

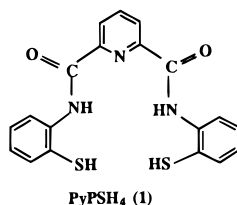
## Co(III) Complexes with Carboxamido N and Thiolato S Donor Centers: Models for the Active Site of Co-Containing Nitrile Hydratases

Juan C. Noveron, Marilyn M. Olmstead, and Pradip K. Mascharak\*

Department of Chemistry and Biochemistry  
University of California  
Santa Cruz, California 96064  
Department of Chemistry, University of California  
Davis, California 95616

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Several microorganisms employ the non-heme iron enzyme nitrile hydratase (NHase) to convert nitriles to amides in the process of assimilation of nitriles as the carbon source.<sup>1</sup> Recent crystallographic studies on two *Rhodococcus* NHases have revealed that the single low-spin Fe(III) site in  $\alpha\beta$  heterodimer is coordinated to two deprotonated carboxamido nitrogens and three Cys-S centers<sup>2</sup> with at least two of them modified to Cys-sulfenic and -sulfenic groups.<sup>3</sup> Some NHases contain a non-corrinoid Co(III) center in place of iron.<sup>1,4</sup> The coordination structure of the Co(III) center is believed to be similar to that of the iron site in other NHases<sup>5</sup> although unlike the iron site, the Co(III) center could be reduced to Co(II) by dithionite and is inhibited by  $\text{CN}^-$  ion.<sup>4</sup> Since Co(III) centers are often kinetically inert, it is not clear how the Co- and Fe-containing NHases hydrate nitriles at comparable speeds. To address the latter issue, we have synthesized the designed pentacoordinate ligand PyPSH<sub>4</sub> (**1**), Hs denote dissociable carboxamide and thiolate protons) and report the reactivities of the mononuclear complex  $(\text{Et}_4\text{N})_2[\text{Co}(\text{PyPS})(\text{CN})]$  (**3**) which in turn has been synthesized from the parent Co(III) complex  $(\text{Et}_4\text{N})_2[\text{Co}_2(\text{PyPS})_2]$  (**2**). The reactivity of complex **3** indicates that substitution reactions are fast at Co(III) centers with carboxamido nitrogens and thiolato sulfurs serving as ligands. The  $\text{p}K_a$  of a water molecule bound to such a Co(III) center has also been determined.  $[\text{Co}(\text{PyPS})(\text{OH})]^{2-}$ , a species derived from **3** in basic aqueous solution, catalyzes hydrolysis of acetonitrile under mild conditions.



The ligand PyPSH<sub>4</sub> (**1**) was synthesized by coupling tritylated 2-aminothiophenol with 2,6-pyridinedicarbonyl dichloride in chloroform in the presence of  $\text{Et}_3\text{N}$  followed by deprotection with  $\text{Et}_3\text{SiH}$  in trifluoroacetic acid.<sup>6</sup> The dimeric Co(III) complex of this pentadentate ligand namely,  $(\text{Et}_4\text{N})_2[\text{Co}_2(\text{PyPS})_2]$  (**2**) was

(1) (a) Yamada, H.; Kobayashi, M. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 1391. (b) Kobayashi, M.; Nagasawa, T.; Yamada, H. *TIBTECH* **1992**, *10*, 402. (c) Nagasawa, T.; Yamada, H. *Trends Biotechnol.* **1989**, *7*, 153.

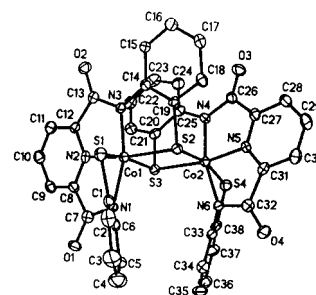
(2) Huang, W.; Jia, J.; Cummings, J.; Nelson, M.; Schneider, G.; Lindqvist, Y. *Structure* **1997**, *5*, 691.

(3) Nagashima, S.; Nakasako, M.; Dohmae, N.; Tsujimura, M.; Takio, K.; Odaka, M.; Yohda, M.; Kamiya, N.; Endo, I. *Nat. Struct. Biol.* **1998**, *5*, 347.

