

The first structurally characterized nitrosyl heme thiolate model complex

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The NO ligand in the formally $\{\text{FeNO}\}^6$ compound $[\text{Fe}(\text{oep})(\text{NO})(\text{thiolate})]$ is bent, and does not impart a significant structural *trans* effect to the Fe–S bond.

Heme thiolate proteins contain the $[\text{Fe}(\text{por})(\text{SR})]$ moiety (por = porphyrinato dianion; SR = cysteinate) and display rich and diverse reactivities that include nitric oxide (NO) biosynthesis, NO reduction, hydroxylation, and detoxification of xenobiotics.^{1,2} The thiolate groups in these proteins serve as axial ligands that influence the reactivities of the iron centers. Crystal structures of the cytochromes P450 (P450) reveal stabilization of the Fe–SR linkage *via* hydrogen bonding between the sulfur atom and neighboring peptide NH groups; in NO synthase (NOS), one of these hydrogen bonds is provided by the indole NH group of a conserved Trp residue.³

NO binds to the iron center in P450 and inhibits the enzyme.⁴ Interestingly, the NO adduct of ferric NOS is an observable intermediate in the catalytic cycle of NOS.⁵ In the case of fungal P450nor (NO reductase), NO binds to the ferric center and is reduced by NADH.² NO reacts with the ferric heme thiolate protein *Cimex nitrophorin* not only by nitrosylation of the iron center, but also by nitrosation of the axial thiolate sulfur atom.⁶ A related nitrosylation–nitrosation double reaction has been proposed as a mechanism of deactivation of liver microsomal P450 by NO;⁴ such processes occur with some ferric thiolate porphyrins.⁷

NO has been shown to bind reversibly to synthetic ferric thiolate porphyrins,^{7,8} but no X-ray structural studies of synthetic nitrosyl heme thiolate compounds have been reported to date. Surprisingly, the Fe–N–O angles in the two structural reports on the NO adducts of ferric heme thiolate proteins range from 100° to 165°.^{2,9} Distal pocket residues are known to affect the FeNO bond geometry in “ferrous” $\{\text{FeNO}\}^7$ nitrosyl heme proteins, but it is not clear to what extent distal residues influence the FeNO geometry in the formally “ferric” $\{\text{FeNO}\}^6$ derivatives.¹⁰ For example, Hu and Kincaid have shown that the FeNO moiety in the NO adduct of ferric P450cam (camphor monooxygenase) is linear in the absence of substrates, whereas it is slightly bent in the presence of substrates.¹¹ Further, Scherlis *et al.* have reported their results of Density-Functional Theoretical calculations on a $[\text{Fe}(\text{porphine})(\text{NO})(\text{cysteinate})]$ model that show that the ground state structure possesses a linear FeNO geometry (178.7°), and that higher energy states of this model may contain bent FeNO geometries.¹²

We were thus interested in structurally characterizing a ferric heme model complex containing mutually *trans* NO and thiolate ligands. Our intent was to examine, for the first time, (i) the FeNO bond geometry in the absence of distal amino acid residues that could influence this geometry, and (ii) the effect of the bound NO group on the *trans* Fe–S bond.

The reaction of the five-coordinate iron porphyrin thiolate complex $[\text{Fe}^{\text{III}}(\text{oep})\{\text{S}-2,6-(\text{CF}_3\text{CONH})_2\text{C}_6\text{H}_3\}]$ (**1**)¹³ (oep = octaethylporphyrinato dianion) with NO in CH_2Cl_2 was monitored by IR spectroscopy. The resulting spectra recorded at different times after NO addition are shown in Fig. 1. A new band at $\sim 1850\text{ cm}^{-1}$ forms immediately after NO addition assigned to ν_{NO} (*cf.*, free NO at 1875 cm^{-1}).¹⁴ However, this band is quickly consumed with the resulting formation of the ν_{NO} band at 1667 cm^{-1} due to the final five-coordinate $[\text{Fe}(\text{oep})(\text{NO})]$ compound.

Also observed is the reduction in intensity of the band at 1720 cm^{-1} due to the ν_{CO} (of the thiolate ligand) of **1** and the generation of a new band at 1743 cm^{-1} coincident with the ν_{CO} of the organic disulfide. We thus assign the new band at $\sim 1850\text{ cm}^{-1}$ to the ν_{NO} of the target six-coordinate compound $[\text{Fe}(\text{oep})(\text{NO})\{\text{S}-2,6-(\text{CF}_3\text{CONH})_2\text{C}_6\text{H}_3\}]$ (**2**). All our attempts, to date, at crystallizing **2** from this reaction mixture have not been successful; only the final $[\text{Fe}(\text{oep})(\text{NO})]$ derivative was obtained, consistent with the ready loss of the thiolate ligand from the six-coordinate compound in solution.

