

Oxygen Binding to Sulfur in Nitrosylated Iron–Thiolate Complexes: Relevance to the Fe-Containing Nitrile Hydratases

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Nitrile hydratase (NHase) is a metalloenzyme which catalyzes the hydrolysis of a variety of nitriles to the corresponding amides.^{1–3} Recent X-ray crystallographic studies on the inactive form of the Fe-containing nitrile hydratase (Fe-NHase) from *Rhodococcus* sp. N-771 revealed that the iron center is coordinated by two deprotonated carboxamido nitrogens, three Cys-S residues with two of them posttranslationally modified to Cys-sulfinic (Cys-SO₂) and Cys-sulfenic (Cys-SO) groups, and a NO molecule.⁴ The iron site of the Fe-NHase coordinated by the modified cysteines residues (sulfinate RSO₂⁻ and sulfenate RSO⁻ groups) through the sulfur atoms is unprecedented.^{1–4} An inactive, NO-bound form is generated in cells grown in the dark, and the active form of the Fe-NHase is regenerated with release of NO molecule upon exposure to light.^{5,6} The role/function of the NO ligand, NO binding to the iron active site before or after Cys-S oxygenation, the oxidant(s) responsible for the posttranslational modifications of the bound Cys-S donors at the active site of the enzyme, the biogenic mechanism and the functional role of the modified cysteine-sulfinic and -sulfenic groups, and the reason(s) for the asymmetric oxygenation of the two Cys-S ligands are the principal questions to be raised in the chemistry of the Fe-NHase.^{7–9} Herein, we report an iron NO-bound complex [(NO)Fe(S,S-C₆H₄)₂]⁻ (**2**) which reacts with molecular oxygen to afford the corresponding S-bonded monosulfinate [(NO)Fe(S,SO₂-C₆H₄)(S,S-C₆H₄)]⁻ (**3**) (Figure 1) and the dimeric S-bonded bis(sulfinate) species [(NO)Fe(SO₂,SO₂-C₆H₄)(S,S-C₆H₄)₂]²⁻ (**4**), respectively. Also, photolysis study reveals that complexes **2** and **3** are photochemically interconvertible.

When [PPN][[(CO)₂(CN)Fe(S,NH-C₆H₄)] (0.4 mmol)¹⁰ was reacted directly with 1,2-benzenedithiol (0.8 mmol) in tetrahydrofuran (THF) at room temperature, the pentacoordinate Fe complex [PPN]-[(C₄H₈O)Fe(S,S-C₆H₄)₂] (**1**) was isolated as a dark red-brown solid after recrystallization from THF/hexane (yield 80%).¹¹ Subsequent addition of NO gas to complex **1** in THF/CH₂Cl₂ produced the thermally stable, dark reddish brown [PPN][(NO)Fe(S,S-C₆H₄)₂] (**2**) complex (Scheme 1a).¹² Obviously, complex **1** serves as an efficient NO trapping agent. Complex **2** (0.1 mmol, 0.0902 g) reacted slowly with molecular oxygen in THF/CH₂Cl₂ solution at room temperature for one week to yield the S-bonded monosulfinate [PPN][(NO)Fe(S,SO₂-C₆H₄)(S,S-C₆H₄)] (**3**) complex (Scheme 1b).^{13–15} The dark purple solid **3** was isolated in 21% yield (0.02 g) after the mixture solution was separated by silica gel chromatography with THF and CH₂Cl₂ as eluant and recrystallized with THF/CH₂Cl₂ and diethyl ether (On the basis of the UV-vis electronic absorption, the actual yield of conversion of **2** to **3** is calculated as 35%).¹⁵ Preliminary study shows that complex **1** does not initiate O₂ activation to yield iron-sulfinate/-sulfenate compound identified by IR ν(SO) spectra; instead, a trace amount of an insoluble yellow solid occurs after the reaction solution is stirred

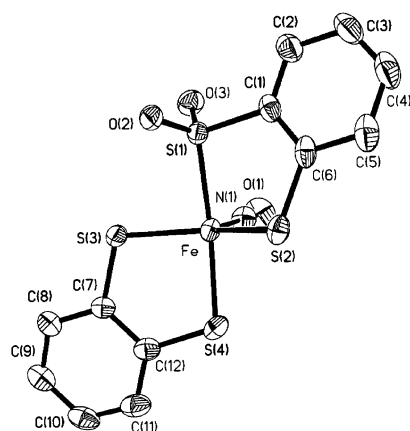


Figure 1. ORTEP drawing and labeling scheme of the [(NO)Fe(S,SO₂-C₆H₄)(S,S-C₆H₄)]⁻ anion. Fe–N(1), 1.629(3); Fe–S(av), 2.219(2); N(1)–O(1), 1.169(4) Å.

