1138 *Inorg. Chem.* **1998,** *37,* 1138−1139

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

Juan C. Noveron,[†] Marilyn M. Olmstead,[‡] and Pradip K. Mascharak*,[†]

Department of Chemistry and Biochemistry, University of California, Santa Cruz, California 95064, and Department of Chemistry, University of California, Davis, California 95616

Received November 5, 1997

The enzyme nitrile hydratase (NHase) which catalyzes conversion of nitriles to amides in several microorganisms contains a non-heme low-spin Fe(III) center at the active site.[1−3] Unlike most other non-heme iron centers in biological systems,[4] the iron center of NHase does not change its oxidation state during turnover; rather it acts as a Lewis acid in the nitrile-to-amide transformation.[1] The iron site of the NHase is quite resistant to reduction[3] and reduction by strong reductants invariably leads to destruction of the enzyme. Earlier spectroscopic studies indicated that the Fe(III) center of NHase is bonded to two to three Cys-S and two to three N,O donors from the side chain residues and a water molecule.[5,6] Recent crystallographic studies on the NHase from *Rhodococcus* sp. R312, however, reveal that the active site iron is coordinated to three Cys-S, two *deprotonated carboxamido nitrogens*, and most possibly a water molecule.[7]

Binding of deprotonated carboxamido N to the Fe(III) center is quite unusual. Indeed, the NHase structure is just the second example of such coordination in biology.[8] The crystallographic results and the general propensity of thiolato groups to stabilize Fe(II) centers led us to hypothesize that coordination by the carboxamido nitrogens is primarily responsible for the unusual stability of the Fe(III) center in NHase. Over the past few years, we[9] and others[10] have synthesized a handful of iron complexes in which deprotonated carboxamido nitrogens are present as donors. However, no iron complex(es) with both thiolato S and carboxamido N donors has been reported so far. In this communication, we report the *first* example of an Fe(III) complex with thiolato S and carboxamido N donors, namely $(Et_4N)[Fe(PyPepS)_2]$ (**1**) where $PyPepSH_2$ = *N*-2-mercaptophenyl-2′-pyridinecarboxamide (Hs denote the dissociable peptide and thiol H). Comparison of the redox potential of this complex with that of $[Fe(PyAS)_2](BPh_4)$ (**2**, PyASH = *N*-2-mercaptophenyl-2′-pyridylmethylenimine, H is the dissociable thiol H)[11] demonstrates that coordinated carboxamido nitrogens indeed provide unusual stability to the Fe(III) center even when thiolato S donors are present in the first coordination sphere.

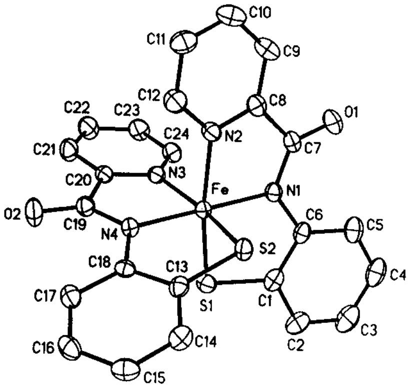
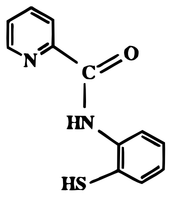
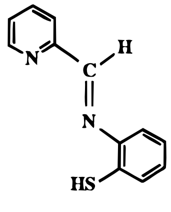


Figure 1. Thermal ellipsoid plot (50% probability level) of the anion of **1**. H atoms are omitted for clarity. Selected bond distances (in Å): Fe−N(1), 1.954 (2); Fe−N(4), 1.954 (2); Fe−N(2), 1.997 (2); Fe−N(3), 2.003 (3); Fe−S(1), 2.2284 (10); Fe−S(2), 2.2297 (11); C(7)−O(1), 1.251 (4); C(7)−N(1), 1.341 (4); C(1)−S(1), 1.761 (3); C(7)−C(8), 1.497 (4). Selected bond angles (in deg): N(1)−Fe−N(2), 81.16 (11); N(1)−Fe−S(1), 86.53 (8); S(2)−Fe−N(4), 86.54 (7); N(3)−Fe−N(4), 81.40 (10); S(1)−Fe−N(2), 165.78 (8), N(1)−Fe−N(4), 178.84 (10); N(4)−Fe−N(2), 98.11 (10); N(1)−Fe−N(3), 99.40 (10).