(4) Nagasawa, T.; Takeuchi, K.; Yamada, H. *Eur. J. Biochem.* **1991**, *196*, 581.

(5) Brennan, B. A.; Alms, G.; Nelson, M. J.; Durney, L. T.; Scarrow, R. C. *J. Am. Chem. Soc.* **1996**, *118*, 9194.

(6) <sup>1</sup>H NMR data ( $\text{CDCl}_3$ , 298 K, ppm from TMS)  $\delta$  3.39 (s, 2H, SH groups), 7.12 (t, 2H), 7.36 (t, 2H), 7.55 (d, 2H), 8.18 (t, 1H), 8.37 (d, 2H), 8.53 (d, 2H), 10.49 (s, 2H, carboxamide groups).



**Figure 1.** Thermal ellipsoid plot (50% probability level) of the anion of **2**. H atoms are omitted for clarity. Selected bond distances (in Å): Co1–S1, 2.2770 (16); Co1–S2, 2.2210 (16); Co1–N1, 1.945 (4); Co1–N2, 1.853 (4); Co1–N3, 1.925 (4); Co1–S3, 2.3156 (15); Co2–S2, 2.2922 (15); Co2–S3, 2.2203 (16); Co2–N4, 1.919 (4); Co2–N5, 1.837 (4); Co2–N6, 1.918 (4); Co2–S4, 2.2865 (15). Selected bond angles (in deg): Co1–S2–Co2, 95.97 (6); Co1–S3–Co2, 95.33 (6); N1–Co1–N2, 82.36 (17); N2–Co1–N3, 82.47 (17); S1–Co1–N1, 83.70 (13); S1–Co1–S3, 165.02 (5); S3–Co1–S2, 84.07 (6); N3–Co1–S1, 93.34 (13); N4–Co2–N5, 83.13 (17); N5–Co2–N6, 83.03 (17); S4–Co(2)–N6, 82.56 (13).

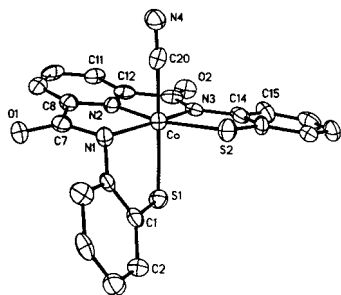
obtained as follows. To a cold (0 °C) mixture of 310 mg (0.8 mmol) of **1** and 80 mg (3.3 mmol) of NaH was added a batch of 200 mg (0.8 mmol) of  $[\text{Co}(\text{NH}_3)_5\text{Cl}]\text{Cl}_2$ , and the mixture was heated to 70 °C for 1 h when a deep brown homogeneous solution resulted. Following addition of 250 mg of  $\text{Et}_4\text{NCl}$ , the solvent was removed in vacuo. The residue was then redissolved in 80 mL of degassed acetonitrile and filtered. The filtrate was concentrated to 40 mL, and 25 mL of diethyl ether was added to it. Storage at –20 °C for 12 h afforded dark crystals of **2** in 60% yield. The mononuclear complex  $(\text{Et}_4\text{N})_2[\text{Co}(\text{PyPS})(\text{CN})]$  (**3**) was synthesized from **2** in the following way. A mixture of 200 mg (0.18 mmol) of **2** and 70 mg (0.44 mmol) of  $(\text{Et}_4\text{N})(\text{CN})$  in 20 mL of acetonitrile was heated to reflux for 15 min, and the reddish brown solution was then concentrated to 7 mL. Crystalline **3** was obtained in 57% yield upon storing this solution at –20 °C for 48 h.

