in making such complexes. Second, coordination of deprotonated carboxamido-N to M(III) centers (especially Fe(III)) was thought to be improbable due to the extraordinarily high pH required for its deprotonation.[15] Indeed, iron precipitates out of solution as hydroxide at pH ≥ 3.[16] Third, coordination of organic thiolates (RS^-) to Fe(III) centers is often beset with autoredox processes in which oxidation of thiolate to thiyl radical by Fe(III) eventually affords disulfide and Fe(II) species.[17] In this case, isolation of the desired Fe(III) complex is nearly impossible. Finally, no example of S-bound −SO (or $-SO_2$) complex of trivalent iron existed in the literature when we began our work. The results of our attempts to overcome these synthetic challenges in the area of Fe(III) and Co(III) model complexes are described herein in two separate sections.

Discussion Part I: Fe(III) Complexes

Coordinatively Saturated Fe(III) Complexes with Mixed Non-Carboxamido Nitrogen and Sulfur Coordination. A limited number of Fe(III) complexes with amine/imine N and thiolato-S donors were already reported[18] when we initiated our research work in 1998. The first coordination spheres of these complexes resembled the biological Fe(III) site of Fe-NHase to some extent and provided initial insight into the properties imparted to Fe(III) centers by a mixed N/S donor set. Take, for instance, the two compounds $[Fe(Ssal{:}N{\cdot}[CH_2]_2{\cdot}NH_2)_2]Cl$ (**1**, Figure 2) and $[Fe(ADIT)_2]Cl$ (**2**, Figure 2), synthesized via Schiff base condensation reactions among ethylenediamine, the appropriate mercaptoaldehyde or ketone, and $FeCl_3$. Chronologically separated by 20 years, these two Fe(III) compounds comprise the exact same coordination sphere, the only difference being the presence of aryl thiolate in **1** versus alkyl thiolate in **2**. Both complexes exhibit short Fe−S distances (average value 2.20 Å) consistent with their low-spin ground states (like Fe-NHase). In addition, the electronic absorption spectrum of **2** displays a low-energy

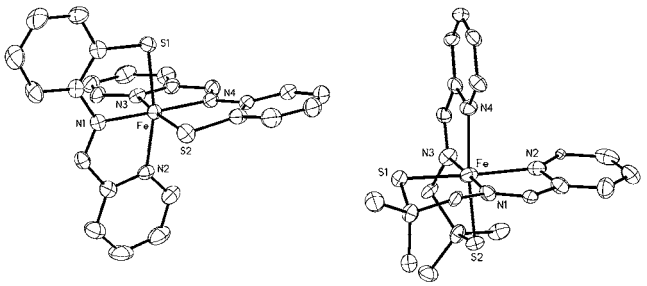


FIGURE 3. Structures of $[Fe(PyAS)_2]^+$ (cation of **3**) and $[Fe(PyMS)_2]^+$ (cation of **4**).

thiolate-to-Fe(III) charge-transfer band near 700 nm very similar to that of Fe-NHase giving **2** its green color.[19]

To determine the effect(s) of the mixed N/S coordination environment on the overall structural, redox, and spectroscopic properties of Fe(III) centers, we designed two sets of tridentate ligands with two ligands in each set. The first set contained ligands with imine N and thiolate S donors. The Fe(III) complexes of the ligands of this set, namely, $[Fe(PyAS)_2]BPh_4$ (**3**, Figure 3) and $[Fe(PyMS)_2]BPh_4$ (**4**, Figure 3) were synthesized via oxidation of the corresponding Fe(II) complexes of the ligands with ferrocenium salts.[20] Simple addition of Fe(III) salts to the deprotonated ligands resulted in formation of intractable solids in both cases presumably due to autoredox processes. Both **3** and **4** comprise low-spin Fe(III) centers and exhibit rhombic EPR spectra and low-energy thiolate-to-Fe(III) charge-transfer bands in the 600 nm range (like Fe-NHase).

Coordinatively Saturated Fe(III) Complexes with Mixed Carboxamido Nitrogen and Sulfur Coordination. The second set of our tridentate ligands contained a carboxamide group in place of the imine group of the first set. This was quite a bold step since before our attempt, no group had synthesized any Fe(III) (or Fe(II)) complex with a mixed donor set that contains both carboxamido-N and thiolato-S donors. The first such Fe(III) complex $Et_4N[Fe(PyPepS)_2]$ (**5**, Figure 4) was synthesized by our group in 1998.[21] It was synthesized by mixing 2 equiv of the deprotonated ligand $PyPepS^{2-}$ with 1 equiv of $Et_4N[FeCl_4]$ in DMF at −40 °C. Unlike similar ligand frames with thiolate group (such as PyASH), the reaction of the deprotonated ligand $PyPepS^{2-}$ with Fe(III) salts does not undergo any autoredox reactions. In fact, no reaction occurs between Fe(II) salts and the deprotonated ligand. The synthesis of **5** demonstrates that addition of carboxamido-N to the ligand frame imparts stability to iron in the +3 oxidation state and shuts off any potential autoredox reaction during the synthesis. Another novel feature of this complex is its remarkable hydrolytic stability. In aqueous solutions, non-heme iron complexes often undergo hydrolytic decomposition leading to formation of rust or other decomposition products.[16] Quite in contrast, **5** can survive for days in degassed water without formation of any visible precipitate. It is therefore evident that Fe(III) centers bound to carboxamido-N are quite resistant to hydrolytic decomposition.