$PyPepSH_2$



PyASH

The ligand $PyPepSH_2$ was synthesized by coupling 2-pyridinecarbonyl chloride with 2,2′-dithiobis(2-aminobenzene) in chloroform in the presence of triethylamine followed by reductive

---

[†] UC−Santa Cruz.

[‡] UC−Davis.

(1) (a) Kobayashi, M.; Nagasawa, T.; Yamada, H. *Trends Biotechnol.* **1992**, *10*, 402. (b) Nagasawa, T.; Yamada, H. *Trends Biotechnol.* **1989**, *7*, 153.

(2) Some NHases contain a non-corrinoid Co(III) center in place of iron. The coordination structure of the Co(III) center is believed to be similar to that of the iron site in other NHases. See: Brennan, B. A.; Alms, G.; Nelson, M. J.; Durney, L. T.; Scarrow, R. C. *J. Am. Chem. Soc.* **1996**, *118*, 9194.

(3) Sugiura, Y.; Kuwahara, J.; Nagasawa, T.; Yamada, H. *J. Am. Chem. Soc.* **1987**, *109*, 5848.

(4) (a) Que, L. Jr.; Ho, R. Y. N. *Chem. Rev.* **1996**, *96*, 2607. (b) Feig, A. L.; Lippard, S. J. *Chem. Rev.* **1994**, *94*, 759.

(5) (a) Scarrow, R. C.; Brennan, B. A.; Cummings, J. G.; Jin, H.; Duong, D. J.; Kindt, J. T.; Nelson, M. J. *Biochemistry* **1996**, *35*, 10078. (b) Brennan, B. A.; Cummings, J. G.; Chase, D. B.; Turner, I. M.; Nelson, M. J. *Biochemistry* **1996**, *35*, 10068. (c) Jin, H.; Turner, I. M., Jr.; Nelson, M. J.; Gurbiel, R. J.; Doan, P. E.; Hoffman, B. M. *J. Am. Chem. Soc.* **1993**, *115*, 5290. (d) Nelson, M. J.; Jin, H.; Turner, I. M., Jr.; Grove, G.; Scarrow, R. C.; Brennan, B. A.; Que, L., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 7072.

(6) Recent pulsed ENDOR results, however, indicate that the ligand derived from water is probably hydroxide. See Doan, P. E.; Nelson, M. J.; Jin, H.; Hoffman, B. M. *J. Am. Chem. Soc.* **1996**, *118*, 7014.

(7) Huang, W.; Jia, J.; Cummings, J.; Nelson, M.; Schneider, G.; Lindqvist, Y. *Structure* **1997**, 5, 691.

(8) Deprotonated carboxamido N-bound iron centers have been discovered in nitrogenase P clusters. See: Peters, J. W.; Stowell, M. H. B.; Soltis, S. M.; Finnegan, M. G.; Johnson, M. K.; Rees, D. C. *Biochemistry* **1997**, *36*, 1181.

(9) (a) Brown, S. J.; Olmstead, M. M.; Mascharak, P. K. *Inorg. Chem.* **1990**, *29*, 3229. (b) Tao, X.; Stephan, D. W.; Mascharak, P. K. *Inorg. Chem.* **1987**, *26*, 754.

(10) (a) Ray, M.; Ghosh, D.; Shirin, Z.; Mukherjee, R. *Inorg. Chem.* **1997**, *36*, 3568. (b) Wocadlo, S.; Massa, W.; Folgado, J.-V. *Inorg. Chim. Acta* **1993**, *207*, 199. (c) Ray, M.; Mukherjee, R.; Richardson, J. F.; Buchanan, R. M. *J. Chem. Soc., Dalton Trans.* **1993**, 2451. (d) Che, C.-M.; Leung, W.-H.; Li, C.-K.; Cheng, H.-Y.; Peng, S.-M. *Inorg. Chim. Acta* **1992**, *196*, 43. (e) Yang, Y.; Diederich, F.; Valentine, J. S. *J. Am. Chem. Soc.* **1991**, *113*, 7195.

(11) Noveron, J. C.; Herradora, R.; Olmstead, M. M.; Mascharak, P. K. *Inorg. Chem.* Submitted.