Reaction of the iron porphyrin thiolate compound **1** as a powder with NO gas in a solvent-free environment produced a derivative with a ν_{NO} band of 1839 cm^{-1} (Nujol mull). This solid product was stable under an atmosphere of NO for several days in

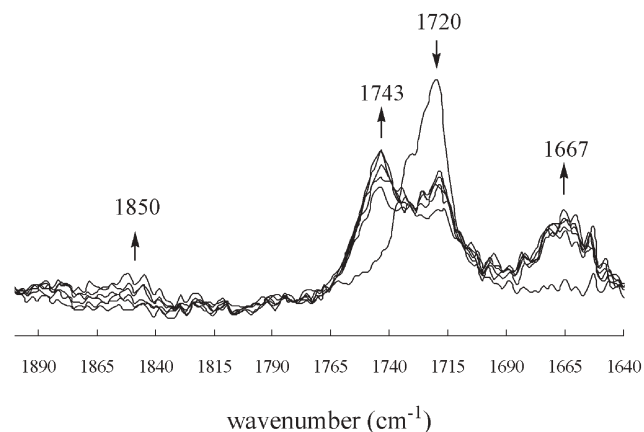


Fig. 1 Infrared spectral monitoring of the reaction of **1** with NO in CH_2Cl_2 ; intervals are 0 s, 10 s, 20 s, 35 s, 60 s, and 120 s.

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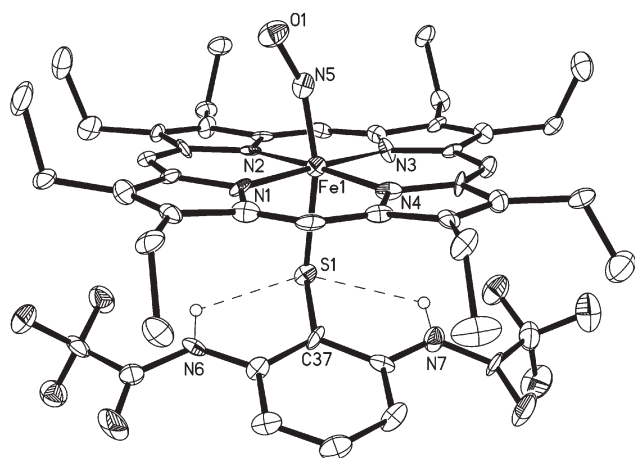


Fig. 2 Molecular structure of $[\text{Fe}(\text{oep})(\text{NO})\{\text{S}-2,6\text{-(CF}_3\text{CONH)}_2\text{C}_6\text{H}_3\}]$ (**2**) with thermal ellipsoids drawn at 35%.

the dark. However, attempted dissolution and crystallization of the nitrosyl product **2** resulted only in the generation of the precursor compound **1** and $[\text{Fe}(\text{oep})(\text{NO})]$.

We then explored the possibility of a heterogeneous reaction of crystals of **1** with NO gas with the hope that product formation would not be accompanied by extensive crystal fragmentation. The crystals used for the reaction were grown from CH_2Cl_2 –hexane (2 : 1) at room temperature.^{†‡} Several crystals of purple **1** were hand-picked and exposed to NO gas at room temperature for several hours to allow for NO diffusion into the crystal lattice. The molecular structure of the dark red nitrosyl product **2** is shown in Fig. 2. Selected structural data of **2** are presented in Table 1, and are compared with the related data from **1**.

There are several interesting features about the structure of **2**. First, the FeNO moiety in **2** is bent with an angle of $159.6(8)^\circ$ in this formally $\{\text{FeNO}\}^6$ compound, and the nitrosyl N-atom is tilted 9.1° from the normal to the 4N (and 24 atom) porphyrin plane. The bent NO geometry is not the result of any close intermolecular contacts; the shortest intermolecular distances are between the nitrosyl O-atom and a thiolate F-atom of another molecule, and between the nitrosyl N-atom and another porphyrin ethyl carbon atom (both distances are ≥ 3.3 Å).

