A Co(III) Complex in a Mixed Sulfur/Nitrogen Ligand Environment: Modeling the Substrate- and Product-Bound Forms of the Metalloenzyme Thio cyanate Hydrolase

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Thiocyanate hydrolase (TCH) is a pink bacterial metalloenzyme found in Thiobacillus thioparus THI 115 that catalyzes the conversion of thiocyanate to carbonyl sulfide and ammonia.1,2 Although TCH has not been well characterized spectroscopically, an analysis of the genes encoding for the enzyme has shown a high active-site sequence homology to the metalloenzyme nitrile hydratase (N thermus), including the residues that bind the metal center.2 NHases catalyze the hydration of nitriles to amides and fall into a rare class of cysteine-ligated non-corrinoid Co(III) or non-heme Fe(III) enzymes.3 NHase has been characterized by a shorter than the average bond length of 2.24 Å found in most Fe-alkyl complexes incorporating cis-thiolates and imine nitrogens.11,12 Furthermore, we were able to model the spin-state and spectroscopic properties of iron-containing NHases can be modeled by imine nitrogens.11,12

or water. Given the large amount of sequence homology, it is likely that the metal center of TCH is ligated in a similar fashion. Previously, our group showed that the spin-state and spectroscopic properties of iron-containing NHases can be modeled by six-coordinate iron complexes incorporating cis-thiolates and imine nitrogens.11,12

Six-coordinate [Co(III)S₂(Me₂N)(Pr,Pr)(SCN)] (I) was synthesized by adding tetrakis-(n-butyl)ammonium thiocyanate to five-coordinate [Co(III)S₁N₁(Pr,Pr)][PF₆] (II) in acetonitrile. This solution was then layered with diethyl ether to afford dark pink crystals of I (Scheme 1). Complex I is diamagnetic (S = 0) and displays an intense absorption band in the UV at 282 nm (ε = 17,000 M⁻¹ cm⁻¹) and another in the visible region at 501 nm (ε = 1000 M⁻¹ cm⁻¹). X-ray quality crystals of I were grown from acetonitrile and diethyl ether at −40 °C. The crystal structure revealed I is in a distorted octahedral geometry with thiocyanate bound linearly to cobalt through its nitrogen (Figure 1). The C−N and C=S bond lengths in the bound SCN⁻ are 1.15(6) and 1.63(5) Å, respectively, which is identical to the bond lengths found in free SCN⁻. The S−Co bond trans to SCN⁻ increases in length from 2.16(2) Å in 2 to 2.22(1) Å in I; however, it is still shorter than the average bond length of 2.24 Å found in most Co(III) thiocyanates.17

Figure 1. ORTEP plot of thiocyanate-bound [Co(III)S₂(N)(Pr,Pr)(SCN)] (I) showing 50% probability ellipsoids. All H atoms, except for the N(2)−H, have been omitted for clarity. Selected bond lengths (Å): Co−N(1), 1.956(4); Co−N(2), 2.070(4); Co−N(3), 1.962(4); Co−N(4), 1.997(4); Co−S(1), 2.218(1); Co−S(2), 2.231(1).

Scheme 1

+ 0

2

X=S(N) (1), NH₂ (3)

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do not change with temperature in the absence of thiocyanate.

Thiocyanate binding to 2 was found to be solvent-dependent. In methane chloride, SCN⁻ binds quantitatively and irreversibly to 2. However, when binding is carried out in solvents that can better support ion separation (acetoniitride and methanol), binding is only detected at low temperature or high SCN⁻ concentrations. This is demonstrated through variable temperature (VT) UV/vis and VT NMR studies. In the VT UV/vis studies, as the temperature is lowered, the band at 284 nm corresponding to 1 starts to grow in while the bands at 358, 445, and 525 nm (corresponding to 2) all diminish, revealing a band at 501 nm (Figure 2). These changes in the electronic spectrum are reversible, indicating that a temperature-dependent equilibrium exists between the bound and unbound states. Further evidence for reversible binding is provided through VT NMR. When the 1H NMR spectrum for 1 is recorded at ambient temperature in MeOH-d₄, a paramagnetic spectrum identical to 2 (S = 1) is observed. If the spectrum is recorded at temperatures below −65 °C, a diamagnetic spectrum is observed that corresponds to 1. Raising the temperature above −65 °C produces a spectrum identical to that of 2, indicating reversible binding, as was demonstrated with the VT UV/vis study. In contrast, the UV/vis and NMR spectrum of 2 do not change with temperature in the absence of thiocyanate. We are currently in the process of investigating the thermodynamics and kinetics of SCN⁻ binding to 2.

Variable temperature UV/vis studies also indicate that complex 2 reversibly binds ammonia in methylene chloride, producing [Co(Pr)S₂Me₂N(S(Pr,Pr))(PF₆)](CH₂Cl₂) (3) (Scheme 1). When 2 is placed under an atmosphere of ammonia, a shoulder grows in at 282 nm. As the temperature is lowered, this shoulder becomes a peak with an e of 11 200 M⁻¹ cm⁻¹. Similar to the spectral changes induced by SCN⁻, ammonia causes the peaks at 358, 445, and 525 nm to disappear, revealing a band at 489 (e = 800 M⁻¹ cm⁻¹) (Figure 2). Just as was demonstrated with 1, ammonia binding is reversible and temperature-dependent. If the ammonia atmosphere used to generate 3 is slowly mixed with nitrogen, a gas bubbles from solution, and the spectrum reverts back to that of 2, and only 2 is isolated. This is observed at temperatures as low as −30 °C. Due to the lability of the ammonia ligand, all attempts to isolate 3 have failed thus far.

We have attempted to hydrolyze SCN⁻ with 2 in aqueous solutions of varying pH. Under all conditions explored, no hydrolysis products were detected. Instead complete decomposition of 2 is observed after 20 min. This result is consistent with attempted nitrile hydrolyses involving either 2 or its iron analogue and is likely caused by hydrolysis of the imine C–N double bond contained in the ligand.

Although we do not see catalytic activity with these models, two important conclusions can be reached from this work. Low-spin d⁶ Co(III) is not often utilized in a metalloenzyme that involves substrate binding to the metal center. This is presumably a consequence of the difficulty in displacing the product from a substitutionally inert metal center. However, we have demonstrated that there is an equilibrium between 1 and 2 (and 3 and 2), indicating that low-spin d⁶ Co(III) is not so inert that ligand displacement is precluded when it is in this ligand environment. Previous studies by Deutsch and Carrano have demonstrated that thioulate has a strong trans labilizing effect on low-spin d⁶ Co(III). In the enzyme, utilization of a cysteinate trans to the open site could facilitate product release. Furthermore, we have shown that Co(III) reversibly binds both the substrate and product of TCH, suggesting a possible mechanism (Scheme 2).

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Supporting Information Available: A detailed preparation, variable temperature NMR spectrum (Figure S-1), and crystallographic data for 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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