Much like the Schiff base analogue **3** discussed earlier, the Fe(III) center in **5** is coordinated in a distorted octa-

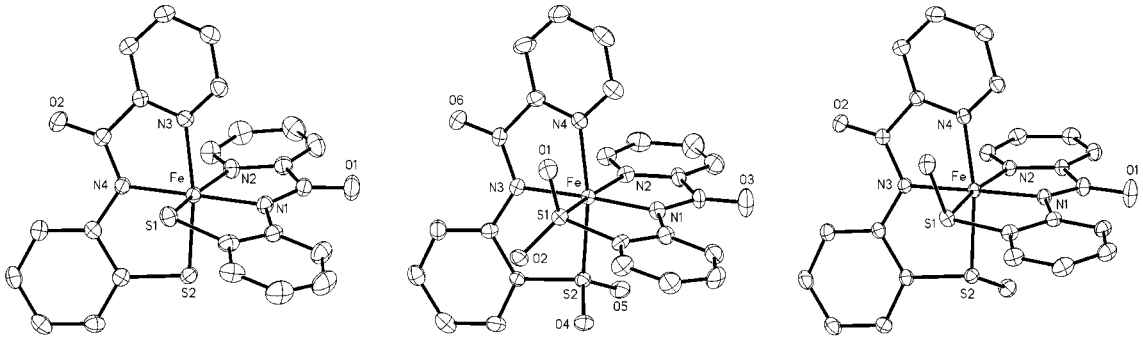


FIGURE 4. Structures of $[Fe(PyPepS)_2]^-$ (anion of **5**), $[Fe(PyPepSO_2)_2]^-$ (anion of **6**), and $[Fe(PyPepSMe)_2]^+$ (cation of **7**).

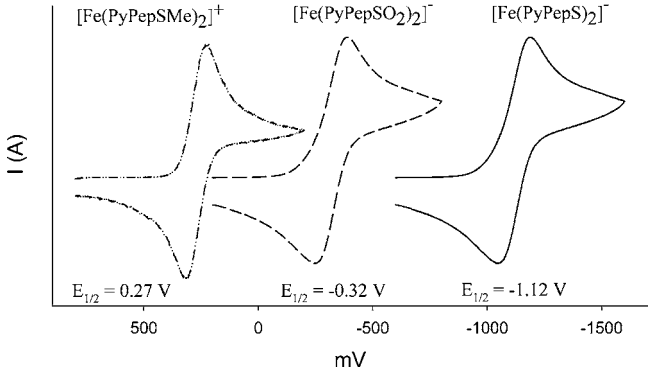


FIGURE 5. Cyclic voltammograms of $Et_4N[Fe(PyPepS)_2]$ (**5**), $Na[Fe(PyPepSO_2)_2]$ (**6**), and $[Fe(PyPepSMe)_2]ClO_4$ (**7**) in DMF (0.1 M $(Et_4N)(ClO_4)$, Pt electrode, 50 mV/s scan rate). Potentials are shown vs aqueous SCE.

hedral geometry with both sulfurs positioned cis to each other (like NHase). In addition, the Fe(III) center is low-spin and exhibits a low-energy thiolate-to-Fe(III) charge-transfer band at 850 nm. Comparison of the redox properties of **3** and **5** reveals another very important effect of ligation of carboxamido-N to Fe(III) center. In DMF, the carboxamido complex **5** affords a half wave potential ($E_{1/2}$) value of −1.12 V (vs SCE, Figure 5) for the Fe(III)/Fe(II) couple, a value that differs by nearly 1 V from the $E_{1/2}$ value of −0.13 V (vs SCE, DMF) of the Schiff base analogue **3**. This result confirms that in six-coordinate Fe(III) complexes, the combination of carboxamido-N and thiolato-S coordination provides remarkable stability to the +3 oxidation state of iron, and this in turn explains why the Fe(III) center of Fe-NHase does not shuttle between different oxidation states but simply acts as a Lewis acid in the hydrolytic process of nitrile-to-amide conversion.

To understand the effects of the post-translational modification of the Cys-S to Cys-SO_x ($x = 1, 2$) groups in complexes such as **5** (and in NHase), we also synthesized the corresponding sulfinato and thioether complexes. As shown in Figure 4, $Na[Fe(PyPepSO_2)_2]$[22] (**6**) contains S-bound sulfinato groups, while $[Fe(PyPepSMe)_2]ClO_4$[23] (**7**) includes thioether S groups ligated to Fe(III) centers. Complex **6** was synthesized by H_2O_2 oxidation of the parent thiolate complex $Et_4N[Fe(PyPepS)_2]$ (**5**) in DMF at −40 °C followed by counterion exchange with $NaClO_4$. This oxidation reaction is surprisingly clean, and no other side product is observed in the reaction mixture. Similar attempts of thiolate oxidation in $[Fe(PyAS)_2]BPh_4$ always result in intractable solids and *not* the sulfinato species. It therefore appears that smooth oxidation of thiolato sulfur to S-bound sulfinate at the Fe(III) center is possible only when the metal is also coordinated to carboxamido nitrogen(s). The metric parameters, low-spin ground state, and hydrolytic stability of the Fe(III) center of **5** do not change upon S-oxidation. Interestingly, the low-energy charge-transfer band of **5** at 850 nm is blue-shifted to 690 nm in the sulfinato complex **6** thereby giving rise to a deep green color much like Fe-NHase. Indeed, the electronic absorption spectrum of **6** is essentially identical to that of Fe-NHase both with the major band at ∼700 nm and a shoulder at ∼400 nm. The $E_{1/2}$ value of **6**, −0.32 V vs SCE (in DMF), is shifted cathodically by 0.80 V from the $E_{1/2}$ value of **5** (Figure 5). In a similar fashion, when the thiolato-S donor of $Et_4N[Fe(PyPepS)_2]$ (**5**) is changed to the corresponding methyl thioether complex in $[Fe(PyPepSMe)_2]ClO_4$ (**7**), the metric parameters, spin state, and electronic absorption spectrum all resemble those of the sulfinato species $Na[Fe(PyPepSO_2)_2]$ (**6**). The only major difference is noted in the $E_{1/2}$ values. In DMF, the $E_{1/2}$ value of **7** is 0.27 V vs SCE (Figure 5). Collectively, these results demonstrate that change of the coordinated thiolato-S to sulfinato or thioether S at the Fe(III) center results in no significant changes in the metric parameters, spin state (all low-spin), and color (all green solutions with S-to-Fe(III) charge-transfer bands in the 700 nm region). However, the reduction potential of the Fe(III) center sequentially shifts to more positive values (Figure 5). One can therefore conclude that while thioether S groups may serve as good structural mimics of the modified S groups in Fe-NHase, they fail to mimic the electronic effect(s) of the thiolato-S or the S-bound sulfinato group.

