Synthesis and Characterization of a Linear Dinitrosyl-Triiron Complex; Comparison to a Flavodiiron Nitric Oxide Reductase Intermediate

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Nitric oxide is released during the immune response by the host during bacterial infection. To counteract this response, bacteria have evolved nitric oxide reductases to convert NO to N₂O. Some of these nitric oxide reductases contain a flavodiiron active site that have bridging carboxylates and hydroxides. Only a handful of synthetic complexes currently exist as models for the protein reactivity. Here we report the reaction of [Fe₂(μ-OH)(μ-Ph₃DBA)](TMEDA)₂(OTf) (4) with NO(g) and Ph₂CSNO to prepare the dinitrosyl-triiron complex [Fe₂(μ-OH)₂(μ-Ph₃DBA)₂(TMEDA)₃(NO)₂](OTf) (5). The reaction was monitored by UV-Vis and ReactIR spectroscopy and compound 5 was characterized by X-ray crystallography, Fe Mössbauer spectroscopy, Evan’s method, and FTIR spectroscopy. The IR spectrum of compound 5 compares favorably to experimental spectroscopic data obtained for the proposed mononitrosylated intermediate of the protein.

Introduction

Nitric oxide (NO) is encountered by bacteria following reduction of nitrate or nitrite during denitrification, or from a host immune response during infection.[1] Multiple forms of nitric oxide reductases (NORs) exist that contain heme and/or non-heme iron active sites (Chart 1).[2] These NORs can be membrane-bound or dissolved in the cytosol. Flavodiiron proteins (FDP) have been proposed to serve as scavenging NORs in the cytosol by reducing NO to N₂O.[3] FDPs contain non-heme, non-sulfur diiron sites where the iron atoms are five- and six-coordinate and are bridged by a solvent-derived ligand (H₂O/OH⁻) and by an aspartate.[4] Each iron atom binds two histidine residues and a terminal oxygen atom from either aspartate or glutamate residues. The open coordination sites on the iron atoms are syn with respect to one another.

Synthetic analogues capable of reproducing this chemistry have not been well explored. The first published carboxylate-bridged diiron(II)-dinitrosyl species was the diiron complex [Fe₂(η-EtHPTB)(O₂CPh)(NO₂)](BF₄)₂ (1; Chart 1).[5] The related compound [Fe₂(BPMP)(O₂CC₃H₃)(NO₂)](BPh₄)₂ (2) forms N₂O upon two-electron reduction by bulk electrolysis, making it the first functional FDP model.[6] Recently the synthetic mononitrosyl-diiron complex [Fe₂(η-EtHPTB)(L)(NO)(DMF)] (3), where L is either hydroxide or N-coordinated DMF, was reported.[7] Complex 3 forms from [Fe₂(η-EtHPTB)(DMF)]³⁻ by one-electron oxidation with NOBF₄ or by addition of ferrocenium ion and Ph₂CSNO.

We investigated the NO reactivity of the site-differentiated diiron complex, [Fe₂(μ-OH)(μ-Ph₃DBA)](TMEDA)₂(OTf) (4), which more closely resembles the coordination sphere of the protein active site.[8] This complex contains both a five- and a six-coordinate iron atom. The product of nitrosylation is [Fe₆(μ-OH)₂(μ-Ph₃DBA)₂(TMEDA)₃(NO)₂](OTf) (5), which was characterized by X-ray crystallography, Mössbauer, Evan’s method, FTIR, and UV-Vis spectroscopy (Scheme 1).

Results and Discussion

To further understand the reactivity of reactive nitrogen oxide species with carboxylate-bridged diiron complexes, we wished to exploit differences in the coordination environment of the two iron centers of 4 with the goal of forming a mononitrosyl-diiron species; however, upon reaction with NO(g) or Ph₂CSNO complex 5 was formed.

Scheme 1. Reaction of 4 with NO(g) or Ph₂CSNO forms 5.