The structure<sup>7</sup> of the dimeric anion of **2** is shown in Figure 1. Each Co(III) center is bonded to one pyridine and two deprotonated carboxamido nitrogens and two thiolato sulfurs of the deprotonated PyPS<sup>4-</sup> ligand. The sixth site on each cobalt is occupied by one thiolato sulfur from the other  $[\text{CoPyPS}]$  moiety. The PyPS<sup>4-</sup> ligand frame is wrapped around both metal centers in the same way, and the two phenyl rings of the two bridging thiolates are almost parallel to each other. The metric parameters of the  $[\text{CoPyPS}]$  moiety are comparable to other known peptide and thiolato complexes of Co(III).<sup>8</sup> In **3**, the PyPS<sup>4-</sup> ligand also employs one pyridine nitrogen, two carboxamido nitrogens, and two thiolato sulfurs to bind Co(III) (Figure 2).<sup>9</sup> One cyanide ion occupies the sixth site, and the geometry around cobalt is distorted octahedral. The Co–C and C–N distances of the Co–CN unit (1.896 (12) and 1.149 (13) Å respectively) compares well with those in other Co(III) complexes with ligated cyanide ions.<sup>10</sup>

(7) X-ray analysis, dark red prisms of  $2 \cdot \text{CH}_3\text{CN} \cdot 0.3\text{H}_2\text{O}$  from acetonitrile,  $\text{C}_{36}\text{H}_{65.60}\text{Co}_2\text{N}_9\text{O}_4\text{S}_4$ , triclinic space group P1,  $a = 12.309$  (2) Å,  $b = 15.395$  (4) Å,  $c = 16.132$  (4) Å,  $\alpha = 105.21$  (2)°,  $\beta = 102.43$  (2)°,  $\gamma = 102.51$  (2)°,  $V = 2757.1$  (11) Å<sup>3</sup>,  $Z = 2$ ,  $d_{\text{calc}} = 1.421$  gm/cm<sup>3</sup>,  $R1 = 5.89\%$ ,  $wR2 = 11.05\%$ . The structure was solved by direct methods (SHELXS-97).

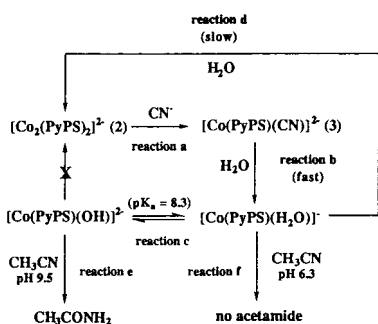
(8) (a) Ray, M.; Ghosh, D.; Shirin, Z.; Mukherjee, R. *Inorg. Chem.* **1997**, *36*, 3568 and references therein. (b) Freeman, H. C.; Moore, C. J.; Jackson, W. G.; Sargeant, A. M. *Inorg. Chem.* **1978**, *17*, 3513. (c) Helis, H. M.; de Meester, P.; Hodgson, D. J. *J. Am. Chem. Soc.* **1977**, *99*, 3309.

(9) X-ray analysis, black parallelepiped of  $3 \cdot \text{CH}_3\text{CN} \cdot \text{H}_2\text{O}$  from acetonitrile,  $\text{C}_{38}\text{H}_{56}\text{CoN}_7\text{O}_3\text{S}_2$ , monoclinic space group P2<sub>1</sub>,  $a = 9.224$  (4) Å,  $b = 22.605$  (8) Å,  $c = 9.976$  (3) Å,  $\alpha = 90^\circ$ ,  $\beta = 106.00$  (3)°,  $\gamma = 90^\circ$ ,  $V = 1999.5$  (13) Å<sup>3</sup>,  $Z = 2$ ,  $d_{\text{calc}} = 1.299$  gm/cm<sup>3</sup>,  $R1 = 6.77\%$ ,  $wR2 = 13.57\%$ . The structure was solved by direct methods (SHELXS-97).



**Figure 2.** Thermal ellipsoid plot (50% probability level) of the anion of **3**. H atoms are omitted for clarity. Selected bond distances (in Å): Co–N1, 1.937 (8); Co–N2, 1.858 (7); Co–N3, 1.930 (8); Co–S1, 2.325 (3); Co–S2, 2.249 (3); Co–C20, 1.896 (12); C20–N4, 1.149 (13); C7–O1, 1.251 (12). Selected bond angles (in deg): N1–Co–S1, 81.9 (3); S1–Co–C20, 168.8 (3); N1–Co–N2, 81.8 (3); N2–Co–N3, 82.7 (4); N3–Co–S2, 88.4 (3); S1–Co–S2, 88.07 (11); S2–Co–C20, 86.4 (3); N1–Co–C20, 90.5 (4); N3–Co–C20, 95.6 (4).