Taken together, the model complexes $Et_4N[Fe(PyPepS)_2]$ (**5**), $Na[Fe(PyPepSO_2)_2]$ (**6**), and $[Fe(PyPepSMe)_2]ClO_4$ (**7**) have provided valuable insights into the intrinsic properties of the biological non-heme iron site in NHase. These cleverly designed yet simple model complexes (Figure 4) reveal that (a) coordination of carboxamido-N and thiolato/sulfinato/thioether-S donors results in low-spin Fe(III) complexes with low-energy charge-transfer bands in the 690−850 nm range, (b) coordination of carboxamido-N and thiolato/sulfinato-S results in a stabilized Fe(III) center devoid of any redox activity, (c) clean oxi-

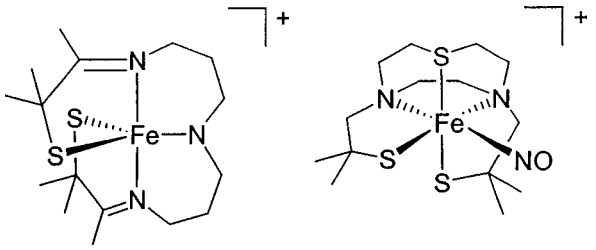


FIGURE 6. Structures of $[Fe(S_2^{Me2}N_3(Pr,Pr)]^+$ (cation of **8**) and $[(bmmp\text{-}TASN)FeNO]^+$ (cation of the NO-adduct of **9**).

dation of Fe−S bonds to Fe−SO_2 can be achieved with retention of the Fe−S bond, and (d) such Fe(III) species do not undergo hydrolytic decomposition.

Coordinatively Unsaturated Fe(III) Complexes with Mixed Non-Carboxamido Nitrogen and Sulfur Coordination. Since insight into the mechanism of NHases could only be obtained from models with at least one labile (solvent-bound) or vacant coordination site, different groups have attempted synthesis of coordinatively unsaturated model complexes with similar coordination structures. Two models, namely, $[Fe(S_2^{Me2}N_3(Pr,Pr)]PF_6$ (**8**)[24] and [(bmmp-TASN)FeCl] (**9**)[25] deserve attention in this regard (Figure 6). The five-coordinate complex **8** comprises one secondary amine N, two imine N, and two alkyl thiolato-S donors wrapped in a distorted trigonal bipyramidal fashion. Surprisingly, unlike its coordinatively satutrated bis analogue $[Fe(ADIT)_2]Cl$ (**2**), $[Fe(S_2^{Me2}N_3(Pr,Pr)]^+$ (**8**) displays $\lambda_{max}(CH_3OH)$ at 416 nm despite the presence of similar donor groups. Complex **8** binds azide to afford $[Fe(S_2^{Me2}N_3$-$(Pr,Pr)(N_3)]$ (azide trans to thiolato-S), a low-spin Fe(III) center with electronic absorption spectrum similar to that of Fe-NHase. It also binds nitric oxide (NO), a photoregulator of Fe-NHase.[26] The Fe(III) center of **9** is ligated to tertiary amine N and thioether S donors (Figure 6), and it also binds NO. Both $[Fe(S_2^{Me2}N_3(Pr,Pr)(NO)]PF_6$ and $[(bmmp\text{-}TASN)FeNO]BPh_4$ exhibit linear Fe−NO bond angles and are diamagnetic with ν_{NO} stretch at 1822 and 1856 cm^{-1}, respectively, typical for {FeNO}^{6} complexes. However, the bound NO in both complexes is lost only upon prolonged exposure to strong UV light with concomitant decomposition of the Fe(III) species. This lack of NO photolability could be due to the absence of key carboxamido-N and/or sulfe(i)nato-S coordination to the metal center. Interestingly, these model complexes show no affinity for nitriles suggesting that nitriles may not bind to the Fe(III) center in the enzyme as well.

Coordinatively Unsaturated Fe(III) Complexes with Mixed Carboxamido Nitrogen and Sulfur Coordination. As part of our modeling work, we have synthesized a five-coordinate model, $(Et_4N)[Fe(PyPS)]$ (**10**, Figure 7),[27] in which the Fe(III) center is coordinated to one pyridine N, two carboxamido-N, and two thiolato-S donors. Two additional model complexes with carboxamido-N donors, namely, $(Et_4N)_2[Fe(L{-}O_2)]$ (**11**)[28] and $(Et_4N)_2[Fe(N_2S_2)Cl]$ (**12**),[29] have also been reported by two other groups (Figure 8). The spectroscopic properties and chemical reactivities of these complexes are quite intriguing and serve as valuable guidelines for future modeling of NHase. For example, complex **11** comprises two carboxamido-N, two thiolato-S, and one sulfinato-O in the first coordination sphere. This coordination sphere mimics the donor atom arrangement observed in the enzyme to a remarkable extent. Despite this fact, its physical parameters ($S = {}^3/_2$, and $\lambda_{max} = 475$ nm) are quite different from those of Fe-NHase, and the Fe(III) center does *not* bind any biologically relevant ligand at the sixth site. Complex **12** also exhibits spectroscopic parameters very different from the enzyme ($S = {}^3/_2$, $\lambda_{max} = 475$ nm). It however, binds NO at the fifth site via replacement of chloride although photolability of NO is not observed in this complex.[30] Clearly, the two model complexes **11** and **12** fail to mimic several characteristics of the iron site in Fe-NHase even after incorporation of biologically relevant ligand frames. This failure strongly suggests that the presence of a thiolato-S trans to the vacant site and sulfe(i)nato moieties are further required for a synthetic model to mimic the spectroscopic properties and the chemical reactivity of the enzyme.