S0020-1669(97)01388-8 CCC: $15.00

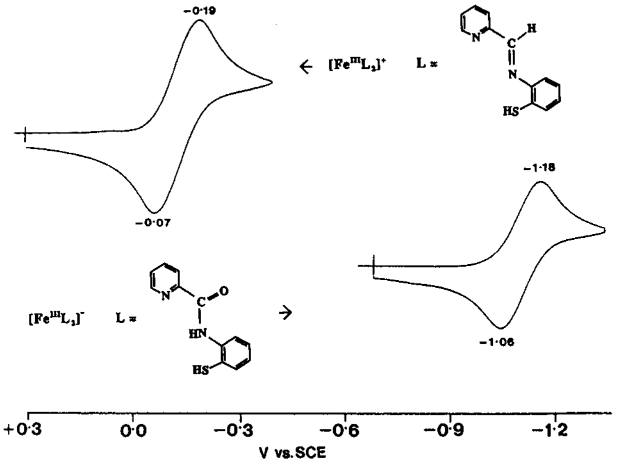


Figure 2. Cyclic voltammograms of $(Et_4N)[Fe(PyPepS)_2]$ (**1**) and $[Fe(PyAS)_2](BPh_4)$ (**2**) in DMF (0.1 M $(Et_4N)ClO_4$, Pt electrode, 50 mV scan rate). Potentials are shown vs aqueous saturated calomel electrode.

cleavage of the product with $NaBH_4$ in THF.[12] The Fe(III) complex $(Et_4N)[Fe(PyPepS)_2]$ (**1**) was synthesized as follows. A solution of 230 mg (1 mmol) of $PyPepSH_2$ and 48 mg (2 mmol) of NaH in 15 mL of dry DMF was cooled to 0 °C, and to it was slowly added with stirring a solution of 163 mg (0.5 mmol) of $(Et_4N)[FeCl_4]$ in 3 mL of DMF. The deep red reaction mixture was stirred for 30 min, and then 15 mL of anhydrous diethyl ether was added to it. Dark crystals of **1** were separated from this mixture upon cooling at -20 °C for 24 h.

The fact that no reduction of iron takes place upon mixing of $PyPepS^{2-}$ with Fe(III) salts is noteworthy. Under similar reaction conditions, addition of thiolate ligands such as $PyAS^-$ to Fe(III) salts causes immediate reduction of iron and only the Fe(II) complex is obtained from the reaction mixture.[11] Quite in line with this observation, $PyPepSH_2$ does not react with Fe(II) sources and all attempts to isolate the Fe(II) complex of this ligand have failed so far. It is therefore possible that ligands such as $PyPepSH_2$, which employ deprotonated carboxamido nitrogens along with thiolato sulfurs to bind iron, do not promote reduction of Fe(III) centers.

The structure[13] of the anion of **1** is shown in Figure 1. The coordination geometry around iron is distorted octahedral with two deprotonated carboxamido nitrogens, one pyridine N, and a thiolato S constituting the basal plane while the other pyridine N and the second thiolato S occupy the axial positions. The two doubly deprotonated $PyPepS^{2-}$ ligands are coordinated in a *mer* fashion. Both ligand frames are essentially planar due to extensive delocalization. Complex **1** exhibits an $Fe(III)-N_{amid}$ (amid = carboxamido) distance of 1.954 (2) Å which is very similar to the $Fe(III)-N_{amid}$ distances observed with other Fe(III) complexes with coordinated carboxamido nitrogens.[9,10] The $Fe(III)-N_{py}$ (py = pyridine) and $Fe(III)-S_{thio}$ (thio = thiolate) distances of **1** (average values of 2.000 (2) and 2.229 (2) Å, respectively) also compare well with analogous complexes of tervalent iron.[9b,14]

Coordination of deprotonated carboxamido N to the Fe(III) center in **1** is readily indicated by the ν_{CO} frequency in its IR spectrum. Upon complexation, ν_{CO} shifts to 1612 cm^{-1} from 1688 cm^{-1} in free $PyPepH_2$. Similar red shift of ν_{CO} has been noted with other Fe(III) complexes with coordinated carboxamido nitrogens.[9,10] In MeCN, **1** exhibits an absorption band at 850 nm of moderate intensity ($\epsilon = 1900$ M^{-1} cm^{-1}) which is tentatively assigned to a thiolate-to-metal charge-transfer transition. In addition, Complex **1** shows several strong overlapping absorption bands in the region 500−350 nm which are responsible for its red color. The low-spin configuration of the Fe(III) center in **1** is confirmed by its effective magnetic moment of $1.98\mu_B$ (polycrystalline) at 298 K as well as its rhombic EPR spectrum ($g = 2.22, 2.14, 1.98$) at 100 K in DMF glass (Supporting Information). The EPR parameters of **1** are very similar to those of other low-spin Fe(III) complexes with N,S coordination[15] including $[Fe(PyAS)_2](BPh_4)$ (**2**).[11] As expected, the *g* values of these complexes are all very close to the *g* values of the enzyme ($g = 2.28, 2.14, 1.97$).[3]

