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 nitrite-to-amide transformation.1 The iron site of the NHase is quite resistant to reduction5 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 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 nitrogen donors and 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 nitrogen is primarily responsible for the unusual stability of the Fe(III) center in NHase. Over the past few years, we9 and others10 have synthesized a handful of iron, for which deprotonated carboxamido nitrogen donors are present. However, no iron complex(ies) 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 [Et4N][Fe-PyPepS2]2 (I) where PyPepSH2 = 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][BPh4] (2. PyASH = N-2-mercaptophenyl-2′-pyridylmethylenamine, H is the dissociable thiol H)11 demonstrates that coordinated carboxamido nitrogen indeed provide unusual stability to the Fe(III) center even when thiolato S donors are present in the first coordination sphere.

The ligand PyPepSH2 was synthesized by coupling 2-pyridinecarboxyl chloride with 2,2′-dithiobis(2-aminobenzene) in chloroform in the presence of triethylamine followed by reduction of [Fe(PyAS)2][BPh4] (2. PyASH = N-2-mercaptophenyl-2′-pyridylmethylenamine, H is the dissociable thiol H)11 demonstrates that coordinated carboxamido nitrogen indeed provide unusual stability to the Fe(III) center even when thiolato S donors are present in the first coordination sphere.

1 UC—Santa Cruz.
2 UC—Davis.
4 Some NHases contain a non-corrinoid Co(III) center in place of iron.2

5 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.

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.


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


cleavage of the product with NaBH₄ in THF. The Fe(III) complex (Et₄N)[Fe(PyPepS)₂] (1) was synthesized as follows. A solution of 230 mg (1 mmol) of PyPepH₂ 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 (Et₄N)[FeCl₃] 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 reaction mixture upon cooling at −20 °C for 24 h.

The fact that no reduction of iron takes place upon mixing of PyPepS²⁻ 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. Quite in line with this observation, PyPepSH₂ does not react with Fe(II) sources failed so far. It is therefore possible that ligands such as (PyAS)²⁻ which contain imidazole N as donor in place of thiolato S in its coordination sphere. In two recent studies, Sellmann and co-workers have also reported stabilization of other low-spin Fe(III) complexes with N,S coordination including [Fe(PyAS)₂](BPh₄) (2). 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).

![Figure 2](image.png)

Figure 2. Cyclic voltammograms of (Et₄N)[Fe(PyPepS)₂] (1) and [Fe-(PyAS)₂](BPh₄) (2) in DMF (0.1 M Et₄NClO₄, Pt electrode, 50 mV scan rate). Potentials are shown vs aqueous saturated calomel electrode.

Coordination of deprotonated carboxamido N to the Fe(III) center in 1 is readily indicated by the νCO frequency in its IR spectrum. Upon complexation, νCO shifts to 1612 cm⁻¹ from 1688 cm⁻¹ in free PyPepH₂. Similar red shift of νCO has been noted with other Fe(III) complexes with coordinated carboxamido nitrogens. In MeCN, 1 exhibits an absorption band at 850 nm of moderate intensity (ε = 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 1.98μB (polycrystalline) at 298 K as well as its rhombohedral 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 including [Fe(PyAS)₂](BPh₄) (2). 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).

The metric and spectroscopic parameters of the two low-spin Fe(III) complexes (Et₄N)[Fe(PyPepS)₂] (1) and [Fe(PyAS)₂](BPh₄) (2) are very comparable except for the fact that 1 contains coordinated carboxamido nitrogens in place of imine nitrogens in 2. 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 (E½) of (Et₄N)[Fe(PyPepS)₂] (1) and [Fe(PyAS)₂](BPh₄) (2) are −1.12 and −0.13 V (vs SCE, saturated calomel electrode), respectively. This dramatic shift of 1 in E½ clearly indicates that coordination of negatively charged strong-field carboxamido nitrogens provides significant stabilization 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 E½ 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) 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.

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) 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 I (11 pages). Ordering information is given on any current masthead page.

IC971388A

(11) NMR data (CDCl₃, 298 K, ppm from TMS): ³¹H 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); ¹³C 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: νCO 1688 cm⁻¹.

(12) X-ray analysis, dark blocks of 1 from DMF/diethyl ether, Fe₂Cu₃Na₃O₃S₄, monoclinic space group P2₁/a, a = 12.539 (3) Å, b = 18.353 (6) Å, c = 13.893 (4) Å, α = 90°, β = 108.84 (2)°, γ = 90°, V = 3026 (2) Å³, Z = 4, d纽带 = 1.411 g/cm³, R₁ = 5.05%, wR₂ = 12.29%. The structure was solved by direct methods (SHELXTL 5.03).

(13) 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 I (11 pages). Ordering information is given on any current masthead page.

IC971388A