Our model complex $(Et_4N)[Fe(PyPS)]$ (**10**) was designed to meet these requirements. In **10**, the Fe(III) center is bound to the tetraanionic $PyPS^{4-}$ ligand via one pyridine N, two carboxamido-N, and two thiolato-S in a helical fashion (Figure 7) and the overall geometry is distorted trigonal bipyramidal. The most important feature of **10** is its ability to form [Fe(PyPS)L]-type adducts with (a) in-plane coordination of carboxamido-N, (b) two thiolato-S at cis disposition, and (c) a thiolato-S trans to the sixth ligand L (Figure 7). The $E_{1/2}$ value (−0.65 V vs SCE) of **10** in DMF (blue-green solution) is close to the $E_{1/2}$ value of the enzyme (−0.48 V vs SCE) measured in aqueous buffer. The Fe(III) center in **10** reversibly binds ligands (L) such as H_2O, CH_3OH, Py, and CN^- to form six-coordinate [Fe(PyPS)L]-type adducts. Binding of these ligands depends on both the temperature and the nature of the solvent. In every case, the six-coordinate $[Fe(PyPS)L]^{n-}$ species exhibits a low-spin EPR spectrum almost identical to that of the enzyme ($g = 2.28$, 2.14, and 1.97), and the green solution displays one broad and strong absorption band near 700 nm.[27] Collectively, these results attest that **10** is an excellent model of the iron site in Fe-NHase.

Although metal-bound nitriles have been shown to be susceptible to hydrolysis, it has been argued that in NHase, nitriles might not bind the metal site directly and a metal-bound hydroxide could be the active species that catalyzes hydrolysis of nitriles nested at the active site.[4,18,27] Indeed, the Fe(III) center of **10** displays *no affinity for nitriles* even at 173 K but binds H_2O reversibly below 243 K. Also, the pK_a of the bound water in $[Fe(PyPS)(H_2O)]^-$ (6.3 ± 0.4 at 243 K) is very close to the optimum pH of hydrolysis by the enzyme, a fact that supports the "hydrolysis by a metal-bound hydroxide" hypothesis. Because of complications arising from hydrolytic decomposition of $[Fe(PyPS)(H_2O)]^-$ in water at room temperature, we could not employ this species for carrying out hydrolysis of nitriles. We have however tested this hypothesis via the use of the corresponding Co(III) complex $[Co(PyPS)(H_2O)]^-$ (vide infra).

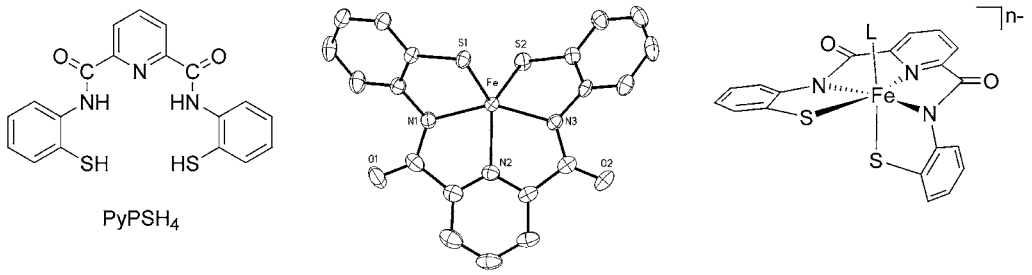


FIGURE 7. The designed ligand $PyPSH_4$, the structure of $[Fe(PyPS)]^-$ (anion of **10**), and the green six-coordinate [Fe(PyPS)L]-type adducts.

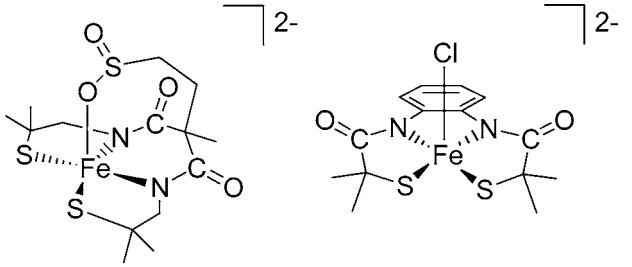


FIGURE 8. Structures of $[Fe(L-O_2)]^{2-}$ (anion of **11**) and $[Fe(N_2S_2)Cl]^{2-}$ (anion of **12**).

Discussion Part II: Co(III) Complexes

Structural Models of Co-NHase: Co(III) Complexes with Mixed Carboxamido Nitrogen and Sulfur Coordination. Although early spectroscopic data suggested that the structure of the Co(III) site in Co-NHase would be very similar to that of the Fe(III) site in Fe-NHase, the structure of Co-NHase, determined only recently, confirmed this similarity.[5] The only exception is the presence of a water molecule at the sixth site of Co(III) instead of NO in case of Fe-NHase (Figure 1). This is indeed what one expects; Co-NHase is not regulated by NO. Since Co-NHases (such as that from *Rhodococcus rhodochrous* J1) have been successfully used in the industrial manufacture of acrylamide and nicotinamide, our aim from the very beginning was to synthesize relevant model Co(III) complexes that serve as functional catalysts for nitrile-to-amide conversion.

When we began our modeling work, there was no example of a Co(III) model complex containing both carboxamido-N and thiolato/sulfinato-S donors in its coordination sphere. The first examples come from research done in our lab, which include $Me_4N[Co(PyPepS)_2]$ (**13**) and $Me_4N[Co(PyRPepS)_2]$ (**14**).[31] Much like the Fe(III) analogues, the Co(III) complex **13** affords a low-spin diamagnetic Co(III) center in a distorted octahedral field with cis thiolato-S donors. This red-brown complex exhibits absorption with λ_{max} at 450 nm similar to the 410 nm band of Co-NHase. In DMF, no reduction of the Co(III) center of **13** is observed up to −1.80 V vs SCE, a fact that demonstrates the stability carboxamido-N imparts on the +3 oxidation state. By comparison, the corresponding Schiff base complex $[Co(PyAS)_2]Cl$ is readily reduced in DMF ($E_{1/2} = -0.40$ V vs SCE).[32] Since the metric and spectroscopic parameters of **14** with alkyl thiolates are very similar to those of **13** with aryl thiolates, our work showed that aryl thiolates could substitute for the Cys-S donors in models of Co-NHase. Overall, the structural and spectral features and the chemical reactivity of the Co(III) model complexes are parallel to those of the Fe(III) analogues. For example, reaction of H_2O_2 with **13** or **14** in CH_3OH affords the corresponding orange S-bound sulfinato species $Na[Co(PyPepSO_2)_2]$ (**15**) and $Na[Co(PyRPepSO_2)_2]$ (**16**), respectively.[31] This suggests that the intrinsic properties of Fe(III) and Co(III) centers ligated to carboxamido-N and thiolato-S donors are very similar and in turn provides an explanation for the occurrence of either Fe(III) or Co(III) at the active site of NHase.