Chart 1. Active site of FDP and previously reported synthetic nitrosylated models.
We were able to understand this result following an X-ray structural investigation of the product. Two molecules of 5 and disordered molecules of ether and methylene chloride fill the asymmetric unit of crystals grown by vapor diffusion of diethyl ether into a methylene chloride solution of 5 (Figure 1). The three iron atoms form a chain with adjacent iron atoms that are bridged by two carboxylate ligands and a hydroxide ion. This triiron motif containing bridging-carboxylate ligands was observed previously, but never with bound nitrosyls.[9] The bridging oxygen atoms were assigned as HO\(^-\) rather than O\(^2-\) based on the Fe–O\(_{\text{bridge}}\) distances that range from 1.95–2.03 Å (see Table 1), and the fact that the hydroxide hydrogen atoms could be found in the electron density map. The central iron is bound only to oxygen atoms of the Ph\(_4\)DBA\(^{2-}\) ligands and the bridging oxygen atoms. The outer iron atoms are bound to a bent nitrosyl and chelated by a TMEDA ligand. The Fe–N–O bond angles of 5 are more acute than the angles of other published nitrosyl-iron complexes. The orientation of the nitrosyls differs for each iron. One nitrosyl points towards the central iron atom and the other is directed perpendicular to the Fe–Fe vector.

![Figure 1](image1.png)

**Figure 1.** ORTEP representation of the inner shell coordination environment of the triiron chain in 5. Thermal ellipsoids are shown at 50% probability. The non-coordinating portions of the Ph\(_4\)DBA\(^{2-}\) ligands, hydrogen atoms, anions, and solvent molecules are omitted for clarity. Color scheme: iron, orange; nitrogen, blue; oxygen, red; carbon, colorless.

The \(^{57}\)Fe Mössbauer spectrum of 5 was fit with three sites (Figure 2). The first and second have similar isomer shifts of 0.70 and 0.68 mm/s, but different quadrupole splitting parameters of 1.02 and 1.47 mm/s, respectively. These sites are consistent with \{Fe(NO)\}\(^2-\) centers. For instance, the second site fit matches well with the value published for 1 (δ, 0.67 mm/s; ΔE\(_Q\), 1.44 mm/s).[10] The third site fit (δ, 0.51 mm/s; ΔE\(_Q\), 0.95 mm/s) matches closely published values for high spin iron(III) atoms in the oxygen-rich coordination environment of the complex [Fe\(_4\)(μ-OH)\(_2\)(PIM)(Ph\(_3\)CCO\(_2\))\(_2\)] (δ, 0.51(2) mm/s; ΔE\(_Q\), 1.06(2) mm/s) and is similar to that of the species formed in the reaction of 4 and O\(_2\) at low temperature (δ, 0.53(1) mm/s; ΔE\(_Q\), 1.28(3) mm/s), suggesting that this site corresponds to the central iron.[8,13] The Mössbauer data are therefore consistent with the assignment of one high spin iron(III) and two \{Fe(NO)\}\(^2-\) sites.

**Table 1.** Selected geometric and vibrational parameters of 1–3 and 5.

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>bond length, Å</td>
<td>1.749(8)</td>
<td>1.777(2)</td>
<td>1.787(2)</td>
</tr>
<tr>
<td>Fe–N(_{\text{no}})</td>
<td>1.750(7)</td>
<td>1.796(3)</td>
<td>1.799(5)</td>
</tr>
<tr>
<td>N–O</td>
<td>1.156(8)</td>
<td>1.156(3)</td>
<td>1.109(6)</td>
</tr>
<tr>
<td>Fe–O(_{\text{bridge}})</td>
<td>2.017(5)</td>
<td>2.057(19)</td>
<td>1.952(2)</td>
</tr>
<tr>
<td>Fe–O(_{\text{edge}})</td>
<td>2.006(5)</td>
<td>2.020(2)</td>
<td>2.044(2)</td>
</tr>
<tr>
<td>bond angle, °</td>
<td>166.6(7)</td>
<td>155.4(2)</td>
<td>171.0(30)</td>
</tr>
<tr>
<td>Fe–N–O</td>
<td>168.3(7)</td>
<td>144.7(2)</td>
<td>139.8(7)(^a)</td>
</tr>
<tr>
<td>Fe–O–Fe</td>
<td>117.2(2)</td>
<td>116.7(9)</td>
<td>130.0(1)</td>
</tr>
<tr>
<td>v(NO) cm(^{-1})</td>
<td>1785</td>
<td>1760</td>
<td>1792</td>
</tr>
</tbody>
</table>

\(^a\)From the major component of the disordered NO.

