A Synthetic Model for the NO-Inactivated Form of Nitrile Hydratase

Dirk Schweitzer, Jeffrey J. Ellison, Steven C. Shoner, Scott Lovell, and Julie A. Kovacs*  
Department of Chemistry  
University of Washington  
Seattle, Washington 98195  
Received April 3, 1998

Nitric oxide (NO)1 has been shown to perform a number of important functions in medicine and biology, including blood pressure control, neurotransmission, and inhibition of tumor growth.2,3 More recently, NO was found to play a regulatory role in the metalloenzymatic nitrile hydratase (NHase)4 by reversibly binding to its iron active site.6 Nitrile hydratases3,7-12 are non-heme, low-spin Fe(III)-containing microbial enzymes which promote the hydration of nitriles to amides. Three facial cysteinates, two cis peptide nitrogens,11 and a hydroxide13 ligate the iron in its active form (NH₄). An inactive, NO-bound form (NH₄(NO)) is generated in whole cell bacterial samples stored in the dark.14 Activity is restored upon exposure to light.1-6 The presence of an IR band at 1853 cm⁻¹ suggests that NO coordinates to the iron site.14 Spin-trapping4 and quantitative NO titration experiments6 show that one NO is released per iron during photoactivation. Initial studies5,14 suggested that NH₄ contained NO species. The EPR silence15 of inactivated NH₄ was, however, inconsistent with this proposal. A recent X-ray structure16 of NH₄ from Rhodococcus sp. N-771 showed that two of the ligated cysteinates had been oxidized to sulfenate and sulfinate groups. However, it was not mentioned whether the crystals used in this study (a) could be photolytically reactivated or (b) displayed the ν(NO) characteristic of NH₄. Sulfinate groups are not detected by resonance Raman, in NH₄ from Rhodococcus sp. R312.17 Herein, we report a synthetic, NO-bound, bis(thiolate)-ligated iron complex which closely matches the properties of the NH₄ iron site, despite the absence of sulfinate groups.

(3) Traylor, T. G.; Sharma, V. S. Biochemistry 1992, 31, 2847-2849.  
(15) Mark Nelson, personal communication. It is possible that S=O vibrations would go undetected if the ~700-nm excitation band does not involve the modified cysteine.

This represents the first structure of an Fe(III)−NO compound containing thiolate ligands. Five-coordinate [Fe(III)S₂(NO)₃(C₆H₄Ph₂)⁺ (I)18] is coordinatively unsaturated with an open S(1)−Fe−N(2) angle (132.3(3)°) suggesting that the metal ion should be accessible to small molecules. Azide binds to this site to afford [Fe(III)S₂(NO)₃(C₆H₄Ph₂)(N₃)] (2),18 which displays an S = 1/2 EPR signal (g = 2.23, 2.16, 1.99) nearly identical to that of azide-inhibited NH₄ (g = 2.23, 2.14, 1.99).19 This suggests that the iron site of our model closely resembles that of the enzyme. Addition of NO₈⁺ causes the paramagnetic NMR signal of I (Figure 1) to collapse to a diamagnetic signal, indicating that the two S = 1/2 radicals18 couple. The product of this reaction,20 [Fe(III)S₂(NO)₃(C₆H₄Ph₂)(N₃)] (3), displays an ν(NO) IR stretch at 1822 cm⁻¹ close to that of NH₄ (1853 cm⁻¹) in the range (1857−1937 cm⁻¹) expected for an [FeNO]complex,21,22 but well outside the range of Fe(II)−NO species. Sulfinate groups. However, it was not mentioned whether the crystals used in this study (a) could be photolytically reactivated or (b) displayed the ν(NO) characteristic of NH₄. Sulfinate groups are not detected by resonance Raman, in NH₄ from Rhodococcus sp. R312.17 Herein, we report a synthetic, NO-bound, bis(thiolate)-ligated iron complex which closely matches the properties of the NH₄ iron site, despite the absence of sulfinate groups.

Figure 1. (a) ¹H NMR spectrum (200 MHz) of [Fe(III)S₂(NO)₃(C₆H₄Ph₂)]₊ (1) vs (b) [Fe(III)S₂(NO)₃(C₆H₄Ph₂)(NO)]− (3) in CD₂CN solution at 297 K.


10.1021/ja981117e CCC: $15.00 © 1998 American Chemical Society Published on Web 10/08/1998
Table 3 listing... Sigzag and...<ref>

(38) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(39) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(40) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(41) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(42) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(43) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(44) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(45) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(46) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(47) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(48) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(49) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(50) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(51) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(52) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(53) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(54) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(55) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(56) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(57) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(58) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(59) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(60) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(61) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(62) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(63) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(64) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(65) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(66) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(67) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(68) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(69) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(70) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(71) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(72) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(73) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(74) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(75) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(76) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(77) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(78) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(79) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(80) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(81) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(82) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(83) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(84) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(85) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(86) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(87) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(88) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(89) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(90) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(91) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(92) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(93) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(94) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(95) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(96) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(97) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(98) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(99) Photolysis reactions were monitored by 1 H NMR and UV/vis.

(100) Photolysis reactions were monitored by 1 H NMR and UV/vis.