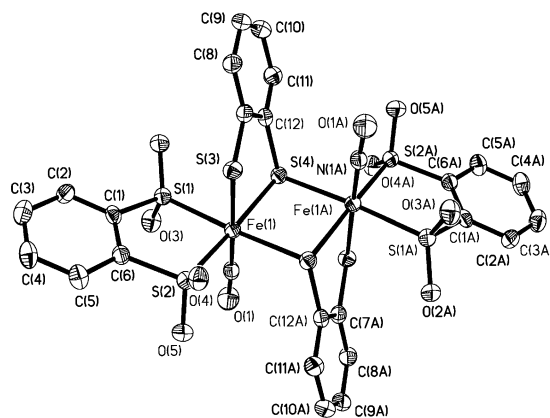


Figure 2. ORTEP drawing and labeling scheme of the [(NO)Fe(SO₂,SO₂-C₆H₄)(S,S-C₆H₄)₂]²⁻ anion. Fe(1)–N(1), 1.647(3); Fe–S(av), 2.2785(9); N(1)–O(1), 1.153(3) Å.

in THF/CH₂Cl₂ for 6 days. Thus, the presence of NO binding to the Fe center appears crucial in promoting oxygenation at sulfur and resulting in the formation of the thermally stable iron–monosulfinate complex **3**.

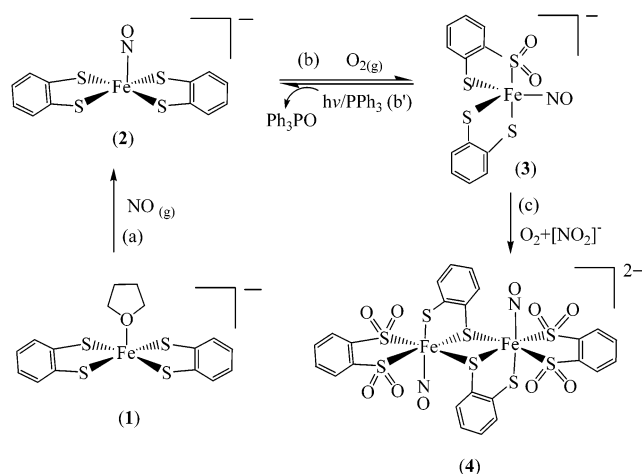
The infrared spectrum of complex **3** shows strong bands at 1779 and 1196, 1060 cm⁻¹ (KBr), corresponding to the ν(NO) and ν-(SO) stretching frequencies of the S-bonded sulfinate group, respectively.¹³ Reactions carried out by using ¹⁸O demonstrate that O₂ is the source of the sulfinate oxygen atoms. Infrared spectrum displays ν(SO) vibrational bands at 1154, 1019 cm⁻¹ (KBr) in the ¹⁸O-labeled complex **3**. Uptake of oxygen atoms by complex **2**, to yield complex **3**, was followed by UV-vis spectrophotometry. Complex **2** has bands in the electronic absorption spectrum at 497 nm (THF). Upon sulfur oxygenation, the color of the complex solution changed from dark reddish brown to purple, and the band

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Scheme 1



at 497 nm disappeared with the formation of two intense absorption bands at 525 and 980 nm. The ^1H NMR spectrum of complex **3**, showing the expected signals for the $[\text{S}_2\text{SO}_2\text{-C}_6\text{H}_4]$ and $[\text{S}_2\text{S-C}_6\text{H}_4]$ ligands, is consistent with a diamagnetic species.¹⁵ The electrochemistry of complex **3**, measured in CH_3CN with 0.1 M $[\text{n-Bu}_4\text{N}][\text{PF}_6]$ as supporting electrolyte (scan rate 100 mV/s), reveals two quasi-reversible oxidation–reduction processes at -0.62 and -1.21 V ($E_{1/2}$) (vs Ag/AgClO_4), as compared to -0.81 and -1.16 V ($E_{1/2}$) for complex **2**.¹³

The S-bonded monosulfinate complex **3** undergoes oxygen transfer reaction in $\text{THF}/\text{CH}_2\text{Cl}_2$ solution with 2 equiv of PPh_3 (expected to be O-atom abstracting agent) over the course of 5 days to yield complex **2** and triphenylphosphine oxide identified by ^{31}P NMR spectroscopy (Scheme 1b').¹⁶ The conversion of complex **3** to complex **2** was also displayed when CH_2Cl_2 solution (10 mL) of complex **3** (0.05 mmol) was photolyzed under N_2 purge at ambient temperature for 60 min; the shift in electronic absorptions at 525 and 980 nm to 497 nm is accompanied by a change in color of the solution from purple to dark reddish brown which is in accord with the formation of complex **2** (Scheme 1b'). During this transformation, no intermediate was detected spectrally. The reversibility of [O] atom binding demonstrates that complexes **2** and **3** are photochemically interconvertible.¹⁷

