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

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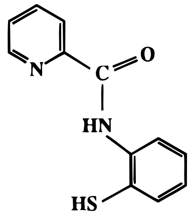
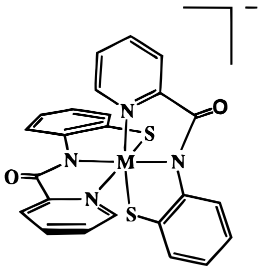
Assimilation of nitriles by several microorganisms is facilitated by the non-heme iron enzyme nitrile hydratase (NHase).[1] The enzyme catalyzes conversion of nitriles to amides and has found use in industrial production of amides and in bioremediation. Recent crystallographic studies on the NHase from *Rhodococcus sp.* R312 have revealed that the single iron site in the $\alpha\beta$ heterodimer is coordinated to two deprotonated carboxamido nitrogens and three Cys-S centers.[2] A more precise structure of the NHase from *Rhodococcus sp.* N-771 shows that at least two of the bound Cys-S centers are post-translationally modified to Cys-sulfenic and -sulfinic groups.[3] The EPR spectrum of the enzyme (g = 2.28, 2.14, 1.97) clearly indicates that the iron is in the +3 oxidation state and exists in a low-spin configuration.[4] Active sites of some NHases contain Co(III) in place of Fe(III).[1,5] The coordination structure around cobalt in these enzymes is believed to be similar to that of the iron site in other NHases.[6] A close scrutiny of the literature reveals that M(III) (M = Fe, Co) complexes with carboxamido nitrogens and thiolato sulfurs as donors are rare and the reactivities of such sites have not been studied at all. Recently, we have reported the synthesis, structure, and properties of the first example of an Fe(III) complex with such coordination, namely, $(Et_4N)[Fe(PyPepS)_2]$ (**1**), where $PyPepSH_2$ = *N*-2-mercaptophenyl-2′-pyridinecarboxamide (H's denote the dissociable peptide and thiol H).[7]



$PyPepSH_2$



$[M^{III}(PyPepS)_2]^-$
M = Fe, Co

We have now isolated the Co(III) complexes $(R_4N)[Co(PyPepS)_2]$ (R = Et, **2a**; R = Me, **2b**) from reaction between $[Co(NH_3)_5Cl]Cl_2$ and $PyPepS^{2-}$ in DMF. Crystallographic study on $(Me_4N)[Co(PyPepS)_2]\cdot 0.5H_2O$ ($\mathbf{2b}\cdot 0.5H_2O$)[8] reveals that the coordination geometry around Co(III) in $[Co(PyPepS)_2]^-$ (structure included in Supporting Information) is distorted octahedral with two doubly deprotonated $PyPepS^{2-}$ ligands ligated in a *mer* fashion. The N_4S_2 chromophore around the Co(III) center consists of two deprotonated carboxamido nitrogens, two pyridine nitrogens, and two thiolato sulfur donors. The two $[M^{III}(PyPepS)_2]^-$ (M = Fe, Co) complexes, schematically shown herein, are structurally quite similar, and together they comprise hitherto unknown examples of M(III) complexes with carboxamido nitrogens and thiolato sulfurs as donors. Side by side, they also provide, for the first time, the opportunity for exploring the possibility of oxidation of the bound thiolato sulfur centers in relation to the post-translational modification of the cysteinate sulfurs at the active site of the *Rhodococcus sp.* N-771 NHase. In this Communication, we report the reactions of the bound thiolato sulfurs of **1** and **2** with H_2O_2. Spectroscopic data on the oxidation products and the crystal structures of $Na[Fe(PyPepSO_2)_2]$ (**3**) and $Na[Co(PyPepSO_2)_2]$ (**4**) clearly show that oxidation of the bound thiolato sulfurs in such coordination gives rise to metal-bound sulfinic groups.

When H_2O_2 is added to a cold (−40 °C) solution of **1** in DMF, the initial red color sharply turns to green. The electronic absorption spectrum of the green species[9] exhibits a band with λ_{max} at 690 nm in solvents such as DMF and water. Overall, the spectrum in water or DMF is very similar to the absorption spectrum of the enzyme (Supporting Information). Interestingly, the strong thiolate-to-Fe(III) charge transfer bands of **1** around 850 and 500 nm are all absent in the spectrum of the green species (Supporting Information), a fact that strongly suggests that the thiolato sulfurs have been modified. Evaporation of the green solution affords a green solid which exhibits a new and strong band at 1184 cm^{-1} in its IR spectrum. This band indicates oxidation of the thiolato sulfurs to sulfinates.[10] The electrospray mass spectrum of the green complex also demonstrates that the oxidized green complex is the sulfinato species $(Et_4N)[Fe(PyPepSO_2)_2]$ (Supporting Information). The oxidation reaction is clean, and no other product is noted in the mass spectrum.