Functional Models of Co-NHase. Coordinatively Unsaturated Co(III) Complexes with Mixed Carboxamido Nitrogen and Sulfur Coordination. To synthesize a functional model of Co-NHase containing a vacant coordination site, we employed the ligand $PyPSH_4$. However, there were some surprises. Unlike the case with Fe(III), reaction of $PyPS^{4-}$ with Co(III) starting material $[Co(NH_3)_5Cl]Cl_2$ resulted in the formation of the dimeric species $(Et_4N)_2[Co_2(PyPS)_2]$ (**17**, Figure 9). Each Co(III) center in this dimeric species exists in an N_3S_3 chromophore, the third sulfur arising from a bridging thiolato moiety from the neighboring $PyPS^{4-}$ ligand.[33] Although the dimeric structure of **17** is quite robust, we were able to cleave the thiolato bridges by the addition of 2 equiv of CN^- in a refluxing CH_3CN medium, a reaction that afforded $(Et_4N)_2[Co(PyPS)(CN)]$ (**18**, Figure 9).[33,34] The reaction was clean, and we finally isolated a monomeric Co(III) species with proper orientation of donor atoms with respect to Co-NHase: two carboxamido nitrogens and one thiolato sulfur in the basal plane, as well as one thiolato sulfur trans to the bound cyanide (sixth ligand). This was the first example of a good model of the Co(III) site in Co-NHase (more specifically, the cyanide-bound Co-NHase). Kovacs and co-workers have reported a second Co(III) model complex, $[Co(S_2^{Me2}N_3(Pr,Pr)]PF_6$ (**19**), with imine N and thiolato-S donors.[35] Like its iron analogue **8** (Figure 6), this Co(III) complex binds azide and thiocyanate and shows no affinity for nitriles. Unfortunately, no further work in terms of nitrile hydrolysis has been reported with **19**.

With the goal of making a functional model of Co-NHase in mind, it was of interest to us to test the utility of $(Et_4N)_2[Co(PyPS)(CN)]$ (**18**) in nitrile hydrolysis. Our main concern was whether the cyanide will leave the kinetically inert low-spin d^6 Co(III) center to open up a position for water (or nitrile) to bind and allow further reaction. To our pleasant surprise, when **18** was dissolved in water, CN^- was immediately lost to generate the

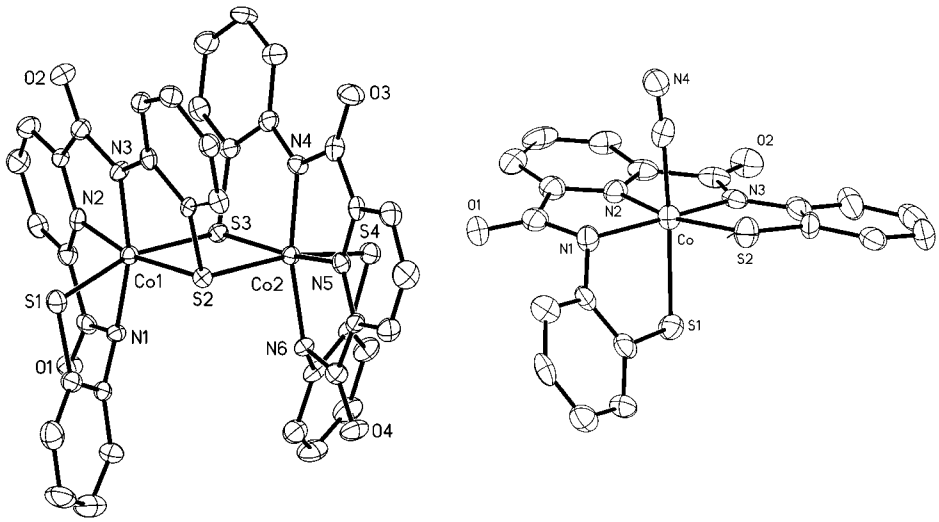
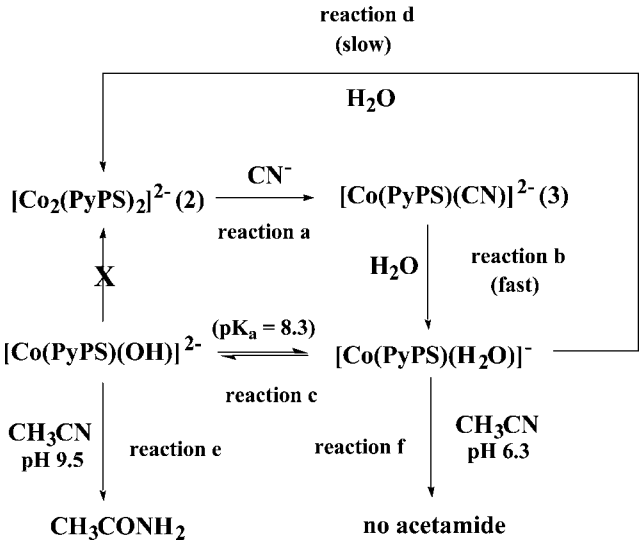


FIGURE 9. Structures of $[Co_2(PyPS)_2]^{2-}$ (anion of **17**) and $[Co(PyPS)(CN)]^{2-}$ (anion of **18**).