### Scheme 1



Complex **3** is the first example of a Co(III) center bonded to carboxamido nitrogens, thiolato sulfurs, and cyanide and resembles the proposed cyanide-bound Co(III) site of Co–NHases in terms of coordination structure.

Complex **2** dissolves in DMF, methanol, and H<sub>2</sub>O, and the dark reddish brown solutions exhibit strong thiolate-to-Co(III) charge-transfer bands around 425 nm.<sup>11</sup> Although the bridged structure is retained in donor solvents such as pyridine even at elevated temperatures, strong ligands such as CN<sup>−</sup> cause scission of the thiolate bridges (reaction a, Scheme 1). Conversion of **2** to **3** takes approximately 30 min at 60 °C, and the process can be followed by electronic absorption and NMR spectroscopy (Supporting Information). Complex **3** displays  $\nu_{\text{CN}}$  at 2111 cm<sup>−1</sup> and is stable when dissolved in aprotic solvents such as DMSO, DMF, and acetonitrile.<sup>11</sup> However, when **3** is dissolved in H<sub>2</sub>O (pH 7), CN<sup>−</sup> is immediately lost<sup>12</sup> and [Co(PyPS)(H<sub>2</sub>O)]<sup>−</sup> is formed (reaction b, Scheme 1). The facile substitution reaction b indicates that the enzyme-like coordination structure of the Co(III) center of **3** is not substitutionally inert.<sup>13</sup>

Formation of [Co(PyPS)(H<sub>2</sub>O)]<sup>−</sup> in aqueous solution of **3** has provided us for the first time the opportunity of determining the p*K*<sub>a</sub> of bound water at Co(III) center with carboxamido nitrogens and thiolato sulfurs as donors. We have measured the p*K*<sub>a</sub> of the

bound water by absorption spectroscopy. As the pH of the solution of [Co(PyPS)(H<sub>2</sub>O)]<sup>−</sup> in aqueous buffer is raised, the peak at ~420 nm is split into two (at ~390 and 450 nm) presumably due to formation of [Co(PyPS)(OH)]<sup>2−</sup> (reaction c, Scheme 1). The plot of the absorption at 400 nm vs pH (Supporting Information) affords a p*K*<sub>a</sub> value of 8.3 for the bound water in [Co(PyPS)(H<sub>2</sub>O)]<sup>−</sup>. Previous work from this laboratory has demonstrated that the bound water in Co(III) complexes of the type [Co(L)(H<sub>2</sub>O)]<sup>+</sup> (L = very similar pentadentate peptide ligands with all nitrogen donors) has p*K*<sub>a</sub> values close to 7.<sup>14</sup> The present result now indicates that thiolato sulfur donors around Co(III) (as in [Co(PyPS)(H<sub>2</sub>O)]<sup>−</sup>) raise the p*K*<sub>a</sub> of bound water.

In neutral or acidic aqueous solution, [Co(PyPS)(H<sub>2</sub>O)]<sup>−</sup> is slowly converted into **2** (reaction d, Scheme 1). The conversion can be prevented when the pH is raised above 9. This shows that [Co(PyPS)(OH)]<sup>2−</sup> is more stable and does not revert back to **2**. It is important to note that reaction d can be prevented at pH 7 by excess CN<sup>−</sup>. Finally, the availability of [Co(PyPS)(OH)]<sup>2−</sup> prompted us to check whether it reacts with RCN to afford RCONH<sub>2</sub>. Our initial results show that when [Co(PyPS)(OH)]<sup>2−</sup> (generated by dissolving **3** in aqueous solution of pH 9.5) is warmed (50 °C) with acetonitrile, acetamide is progressively formed in the reaction mixture (reaction e, Scheme 1). The rate of formation of acetamide, as followed by NMR spectroscopy (Supporting Information) and HPLC, is faster than the rate of hydrolysis of nitrile to amide by polyamine complexes of Co(III) with bound hydroxide.<sup>15</sup> For example, in a reaction mixture containing 0.032 mmol of **3** in 5 mL of Tris buffer (10 mM, pH 9.5) and 1.5 mL of acetonitrile and kept at 50 °C, 0.48 mmol (15 turnovers) and 0.56 mmol (18 turnovers) of acetamide was produced after 2 and 4 h, respectively. No acetamide was formed in the absence of **3**. It is also important note that [Co(L)(H<sub>2</sub>O)]<sup>+</sup> complexes with all nitrogen donors<sup>14</sup> do not afford any acetamide at pH 9.5. Thus, the presence of thiolato sulfurs around Co(III) appears crucial in promoting hydrolysis of nitrile by such centers.