Structural characterization of the sulfinato species has been possible following isolation of crystalline $Na[Fe(PyPepSO_2)_2]$ (**3**). When an equivalent amount of $NaClO_4$ in methanol is added to the green solution of $[Fe(PyPepSO_2)_2]^-$ in 1:1 DMF/ether and

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[9] Once formed, the green species is quite stable both in solution and in the solid state and can be kept at room temperature for a long time without decomposition.

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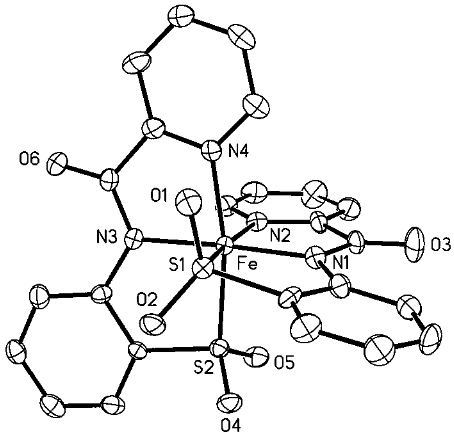

Figure 1. Thermal ellipsoid plot (50% probability level) of the anion of **3**. H atoms are omitted for clarity. Selected bond distances (Å): Fe−N(1),1.894(5); Fe−N(2), 1.995(5); Fe−N(3), 1.968(5); Fe−N(4), 1.969(5); Fe−S(1), 2.1877(17); Fe−S(2), 2.2232(17); S(1)−O(1), 1.485(4); S(2)−O(5), 1.465(4); C(7)−O(3), 1.212(8); C(1)−S(1), 1.793(6); C(13)−S(2), 1.788(6). Selected bond angles (deg): N(1)−Fe−N(2), 84.2(2); N(3)−Fe−N(4), 81.6(2); N(1)−Fe−N(3), 175.4(2); N(2)−Fe−S(1), 169.59(16); N(1)−Fe−S(2), 89.84(16); N(3)−Fe−S(2), 86.15(15); S(1)−Fe−S(2), 94.07(6); O(1)−S(1)−O(2), 111.7(3); O(4)−S(2)−O(5), 112.1(3).

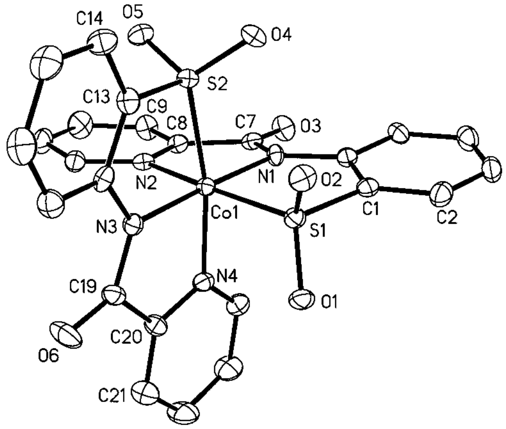

Figure 2. Thermal ellipsoid plot (50% probability level) of the anion of **4**. H atoms are omitted for clarity. Selected bond distances (Å): Co−N(1), 1.924(3); Co−N(2), 1.958(3); Co−N(3), 1.917(3); Co−N(4), 1.971(3); Co−S(1), 2.1646(10); Co−S(2), 2.1666(10); S(1)−O(1), 1.468(2); S(2)−O(5), 1.477(2); C(7)−O(3), 1.240(4); C(1)−S(1), 1.776(3). Selected bond angles (deg): N(1)−Co−N(2), 83.33(11); N(3)−Co−N(4), 83.42(11); N(3)−Co−N(2), 98.27(11); N(1)−Co−S(1), 88.39(8); N(3)−Co−S(2), 87.44(8); S(1)−Co−S(2), 94.51(4); O(1)−S(1)−O(2), 112.44(13); O(4)−S(2)−O(5), 112.06(14).