Scheme 1



corresponding aqua adduct $Et_4N[Co(PyPS)(H_2O)]$. The lability observed with **18** suggested for the first time that the Co(III) centers in this kind of coordination environment are not inert and allow ligand substitution.[33] The rapid loss of CN^-, a strong ligand, from the Co(III) center in **18** is particularly noteworthy. We believe that this lability arises from the fact that the CN^- in **18** is situated trans to a thiolato-S donor. Since the situation is similar in the enzyme (water is trans to a Cys-S),[5] it appears that nature has created a Co(III) site in Co-NHase that is capable of catalytic transformation. The common notion that low-spin d^6 Co(III) centers are not a suitable choice for enzyme active sites is therefore not true. Kovacs and co-workers have discussed this issue in a recent paper in some detail.[35]

The easy formation of $Et_4N[Co(PyPS)(H_2O)]$ from **18** provided us for the first time, the opportunity of determining the pK_a of the bound water at a Co(III) center in the presence of carboxamido-N and thiolato-S donors. The pK_a of the bound water molecule in $Et_4N[Co(PyPS)(H_2O)]$ was determined to be 8.3 ± 0.03.[33,34] In neutral or acidic solution, $[Co(PyPS)(H_2O)]^-$ eventually reverts to $[Co_2(PyPS)_2]^{2-}$. However, in basic solution (pH $\geq$ 9), $[Co(PyPS)(OH)]^{2-}$ is very stable and no dimerization is observed. In addition, when a solution of $[Co(PyPS)(OH)]^{2-}$ in aqueous buffer of pH 9.5 is warmed (50 °C) with acetonitrile, acetamide is progressively formed in the reaction mixture (monitored by GC, Scheme 1).[33] Only a trace amount of acetamide is formed in the absence of the cobalt catalyst. The fact that this formation of acetamide is catalytic (15 and 18 turnovers after 2 and 4 h, respectively) demonstrates that $[Co(PyPS)(OH)]^{2-}$ is a functional mimic of the Co(III) site in Co-NHase. It is important to note that only at pH values above the pK_a of the bound water in $Et_4N[Co(PyPS)(H_2O)]$ one observes significant nitrile hydrolysis. This strongly suggests that a Co(III)-OH unit is responsible for the nitrile-to-amide conversion.[4] The alternative mechanism in which nitriles coordinate to Co(III) by replacing water and then get hydrolyzed is not supported by our results; the aqua adduct $[Co(PyPS)(H_2O)]^-$ at pH 6.3 does *not* hydrolyze acetonitrile even under more harsh conditions. Also, $[Co^{III}(L)(H_2O)]^+$ complexes with similar ligand frames (all nitrogen donors, pK_a of $H_2O \approx 7$)[36] do not afford any acetamide at pH 9.5. Clearly, the presence of thiolato-S donors are required for promoting nitrile hydrolysis by Co(III) centers.

Very recently, we have also examined the effect(s) of S-oxygenation on the rate of nitrile hydrolysis by these Co(III) complexes.[34] Oxidation of **17** (Figure 9) with H_2O_2 followed by reaction with CN^- affords $(Et_4N)_2[Co(PyPS(SO_2))(CN)]$ (**20**, Figure 10), a complex with one thiolato-S and one sulfinato-S bound to Co(III). This asymmetrically oxidized species exchanges CN^- for water quite *slowly* and gives rise to $Et_4N[Co(PyPS(SO_2))(H_2O)]$ in aqueous solution.

It is interesting to note here that $K_2[Co(PyPSO_2(OSO_2))(CN)]$ (**21**, Figure 10), another oxidized complex derived from **20**, exhibits no lability of CN^-. This loss of lability has been attributed to the greater Lewis acidity of the Co(III) center following sulfur oxidation.[34] These observa-

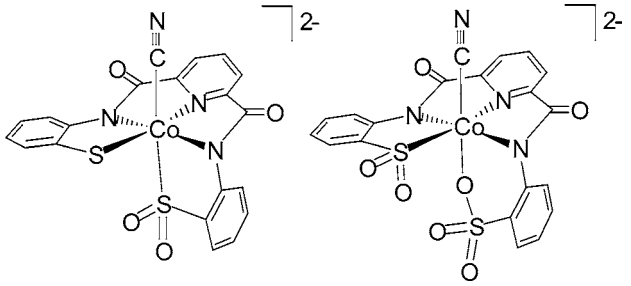


FIGURE 10. Structures of $[Co(PyPS(SO_2))(CN)]^{2-}$ (anion of **20**) and $[Co(PyPSO_2(OSO_2))(CN)]^{2-}$ (anion of **21**).

tions strongly suggest that at least one unmodified S moiety (much like the enzyme) is required for lability in these Co(III) complexes. Also, the lower pK_a value of the bound water in $Et_4N[Co(PyPS(SO_2))(H_2O)]$ (7.2 ± 0.06) compared to $Et_4N[Co(PyPS)(H_2O)]$ (8.3 ± 0.03) indicates that oxidized sulfur moieties increase the acidity of bound water.[34] We believe that one of the roles of the Cys-SO(x) residues in the enzyme is to modulate the pK_a of the bound water such that it is functional at physiological pH via a metal-bound hydroxide. Support for this conclusion comes from our preliminary experiments in the area of nitrile hydrolysis by $[Co(PyPS(SO_2))(OH)]^{2-}$ and $[Co(PyPS)(OH)]^{2-}$. Since the pK_a's of bound water in $[Co(PyPS)(H_2O)]^{-}$ and $[Co(PyPS(SO_2))(H_2O)]^{-}$ are ∼8.3 and ∼7.2, respectively, one expects a faster rate of hydrolysis in the case of $[Co(PyPS(SO_2))(H_2O)]^{-}$ at pH 8. Indeed, $Et_4N[Co(PyPS(SO_2))(H_2O)]$ hydrolyzes acetonitrile *three times faster* than $Et_4N[Co(PyPS)(H_2O)]$ in aqueous buffer of pH 8.[34]