Similarities in structural features and noticeable reactivity toward hydration of nitrile qualify [Co(PyPS)(OH)]<sup>2−</sup> as a good synthetic analogue of the cobalt site in Co–NHases. Also, reaction e suggests that the enzyme-mediated hydration of nitriles could proceed via intermolecular attack of cobalt-bound hydroxide on nitriles nested in the active site pocket. The alternative mechanism in which nitriles first coordinate to Co(III) by replacing water and then get hydrolyzed is not supported by the observation that at pH 6.3 [Co(PyPS)(H<sub>2</sub>O)]<sup>−</sup> does not initiate any hydrolysis of acetonitrile even after heating at 60 °C for 48 h (reaction f). Attempts to isolate and structurally characterize [Co(PyPS)(OH)]<sup>2−</sup> and determine the rate of hydrolysis of different RCNs by [Co(PyPS)(OH)]<sup>2−</sup> are in progress at this time.

**Supporting Information Available:** Changes in the electronic absorption and <sup>1</sup>H NMR spectra during conversion of **2** to **3** (Figures S1 and S2), <sup>1</sup>H NMR spectrum of [Co(PyPS)(py)]<sup>−</sup> in pyridine-*d*<sub>5</sub> (Figure S3), absorption spectrum of [Co(PyPS)(H<sub>2</sub>O)]<sup>−</sup> at different pH and plot of pH vs absorbance at 400 nm (Figure S4), NMR spectra showing formation of acetamide in reaction between [Co(PyPS)(OH)]<sup>2−</sup> and acetonitrile in aqueous solution of pH 12 (Figures S5a and S5b), NMR spectrum of [Co(PyPS)(OH)]<sup>2−</sup> in D<sub>2</sub>O (pD 12) (Figure S6), and crystallographic data for **2** and **3** (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(10) (a) Romero, R. G.; Morales, A. D.; Rodriguez, J. D.; Bertran, J. F. *Transition Met. Chem.* **1992**, *17*, 573. (b) Fronczek, F. R.; Marsh, R. E.; Schaefer, W. P. *J. Am. Chem. Soc.* **1982**, *104*, 3382.

(11) Complex **2** in DMF,  $\lambda_{\text{max}}$  nm ( $\epsilon$ , M<sup>−1</sup> cm<sup>−1</sup> per Co<sub>2</sub>) 540 sh, 430 (25500), 325 (39000). Complex **3** in DMF,  $\lambda_{\text{max}}$  nm ( $\epsilon$ , M<sup>−1</sup> cm<sup>−1</sup>): 600 sh, 470 (4100), 413 (4700).

(12) The electrospray mass spectrum of a freshly prepared (>10 s) solution of **3** in water showed mostly peaks due to [Co(PyPS)(H<sub>2</sub>O)]<sup>−</sup> and [Co<sub>2</sub>(PyPS)<sub>2</sub>]<sup>2−</sup>.

(13) Similarly, addition of **3** to pyridine immediately affords [Co(PyPS)(py)]<sup>−</sup> (Supporting Information).

(14) (a) Chavez, F. A.; Olmstead, M. M.; Mascharak, P. K. *Inorg. Chem.* **1997**, *36*, 6323. (b) Chavez, F. A.; Nguyen, C. V.; Olmstead, M. M.; Mascharak, P. K. *Inorg. Chem.* **1996**, *35*, 6282.

(15) (a) Kim, J. H.; Britten, J.; Chin, J. *J. Am. Chem. Soc.* **1993**, *115*, 3618. (b) Chin, J.; Kim, J. H. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 523.