Subsequent reaction of complex **3** (0.939 g, 1 mmol) with $[\text{PPN}][\text{NO}_2]$ (0.584 g, 1 mmol) in the presence of O_2 in $\text{THF}/\text{CH}_2\text{Cl}_2$ (1:1 ratio) for 10 days at room temperature, as shown in Scheme 1c, produced the dark blue, thermally unstable dimeric bis(sulfinate) $[\text{PPN}]_2[(\text{NO})\text{Fe}(\text{SO}_2\text{SO}_2\text{-C}_6\text{H}_4)(\text{S}_2\text{S-C}_6\text{H}_4)]_2$ (**4**) crystals (yield 3%) after washed with THF and recrystallized from CH_2Cl_2 .¹⁸ Complex **4** displayed three distinct peaks at 1212, 1067, 1057 cm^{-1} (KBr) in the IR $\nu(\text{SO})$ spectrum, consistent with the presence of bis(sulfinate) groups coordinated to iron.¹⁶

Structures of complexes **3** and **4** are presented in Figures 1 and 2, respectively. Analysis of the bond angles for complexes **2** and **3** reveals that iron is best described as existing in a distorted trigonal bipyramidal coordination environment with NO and sulfinate groups occupying equatorial and axial positions, respectively, in complex **3**, whereas the distorted square pyramidal geometry is adopted in complex **2**. Consistent with other published transition-metal sulfinate complexes,¹³ the S–O bond lengths average to ca. 1.464(2) and 1.462(2) Å in complexes **3** and **4**, respectively. The O···O distance is measured at 2.468 Å, while the S(1)···S(2) distance is 3.072 Å (S(1)···S(3), 3.030 Å) in complex **3**.

In summary, the results obtained from this work implicate that binding of one NO molecule to the Fe center promotes sulfur

oxygenation of iron–dithiolates by molecular oxygen and stabilizes the S-bonded monosulfinate iron species, as observed in the inactive, NO-bound form of the Fe–NHase, and the iron–NO–sulfinate species exhibit the photolabilization of sulfur-bound [O] moiety under mild conditions. Studies of the NO/Fe oxidation state(s) of this series of $\{\text{Fe}(\text{NO})\}^6$ -type iron–NO–sulfinate species by XAS,¹⁹ the influence of $\{\text{Fe}(\text{NO})\}^n$ electronic structure on the Fe–N–O bond angle as well as sulfinate ligand(s) on the electronic environment of iron center,¹⁹ the effect of S atom electron density on the sulfur oxygenation of the analogous iron–thiolate–nitrosyl compounds, and mechanistic studies of sulfur oxygenation are ongoing.²⁰

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Supporting Information Available: Crystallographic data in CIF format and additional figures and experimental details (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (11) Complex 1: ^1H NMR (CD_2Cl_2): δ 3.84 (br), 1.98 (br) ($\text{C}_4\text{H}_8\text{O}$), -3.01 (br), -36.79 (br) (S,S-C₆H₄) ppm. Absorption spectrum (THF) [λ_{max} , nm (ϵ , $\text{M}^{-1}\text{cm}^{-1}$): 560(11469), 360(16658), 329(17441), 302(17991)]. Anal. Calcd for $\text{C}_{52}\text{H}_{46}\text{ONP}_2\text{S}_4\text{Fe}$: C, 65.96; H, 4.90; N, 1.48. Found: C, 66.31; H, 4.92; N, 1.79.
- (12) Complex 2: IR: 1789 s (THF) (ν_{NO}) cm^{-1} . ^1H NMR (CDCl_3): δ 6.87 (m), 7.55 (m) (S,S-C₆H₄) ppm. Absorption spectrum (THF) [λ_{max} , nm (ϵ , $\text{M}^{-1}\text{cm}^{-1}$): 320(34925), 497(4400), 610(1010)]. Anal. Calcd for $\text{C}_{48}\text{H}_{38}\text{ON}_2\text{P}_2\text{S}_4\text{Fe}$: C, 63.71; H, 4.23; N, 3.09. Found: C, 63.49; H, 4.70; N, 3.38. The X-ray structure of **2** will be published in the full report.
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- (15) Complex 3: IR: 1789 s (THF), 1791 s (CH_2Cl_2) (ν_{NO}); 1779 (KBr) (ν_{NO}), 1196, 1060 (KBr) (ν_{SO}) cm^{-1} . ^1H NMR (CDCl_3): δ 8.026 (dd), 7.765 (d), 7.17 (dd), 7.08 (t), 6.985 (t) (S,S-C₆H₄, S, S(O)₂-C₆H₄) ppm. Absorption spectrum (THF) [λ_{max} , nm (ϵ , $\text{M}^{-1}\text{cm}^{-1}$): 312(23829), 525-(4750), 625sh(2600), 980(3900)]. Anal. Calcd for $\text{C}_{48}\text{H}_{38}\text{O}_3\text{N}_2\text{P}_2\text{S}_4\text{Fe}$: C, 61.54; H, 4.09; N, 2.99. Found: C, 62.28; H, 4.37; N, 3.15.
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- (18) Complex 4: IR: 1862 (ν_{NO}), 1212, 1067, 1057 (ν_{SO}) (KBr) cm^{-1} . Anal. Calcd for $\text{C}_{96}\text{H}_{76}\text{O}_{10}\text{N}_4\text{P}_4\text{S}_8\text{Fe}_2$: C, 59.505; H, 3.953; N, 2.891. Found: C, 60.20; H, 4.37; N, 2.60.
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