The metric and spectroscopic parameters of the two low-spin Fe(III) complexes $(Et_4N)[Fe(PyPepS)_2]$ (**1**) and $[Fe(PyAS)_2](BPh_4)$ (**2**) are very comparable except for the fact that **1** contains coordinated carboxamido nitrogens in place of imine nitrogens in **2**.[16] We were curious to find out the effect of this difference in the redox behavior of these two complexes. The results are shown in Figure 2. In DMF, the half-wave potentials ($E_{1/2}$) of $(Et_4N)[Fe(PyPepS)_2]$ (**1**) and $[Fe(PyAS)_2](BPh_4)$ (**2**) are -1.12 and -0.13 V (vs SCE, saturated calomel electrode), respectively. This dramatic shift of 1 V in $E_{1/2}$ clearly indicates that coordination of negatively charged strong-field carboxamido nitrogens provide significant stability to the +3 oxidation state of iron in these low-spin non-heme iron complexes. That the combination of deprotonated carboxamido N and thiolato S donor set is especially desirable for the stability of the Fe(III) center is further indicated by the $E_{1/2}$ value (-0.31 V vs SCE) of the low-spin Fe(III) complex $[Fe(Pypep)_2]Cl$ (Pypep = *N*-(2-(4-imidazolyl)ethyl)-pyridine-2-carboxamido)[9b] which contains imidazole N as donor in place of thiolato S in its coordination sphere. In two recent accounts, Sellmann and co-workers have also reported stabilization of iron in +3 and higher oxidation states by combinations of alkylamido N and thiolato S donor centers.[17]

In conclusion, the results of this study demonstrate that simultaneous presence of deprotonated carboxamido nitrogens and thiolato sulfurs in the first coordination sphere provide extra stability to Fe(III) centers in non-heme iron complexes. It is possible that the resistance to reduction of the Fe(III) site in NHase also arises from coordination of such donors (as shown by the crystal structure[7]) to the metal center.

Supporting Information Available: EPR spectrum of **1** in DMF glass (100 K) (Figure S1), structure of **2** (Figure S2), 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 **1** (11 pages). Ordering information is given on any current masthead page.

IC971388A

---

(12) NMR data ($CDCl_3$, 298 K, ppm from TMS): ^1H NMR δ 3.28 (s, 1H), 7.06 (t, 1H), 7.36 (t, 1H), 7.50 (m, 2H), 7.91 (t, 1H), 8.31 (d, 1H), 8.52 (d, 1H), 8.68 (d, 1H), 10.84 (s, 1H); ^{13}C NMR δ 118.0, 121.0, 122.4, 124.3, 126.5, 128.9, 134.6, 137.5, 138.4, 148.2, 149.8, 162.1. IR data: ν_{CO} 1688 cm^{-1}.

(13) X-ray analysis, dark blocks of **1** from DMF/diethyl ether, $FeC_{32}H_{36}N_5O_2S_2$, monoclinic space group $P2_1/n$, $a = 12.539$ (3) Å, $b = 18.353$ (6) Å, $c = 13.893$ (4) Å, $\alpha = 90°$, $\beta = 108.84$ (2)°, $\gamma = 90°$, $V = 3026$ (2) $Å^3$, $Z = 4$, $d_{calc} = 1.411$ g/cm^3, $R1 = 5.05\%$, $wR2 = 12.29\%$. The structure was solved by direct methods (SHELXTL 5.03).

(14) (a) Govindaswamy, N.; Quarless, D. A., Jr.; Koch, S. A. *J. Am. Chem. Soc.* **1995**, *117*, 8468. (b) Beissel, T.; Burger, K. S.; Voigt, G.; Wieghardt, K.; Butzlaff, C.; Trautwein, A. X. *Inorg. Chem.* **1993**, *32*, 124. (c) Fallon, G. D.; Gatehouse, B. M. *J. Chem. Soc., Dalton Trans.* **1975**, 1344.

(15) Shoner, S. C.; Barnhart, D.; Kovacs, J. A. *Inorg. Chem.* **1995**, *34*, 4517.

(16) The structure of $[Fe(PyAS)_2](BPh_4)$ (**2**) and selected bond distances are included in the Supporting Information.

(17) (a) Sellmann, D.; Emig, S.; Heinemann, F. W.; Knoch, F. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1201. (b) Sellmann, D.; Emig, S.; Heinemann, F. W. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1734.