**Figure 2.** \(^{57}\)Fe Mössbauer spectrum of 5 collected at 80 K. Color coding: raw, black; full fit, red; site 1, blue; site 2, green; site 3, pink. Site 1: δ, 0.70(2) mm/s; ΔE\(_Q\), 1.02(2) mm/s; Γ, 0.32(2) mm/s; Area, 0.30%. Site 2: δ, 0.68(2) mm/s; ΔE\(_Q\), 1.47(2) mm/s; Γ, 0.35(2) mm/s; Area, 0.26%. Site 3: δ, 0.51(2) mm/s; ΔE\(_Q\), 0.95(2) mm/s; Γ, 0.49(2) mm/s; Area, 0.44%.

Compound 5 does not display an EPR signal at ambient temperature or at 77 K, similar to results previously obtained for 1–3.[5] Typically high spin Fe\(^3+\) systems exhibit an EPR spectrum from 4–9 G, but such is not observed in this case. The cyclic voltammogram of 5 reveals irreversible electrochemical processes at -1.06 V, -0.69 V, and 1.00 V vs Fe/C.\(^+\) (Supporting Information; Figure S2). At 292 K, the trinuclear compound has a \(\mu_{\text{eff}}\) of 6.30 \(\mu_B\) in solution, which correlates to an S = 5/2 system. The overall spin state is consistent with antiferromagnetic interactions between the three iron atoms, where the outer \{Fe(NO)\}\(^2-\) units have S = 3/2 and the central iron is high spin with S = 5/2. The spin assignment is supported by the \(^{57}\)Fe Mössbauer spectrum, discussed above.

When the reaction of 4 and NO(g) is followed by UV-Vis (Figure 3) in acetonitrile at 25 °C, two absorption bands initially grow in at 434 nm and 617 nm together with a shoulder at 347 nm. The spectrum continues to change, with the shoulder at 347 nm increasing in intensity and the band at 431 nm red-shifting to 452 nm and decreasing in intensity; there is an approximate isosbestic point at 420 nm. These changes suggest that the reaction is multi-phasic with more than one kinetically distinguishable step. One possibility is that the reaction proceeds through oxidation of the iron centers followed by disproportionation into 5 and an unidentified, non-nitrosylated iron species.

![Figure 3](image2.png)

**Figure 3.** UV-Vis spectrum (left) and kinetic traces of selected wavelengths (right) of the reaction of 4 and excess NO(g) in acetonitrile at 25 °C. Scans were collected every 30 seconds.
The reaction can also be followed by ReactIR in CH₂Cl₂ at ambient temperature (Figure 4). Four bands were monitored that change in intensity with different rates. The intensity of bands at 1722 cm⁻¹ and 1602 cm⁻¹ increases, while an initial band at 1627 cm⁻¹ diminishes. A band at 1615 cm⁻¹ initially forms, but begins to decrease after 4 min. The band at 1722 cm⁻¹ is a ν(NO) stretch, whereas the 1602 cm⁻¹, 1615 cm⁻¹, and 1627 cm⁻¹ bands correspond to vibrations from the carboxylate groups. No intermediate iron-nitrosyl species were observed, even at low temperature. The changes in band intensities suggest that oxidation of the iron atoms and rearrangement of the iron–carboxylate coordination environment may occur prior to NO binding. The ν(NO) band is 40–80 cm⁻¹ lower in energy than in other published synthetic nitrosyl-diiron compounds, but closer to the resonance Raman ν(NO) frequency of 1681 cm⁻¹ observed for a mononitrosyliron species, FDP(NO). The FDP(NO) derivative is proposed to have a bent nitrosyl oriented towards the non-nitrosylated iron atom, a geometry that we observe in the crystal structure of 5. The lower ν(NO) energy in the complex may also be attributed to more anionic ligands bound to the {Fe(NO)}²⁻ units in 5 compared to complexes 1–3, a phenomenon previously reported in mononuclear iron-nitrosyls.