Second, the Fe atom in **2** is situated almost in the 4N plane of the porphyrin, with an upward displacement of 0.05 Å towards the NO ligand. Concurrent with this upward movement of the Fe atom upon formation of **2** is the shortening of the Fe– N_{por} bond lengths by ~ 0.05 Å. Considering that the Fe atom was displaced 0.41 Å towards the S atom in the precursor **1**, this represents a

Table 1 Selected structural data (in Å and $^\circ$) for compounds **1** and **2**

	1	2
Fe1–N5	—	1.671(9)
N5–O1	—	1.187(9)
Fe1– N_{por}	2.054(2)–2.064(2)	2.003(8)–2.017(7)
Fe1–S1	2.359(1)	2.356(3)
\angle Fe1–N5–O1	—	159.6(8)
\angle S1–Fe1–N5	—	165.5(3)
\angle C37–S1–Fe1	104.22(11)	110.6(3)
S tilt ^a	3.4 [4.3]	6.3 [6.7]

^a Tilt of the S atom from the normal to the porphyrin 4N [24 atom] plane.

remarkable ~ 0.46 Å apical movement of the Fe atom in this solid-state reaction of **1** with NO gas! Even more striking is that the *trans* Fe–S bond length does not change in going from the five-coordinate **1** to the six-coordinate **2**, implying that there is no significant structural *trans* effect of NO in this six-coordinate iron nitrosyl thiolate porphyrin when prepared from the five-coordinate crystals. This may be compared with the structural *trans* effect observed in the $\{\text{FeNO}\}^7$ compounds $[\text{Fe}(\text{por})(\text{NO})(\text{N-base})]$ which show a ~ 0.2 Å increase in the Fe–N(ax) distance upon NO binding.¹⁵

Third, the FeNO and FeS(thiolate) planes are essentially mutually perpendicular, and these two planes straddle the Fe–N1 bond; C37–S1–Fe1–N1 torsion angle = $-55.6(4)^\circ$, and O1–N5–Fe1–N1 = $43(2)^\circ$. In addition, there is a slight opening of the Fe1–S1–C37 angle (from 104° to 111°) upon NO binding and subsequent movement of the S-atom toward the mean porphyrin plane.

When placed in a broader context, the crystal structure of **2** reveals an intrinsic tilting (of the nitrosyl N-atom from the porphyrin normal) and bending of the NO group in this formally $\{\text{FeNO}\}^6$ species. We have previously reported that such a tilting and bending feature represents a low energy conformation in the related $\{\text{FeNO}\}^6$ compound $[\text{Fe}(\text{oep})(\text{NO})(\text{C}_6\text{H}_4\text{F})]$.¹⁶ Our results suggest that such a tilting and bending of the NO group may be a common feature in the NO adducts of ferric heme thiolate proteins.

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Notes and references

[†] The crystal structure of **1** was reported previously by Ueyama *et al.*¹³ Their crystals were grown from toluene, and the Fe–S and Fe– N_{por} bond lengths in their structure were 2.356(3) and 2.048(average) Å, respectively.

[‡] *Crystal data.* Compound **1**·1/2(hexane): $\text{C}_{49}\text{H}_{56}\text{F}_6\text{FeN}_7\text{O}_3\text{S}$, $M = 962.91$, triclinic, space group $P\bar{1}$, $a = 8.9881(14)$ Å, $b = 13.208(2)$ Å, $c = 19.879(3)$ Å, $\alpha = 100.889(5)^\circ$, $\beta = 91.761(5)^\circ$, $\gamma = 101.373(5)^\circ$, $V = 2266.3(6)$ Å³, $T = 100(2)$ K, $Z = 2$, $Z' = 1$, $D_c = 1.411$ g cm⁻³, $\mu = 0.451$ mm⁻¹, 18050 reflections measured, 8813 unique ($R_{\text{int}} = 0.0260$), $F(000) = 1008$, R_1 (obs. data) = 0.0563, wR_2 (all data) = 0.1645. Compound **2**·1/2(hexane): $\text{C}_{49}\text{H}_{56}\text{F}_6\text{FeN}_7\text{O}_3\text{S}$, $M = 992.92$, triclinic, space group $P\bar{1}$, $a = 9.181(5)$ Å, $b = 12.455(7)$ Å, $c = 21.261(12)$ Å, $\alpha = 98.573(9)^\circ$, $\beta = 94.080(9)^\circ$, $\gamma = 99.425(9)^\circ$, $V = 2360(2)$ Å³, $T = 100(2)$ K, $Z = 2$, $Z' = 1$, $D_c = 1.397$ g cm⁻³, $\mu = 0.438$ mm⁻¹, 9889 reflections measured, 4873 unique ($R_{\text{int}} = 0.0680$), $F(000) = 1038$, R_1 (obs. data) = 0.0833, wR_2 (all data) = 0.2035. CCDC 298938 (**2**) and CCDC 298939 (**1**). For crystallographic data in CIF or other electronic format see DOI: 10.1039/b602611g

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