Summary and Conclusion

It is now quite apparent that Fe(III)/Co(III) complexes with mixed carboxamido-N and thiolato/sulfinato-S donor sets can be synthesized and isolated in crystalline forms. In the case of Fe(III) complexes, coordination of these groups results in low-spin Fe centers with the +3 oxidation state stabilized to a great extent. The electronic absorption spectra of these Fe(III) complexes are remarkably similar to the spectrum of Fe-NHase suggesting that the green color of the enzyme arises primarily from a thiolate-to-metal charge-transfer band. These Fe(III) complexes are very stable in aqueous solution and do not undergo hydrolytic decomposition. In regards to Co(III) complexes, coordination of these donor groups affords the first example of a functional model capable of hydrolyzing nitriles into their corresponding amides. The proper disposition of donor groups, especially the trans thiolato-S donor, allows for rapid lability of the sixth site in these Co(III) complexes. The oxidized sulfur residues modulate the pK_a of the bound water molecule in these species. And finally, nitrile hydrolysis is achieved only with Co(III) model complexes with thiolato- and sulfinato-S donors *and* at pH values above the pK_a of bound water. These results suggest that an M(III)−OH unit is responsible for nitrile hydrolysis by these model complexes. Taken together, the interplay between the unprecedented structural features and chemical behavior of the M(III) (M = Fe, Co) centers in NHases has become quite clear following completion of these modeling studies.

References

(1) Kobayashi, M.; Shimizu, S. Nitrile Hydrolases. *Curr. Opin. Chem. Biol.* **2000**, *4*, 95−102.
(2) Endo, I.; Odaka, M.; Yohda, M. An Enzyme Controlled by Light: the Molecular Mechanism of Photoreactivity in Nitrile Hydratase. *Trends Biotechnol.* **1999**, *17*, 244−248.
(3) Nagasawa, T.; Yamada, H. Microbial Transformations of Nitriles. *Trends Biotechnol.* **1989**, *7*, 153−158.
(4) Huang, W.; Jia, J.; Cummings, J.; Nelson, M.; Schneider, G.; Lindqvist, Y. Crystal Structure of Nitrile Hydratase Reveals a Novel Iron Centre in a Novel Fold. *Structure* **1997**, *5*, 691−699.
(5) Miyanaga, A.; Fushinobu, S.; Ito, K.; Wakagi, T. Crystal Structure of Cobalt-Containing Nitrile Hydratase. *Biochem. Biophys. Res. Commun.* **2001**, *288*, 1169−1174.
(6) Nagashima, S.; Nakasako, M.; Dohmae, N.; Tsujimura, M.; Takio, K.; Masafumi, O.; Yohda, M.; Kamiya, N.; Endo, I. Novel Non-Heme Iron Center of Nitrile Hydratase with a Claw Setting of Oxygen Atoms. *Nat. Struct. Biol.* **1998**, *5*, 347−351.
(7) Endo, I.; Nojiri, M.; Tsujimura, M.; Nakasako, M.; Nagashima, S.; Yohda, M.; Odaka, M. Fe-Type Nitrile Hydratase. *J. Inorg. Biochem.* **2001**, *83*, 247−253.
(8) Odaka, M.; Fujii, K.; Hoshino, M.; Noguchi, T.; Tsujimura, M.; Nagashima, S.; Yohda, M.; Nagamune, T.; Inoue, Y.; Endo, I. Activity Regulation of Photoreactive Nitrile Hydratase by Nitric Oxide. *J. Am. Chem. Soc.* **1997**, *119*, 3785−3791.
(9) Murakami, T.; Nojiri, M.; Nakayama, H.; Odaka, M.; Yohda, M.; Dohmae,; Takio, K.; Nagamune, T.; Endo, I. Post-translational Modification is Essential for Catalytic Activity of Nitrile Hydratase. *Protein Sci.* **2000**, *9*, 1024−1030.
(10) Yamada, H.; Kobayashi, M. Nitrile Hydratase and Its Application to Industrial Production of Acrylamide. *Biosci. Biotech. Biochem.* **1996**, *9*, 1391−1400.
(11) Kobayashi, M.; Nagasawa, T.; Yamada, H. Enzymatic Synthesis of Acrylamide: A Success Story Not Yet Over. *Trends Biotechnol.* **1992**, *10*, 402−408.
(12) Kobayashi, M.; Shimizu, S. Metalloenzyme Nitrile Hydratase: Structure, Regulation, and Application to Biotechnology. *Nat. Biotechnol.* **1998**, *16*, 733−736.
(13) Wyatt, J. M.; Knowles, C. J. Microbial Degradation of Acrylonitrile Waste Effluents: The Degradation of Effluents and Condensates from the Manufacture of Acrylonitrile. *Int. Biodeterior. Biodegrad.* **1995**, *35*, 227−248.
(14) Ibers, J. A.; Holm, R. H. Modeling Coordination Sites in Metallobiomolecules. *Science* **1980**, *209*, 223−235.
(15) Marlin, D. S.; Mascharak, P. K. Coordination of Carboxamido Nitrogen to Tervalent Iron: Insight Into a New Chapter of Iron Chemistry. *Chem. Soc. Rev.* **2000**, *29*, 69−74.
(16) Lippard, S. J. Oxo-Bridged Polyiron Centers in Biology and Chemistry. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 344−361.
(17) Ellis, K. J.; Lappin, A. G.; McAuley, A. Metal-Ion Oxidations in Solution. Part XV. Rate-Determining Dimerisations in Redox Reactions of Iron(III) with Some α-Mercaptocarboxylic Acids. *J. Chem. Soc., Dalton Trans.* **1975**, 1930−1934.
(18) For a complete list, see: Mascharak, P. K. Structural and Functional Models of Nitrile Hydratase. *Coord. Chem. Rev.* **2002**, *225*, 201−214.
(19) Shoner, S. C.; Barnhart, D.; Kovacs, J. A. A Model for the Low-Spin, Non-Heme, Thiolate-Ligated Iron Site of Nitrile Hydratase. *Inorg. Chem.* **1995**, *34*, 4517−4518.
(20) Noveron, J. C.; Herradora, R.; Olmstead, M. M.; Mascharak P. K. Low-Spin Iron(III) Complexes with N,S Coordination: Syntheses, Structures, and Properties of bis(*N*-2-mercaptophenyl-2′-pyridylmethyleniminato)iron(III) Tetraphenylborate and bis(*N*-2-mercapto-2-methylpropyl-2′-pyridylmethyleniminato)iron(III) Tetraphenylborate. *Inorg. Chim. Acta* **1999**, *285*, 269−276.
(21) Noveron, J. C.; Olmstead, M. M.; Mascharak P. K. Effect of Carboxamido N Coordination to Iron on the Redox Potential of Low-Spin Non-Heme Iron Centers with N, S Coordination: Relevance to the Iron Site of Nitrile Hydratase. *Inorg. Chem.* **1998**, *37*, 1138−1139.
(22) Tyler, L. A.; Noveron, J. C.; Olmstead, M. M.; Mascharak P. K. Oxidation of Metal-Bound Thiolato Sulfur Centers in Fe(III) and Co(III) Complexes with Carboxamido Nitrogens and Thiolato Sulfurs as Donors: Relevance to the Active Sites of Nitrile Hydratases. *Inorg. Chem.* **1999**, *38*, 616−617.
(23) Harrop, T. C.; Prapasiri, S.; Olmstead, M. M.; Mascharak, P. K. Fe(III) and Co(III) Complexes with Carboxamido N and Thioether