Conclusions

A novel dinitrosyltriiron complex was prepared by direct reaction of 4 with NO(g) or the S-nitrosothiol, Ph₃CSNO. This complex consists of an arrangement of three iron atoms having two bent nitrosyls bound to the terminal metal ions. The central iron is six-coordinate with only oxygen donor ligands. The vibrational spectroscopic parameters of 5 match those of the mononitrosylated active site of FDP more closely than other previously prepared synthetic model complexes.

Experimental Section

General Comments. All manipulations were performed under an atmosphere of nitrogen gas using an MBraun glovebox. NO (Airgas, 99%) was purified by a literature procedure. The NO gas stream was passed through an Ascarite column (NaOH fused on silica gel) and a 6 ft. coil filled with silica gel that was cooled to -78 °C using a dry ice/acetone bath. NO was maintained in a standard gas storage bulb and transferred via gas tight syringes. Diethyl ether, methylene chloride, and acetonitrile were purified by passage through activated alumina then stored over 4-Å molecular sieves prior to use. Compound 4 and Ph₃CSNO were synthesized as previously published. All reagents were purchased from Sigma-Aldrich and used as received.

Physical Measurements. FTIR spectra were recorded on a Thermo Nicolet Avatar 360 spectrometer running the OMNIS software package. X-Band EPR spectra were obtained with a Bruker EMX EPR spectrometer at ambient temperature or at 77 K using a quartz finger dewar in 2-MeTHF. Low-resolution mass spectra were obtained with an Agilent 1100 Series LC/MSD mass spectrometer. The Evan’s method experiment was performed on a 500 MHz Varian Inova NMR spectrometer in CD₂Cl₂ using hexamethyldisiloxane as the standard. The temperature was determined by measuring the residual peak separation of the H NMR of neat CD₃OD. Diamagnetic corrections were estimated using Pascal’s constants. UV–Vis spectra were recorded on a Cary-50 spectrophotometer in airtight rubber septum-capped quartz cells at 25.0 °C. Samples for Mössbauer studies were prepared by grinding solids with Apiezon-N grease. These Mössbauer samples were placed in an 80 K cryostat during measurement. A ⁵⁷Co/Rh source was moved at a constant acceleration at room temperature against the absorber sample. All isomer shift (δ) and quadrupole splitting (ΔE_Q) values are reported with respect to ⁵⁷Fe-enriched metallic iron foil that was used for velocity calibration. The displayed spectrum was folded to enhance the signal-to-noise ratio. Fits of the data were calculated by the WMROSS plot and fit program, version 2.5. In situ IR spectra were recorded on a ReactIR iC 10 instrument from Mettler Toledo equipped with a 1 in. diameter, 30-reflection silicon ATR (SiComp) probe. In a typical experiment, the instrument was blanked with a solution of 4 in CH₂Cl₂ (30 mM). After the first data collection time point was taken, NO(g) was added to the anaerobic sample compartment through a rubber septum with a gas tight syringe. The data were referenced to the initial scan and imported to Origin 8.0 as a *.spc file to generate the figures. X-Band EPR spectra were recorded on a Bruker EMX EPR spectrometer at ambient or liquid nitrogen temperature using a quartz finger dewar. Electrochemical measurements were performed at ambient temperature in a glovebox on a VersaSTAT3 Princeton Applied Research potentialstat running the V3-Studio electrochemical analysis software. A 3-electrode set-up was employed comprising a glassy carbon working electrode, platinum wire auxiliary electrode, and a Ag(s)/Ag⁺(s) silver wire pseudo-reference electrode. Triply recrystallized (Bu₄N)PF₆ was used as the supporting electrolyte. All electrochemical data were referenced internally to the Fe/Ce²⁺ couple at 0.00 V. X-ray Data Collection, and Structure and Solution Refinement. Crystals of 5 suitable for X-ray diffraction were mounted in Paratone N oil and frozen under a nitrogen cold stream maintained by a KRYO-FLEX low-temperature apparatus. Data were collected on a Bruker APEX CCD X-ray diffractometer with Mo Kα radiation (λ= 0.71073 Å) controlled by the APEX2 software package. Empirical absorption corrections were calculated with SADABS.[22] The structure was solved by direct methods with refinement by full-matrix least-squares based on F² using SHELXTL-97.[23] All non-hydrogen atoms were located and their positions refined anisotropically. Hydrogen atoms were assigned to idealized positions and given thermal parameters equal to either 1.5 (methyl hydrogen atoms) or 1.2 (non-methyl hydrogen atoms) times the thermal parameters of the atoms to which they were attached. Hydrogen atoms of the bridging hydroxide ion were located in a difference electron density map and O-H bond...
distances were fixed to 0.84 Å. Hydrogens were not modeled for the disordered solvent molecules. Two molecules of S occur within the asymmetric unit. All NO units were disordered over two positions. In one molecule of S, the TMEDA unit was disordered across two positions. In the other molecule of S, a phenyl group was disordered over two positions. One molecule of diethyl ether was modeled without disorder and a second was disordered across two positions. A complicated four-part solvent disorder consisting of three methane chloride molecules and one diethyl ether molecule was also modeled. Both trifluor counterions were disordered across two positions. Bond distances, angles, and anisotropic displacement parameters of the disordered atoms were constrained to be similar. The anisotropic displacement parameters of the four-part disorder were constrained to be equal. Space group verification was performed using Platon.[24] Figures were generated using the Olex 2.1 graphical user interface.[25] See Table S1 in the Supporting Information for crystallographic data and refinement details.