the resulting mixture is cooled at −20 °C for 48 h, Na[Fe$(PyPepSO_2)_2$]·H_2O·$2CH_3OH$ (**3**·H_2O·$2CH_3OH$) is isolated in 30% yield. The structure of [Fe$(PyPepSO_2)_2$]$^-$ (Figure 1), the anion of **3**, demonstrates that the process of oxidation indeed affords the sulfinato species.[11] The S−O distance (average 1.472(4) Å) compares well with the same observed with known sulfinato complexes.[10] In the crystal lattice, the sodium ions are coordinated to water (average Na−O distance = 2.371(5) Å) as well as the sulfinato oxygens (average Na−O distance = 2.363(5) Å). The sulfinato oxygens are also hydrogen bonded to the methanol molecules of crystallization. The metric parameters of the first coordination sphere around iron, however, do not undergo significant changes upon conversion of [Fe$(PyPepS)_2$]$^-$ to [Fe$(PyPepSO_2)_2$]$^-$.

The oxidized product of **2** has also been characterized in the present study. Addition of H_2O_2 to a cold (−10 °C) solution of Na[Co$(PyPepS)_2$] in methanol brings about a rapid color change of red to orange, and the resulting solution, upon evaporation at room temperature, affords crystalline Na[Co$(PyPepSO_2)_2$]·H_2O·$2CH_3OH$ (**4**·H_2O·$2CH_3OH$) in 52% yield. The structure of [Co$(PyPepSO_2)_2$]$^-$ (Figure 2), the anion of **4**, also demonstrates the formation of the sulfinato species upon oxidation.[12] The S−O distance (average 1.469(2) Å) is comparable to that in **3**.[13] Complex **3** exhibits a strong band at 1193 cm^{-1} in its IR spectrum due to the presence of the sulfinato moieties, and its yellow solution displays absorption bands with maxima at 377 and 307 nm. Although Co(III) complexes with coordinated sulfinates and sulfenates have been synthesized with H_2O_2 in previous works,[13] structural information on such species has been scarce. Complex **4** merits attention in this regard.

In summary, the present work demonstrates that oxidation of Fe(III) and Co(III) complexes with first coordination spheres resembling the active sites of NHases can be oxidized to give sulfinato species. Also, the similarity between the absorption spectrum of **3** to that of the enzyme suggests that the green color of the enzyme could arise from an Fe(III) center with coordinated carboxamido nitrogens and sulfinato sulfurs around it.

Supporting Information Available: Electronic absorption spectra of [Fe$(PyPepS)_2$]$^-$ and [Fe$(PyPepSO_2)_2$]$^-$ (Figure S1) and of [Fe$(PyPepSO_2)_2$]$^-$ in water (Figure S2), electrospray mass spectrum of [Fe$(PyPepSO_2)_2$]$^-$ (Figure S3), structure of (Me_4N)[Co$(PyPepS)_2$]·$0.5H_2O$ (**2b**·$0.5H_2O$) (Figure S4), H-bonding network in **3**·H_2O·$2CH_3OH$ (Figure S5), analytical and spectroscopic data, and tables of crystal data and intensity collection and refinement parameters, positional coordinates, bond distances and angles, H atom coordinates, and anisotropic and isotropic thermal parameters for **3**·H_2O·$2CH_3OH$ and **4**·H_2O·$2CH_3OH$. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) X-ray analysis: black plate of Na[Fe$(PyPepSO_2)_2$]·H_2O·$2CH_3OH$ (**3**·H_2O·$2CH_3OH$) from methanol, $FeNaC_{26}H_{26}N_4O_9S_2$, monoclinic space group $P2_1/c$, $a = 14.947(3)$ Å, $b = 11.3777(15)$ Å, $c = 16.569(2)$ Å, $\alpha = 90°$, $\beta = 97.554\ (10)°$, $\gamma = 90°$, $V = 2793.3(8)$ Å^3, $Z = 4$, $d_{calc} = 1.620$ g/cm^3, R1 = 7.94%, wR2 = 19.79%. The structure was solved by direct methods (SHELXS-97).

(12) X-ray analysis: orange parallelepiped of Na[Co$(PyPepSO_2)_2$]·$H_2O.2CH_3OH$ (**4**·H_2O·$2CH_3OH$) from methanol, $CoNaC_{26}H_{26}N_4O_9S_2$, monoclinic space group $P2_1/c$, $a = 15.050(3)$ Å, $b = 11.333(2)$ Å, $c = 16.478(3)$ Å, $\alpha = 90°$, $\beta = 97.06(2)°$, $\gamma = 90°$, $V = 2789.2(9)$ Å ^{3}, $Z = 4$, $d_{calc} = 1.630$ g/cm^3, R1 = 4.05%, wR2 = 8.44%. The structure was solved by direct methods (SHELXS-97).

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