S Donor Centers: Relevance to the Active Site of the Nitrile Hydratases, manuscript in preparation, 2004.
(24) Ellison, J. J.; Nienstedt, A.; Shoner, S. C.; Barnhart, D.; Cowen, J. A.; Kovacs, J. A. Reactivity of Five-Coordinate Models for the Thiolate-Ligated Fe Site of Nitrile Hydratase. *J. Am. Chem. Soc.* **1998**, *120*, 5691−5700.
(25) Grapperhaus, C. A.; Patra, A. K.; Mashuta, M. S. First $\{Fe-NO\}^6$ Complex with an N_2S_3Fe-NO Core as a Model of NO-Inactivated Iron-Containing Nitrile Hydratase. Are Thiolates and Thioethers Equivalent Donors in Low-Spin Iron Complexes? *Inorg. Chem.* **2002**, *41*, 1039−1041.
(26) Schweitzer, D.; Ellison, J. J.; Shoner, S. C.; Lovell, S.; Kovacs, J. A. A Synthetic Model for the NO-Inactivated Form of Nitrile Hydratase. *J. Am. Chem. Soc.* **1998**, *120*, 10996−10997.
(27) Noveron, J. C.; Olmstead, M. M.; Mascharak P. K. A Synthetic Analogue of the Active Site of Fe-Containing Nitrile Hydratase with Carboxamido N and Thiolato S as Donors: Synthesis, Structure, and Reactivities. *J. Am. Chem. Soc.* **2001**, *123*, 3247−3259.
(28) Heinrich, L.; Li, Y.; Vaissermann, J.; Chottard, G.; Chottard, J.-C. A Pentacoordinated Di-*N*-Carboxamido-Dithiolato-*O*-Sulfinato-Iron(III) Complex Related to the Metal Site of Nitrile Hydratase. *Angew. Chem., Int. Ed.* **1999**, *38*, 3526−3528.
(29) Artaud, I.; Chatel, S.; Chauvin, A. S.; Bonnet, D.; Kopf, M. A.; Leduc, P. Nitrile Hydratase and Related Non-Heme Iron Sulfur Complexes. *Coord. Chem. Rev.* **1999**, *190*−*192*, 577−586.
(30) Chatel, S.; Chauvin, A.-S.; Tuchagues, J.-P.; Leduc, P.; Bill, E.; Chottard, J.-C.; Mansuy, D.; Artaud, I. Structural and Spectroscopic Characterization of a Five-Coordinate $\{Fe-NO\}^6$ Complex Derived from an Iron Complex with Carboxamido N and Thiolato S Donors. *Inorg. Chim. Acta* **2002**, *336*, 19−28.
(31) Tyler, L. A.; Noveron, J. C.; Olmstead, M. M.; Mascharak P. K. Co(III) Complexes with Coordinated Carboxamido Nitrogens and Thiolato Sulfurs as Models for Co-Containing Nitrile Hydratase and Their Conversion to the Corresponding Sulfinato Species. *Inorg. Chem.* **2000**, *39*, 357−362.
(32) Tyler, L. A.; Olmstead, M. M.; Mascharak P. K. Conversion of Azomethine Moiety to Carboxamido Group at Cobalt(III) Center in Model Complexes of Co-Containing Nitrile Hydratase. *Inorg. Chem.* **2001**, *40*, 5408−5414.
(33) Noveron, J. C.; Olmstead, M. M.; Mascharak P. K. Co(III) Complexes with Carboxamido N and Thiolato S Donor Centers: Models for the Active Site of Co-Containing Nitrile Hydratases. *J. Am. Chem. Soc.* **1999**, *121*, 3553−3554.
(34) Tyler, L. A.; Noveron, J. C.; Olmstead, M. M.; Mascharak P. K. Modulation of the pK_a of Metal-Bound Water via Oxidation of Thiolato Sulfur in Model Complexes of Co(III) Containing Nitrile Hydratase: Insight into Possible Effect of Cysteine Oxidation in Co-Nitrile Hydratase. *Inorg. Chem.* **2003**, *42*, 5751−5761.
(35) Shearer, J.; Kung, I. Y.; Lovell, S.; Kaminsky, W.; Kovacs, J. A. Why is there an "Inert" Metal Center in the Active Site of Nitrile Hydratase? Reactivity and Ligand Dissociation from a Five-Coordinate Co(III) Nitrile Hydratase Model. *J. Am. Chem. Soc.* **2001**, *123*, 463−468.
(36) Chavez, F. A.; Nguyen, C. V.; Olmstead, M. M.; Mascharak, P. K. Synthesis, Properties, and Structure of a Stable Cobalt(III) Alkyl Peroxide Complex and Its Role in the Oxidation of Cyclohexane. *Inorg. Chem.* **1996**, *35*, 6282−6291.

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