\[ \text{[Fe} \text{(OH)}_2 \text{]_2[Fe} \text{P} \text{H}_2 \text{DBA}_2] \text{TMEDA}_2 \text{NO}_2 \text{]} \text{(OTf)]}} \text{, 5. Method A.} \\
In a 50 mL Erlenmeyer flask, compound 4 (150 mg, 137 μmol) was dissolved in methylene chloride (15 mL). In a separate 20 mL vial, PhCSNO (83.6 mg, 274 μmol) was dissolved in methylene chloride (15 mL) and instantly poured into the reaction flask and capped with a rubber septum. Throughout the course of the reaction, 40 – 60 μL aliquots were taken to monitor the reaction by solution IR between CaF\(_2\) salt plates. After no further growth or decay in bands was observed in the 1500 – 1850 cm\(^{-1}\) region (6 h), the reaction mixture was poured into 300 mL pentane and stirred for 10 min. The mixture was filtered over Celite and the remaining solid was extracted with 15 mL methylene chloride. The volume of the methylene chloride extract was reduced to 4 mL. Diethyl ether vapor was diffused into the methylene chloride solution to yield 60.5 mg of dark green crystals of 5 (33.3 μmol, 24.3%) suitable for X-ray diffraction study. Anal. Calc’d for C\(_{30}\)H\(_{28}\)F\(_2\)F\(_4\)Fe\(_2\)N\(_2\)O\(_7\) (CH\(_2\)Cl\(_2\))\(_{23}\): C, 58.95; H, 4.54; N, 4.38. Found: C, 58.84; H, 4.37; N, 4.49. ESL-MS (MeCN, m/z): 148.7 ([OTf\(^{-}\)], 149.0). FT-IR (CH\(_2\)Cl\(_2\), cm\(^{-1}\)): 1722 (ν\(_{\text{νCO}}\), m); 1506 (ν\(_{\text{νCN}}\), m). 1H NMR spectrum, Evans’ method 1H NMR spectrum, the solid-state infrared spectrum are available.

**Acknowledgments**

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[22] G. M. Sheldrick, University of Göttingen; Göttingen, Germany, 2008.


The complex [Fe(μ-OH)₂(μ-PhDBA)₂(TMEDA)₂(NO)]²⁺(OTf) was synthesized and characterized spectroscopically. Its properties resemble those of a proposed intermediate observed during the catalytic reduction of nitric oxide by flavodiiron reductases.