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Electronic Structure of Ferric Heme Nitrosyl Complexes with Thiolate Coordination

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The effect of trans thiolate ligation on the coordinated nitric oxide in ferric heme nitrosyl complexes as a function of the thiolate donor strength, induced by variation of NH–S(thiolate) hydrogen bonds, is explored. Density functional theory (DFT) calculations (BP86/TZVP) are used to define the electronic structures of corresponding six-coordinate ferric [Fe(P)(SR)(NO)] complexes. In contrast to N-donor-coordinated ferric heme nitrosyls, an additional Fe–N(O) σ interaction that is mediated by the d_{z^2}/d_{xz} orbital of Fe and a σ^*-type orbital of NO is observed in the corresponding complexes with S-donor ligands. Experimentally, this is reflected by lower ν(N–O) and ν(Fe–N) stretching frequencies and a bent Fe–N–O moiety in the thiolate-bound case.

It is of great biological importance to explore the effect of trans thiolate coordination to heme nitrosyls in proteins and their corresponding model complexes. Nitric oxide (NO)-bound heme centers with axial cysteinate ligands are present in NO synthase (NOS),[1,2] the Cimex lectularius (bedbug) nitrophorin,[3] and the fungal nitric oxide reductase (P450nor).[4] Comparison of nitrosylation (on/off) rates shows that the thiolate ligand has a profound effect on the $\mathrm{Fe^{III}}$−NO unit, but the electronic structural reasons for this finding are not clear.[5] In terms of other properties, it is known for a long time that ferric heme nitrosyls with trans imidazole or pyridine donors have linear Fe−N−O units and ν(N−O) of $>1900\ \mathrm{cm^{-1}}$. This has been observed in proteins and model complexes.[6] In contrast, thiolate coordination leads to a bent structure [$\angle$(Fe−N−O) = 161−165°], but it was not clear whether this is due to electronic or steric effects.[2,4] Recently, Xu et al. published the first X-ray structural characterization of the ferric nitrosyl heme thiolate model complex [Fe(OEP)(NO){S-2,6-$(\mathrm{CF_3CONH})_2\mathrm{C_6H_3}$}] (**1**; OEP = octaethylporphyrin).[7] Importantly, the Fe−N−O unit is again bent with an angle of 160°, which demonstrates an intrinsic bending of the Fe−N−O moiety *due to electronic effects*. Another interesting feature of thiolate-coordinated ferric heme nitrosyls is the low ν(N−O) stretching frequency of 1800−1850 $\mathrm{cm^{-1}}$. In order to determine the role of thiolate for the functions of the above-mentioned proteins, it is therefore of critical importance to explore the electronic effect of the thiolate on the Fe−NO unit.[8]

In this study, the effect of trans thiolate coordination on the coordinated NO as a function of the thiolate donor strength [induced by variation of NH−S(thiolate) hydrogen bonds] is explored for the first time. For this purpose, density functional theory (DFT) calculations (BP86/TZVP) have been performed on six-coordinate ferric [Fe(P)(SR)(NO)] (P = $\mathrm{porphine^{2-}}$) complexes using benzene[2,6-bis(trifluoroacetamido)]thiolate (S-2,6-$(\mathrm{CF_3CONH})_2\mathrm{C_6H_3}$ = **S1**) with *two hydrogen bonds* (**2**), benzene(2-trifluoroacetamido)thiolate (**S2**) with *one hydrogen bond* (**3**), thiophenolate (**S3**) with *no hydrogen bonds* (**4**), and, additionally, the strong donor methylthiolate (**5**) (cf. Chart 1).

The optimized geometric parameters for model **2** (cf. Figure 1) shown in Table 1 are in very good agreement with the crystal structure of compound **1**. Most notably, in all optimized structures, the Fe−N−O geometry is bent. In contrast, semiempirical SAM1 calculations by Scherlis et al. on the nitrosylated active site of ferric cytochrome P450 show a linear Fe−N−O group for the singlet ground state,[11] indicating that this method does not correctly describe the Fe−N−O geometry.

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Chart 1

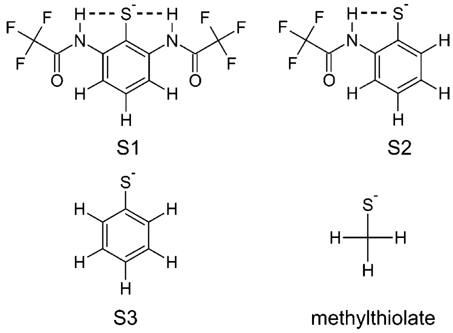
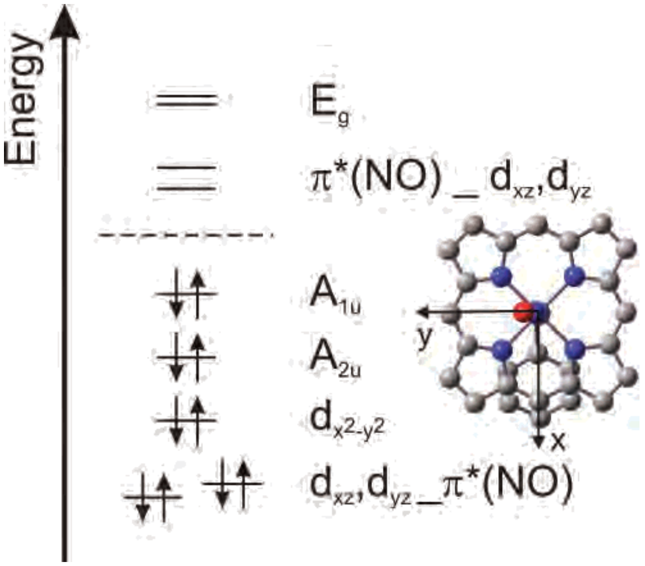


Chart 2



Ligands **S1** and **S2** show two and one hydrogen bond(s) between the thiophenolate S atom and the amido H atom(s), respectively [calcd $\Delta(S-H) \approx 2.37$ Å]. Corresponding hydrogen-bonding interactions in P450 enzymes are mediated by the cysteinate S atom and the protein backbone NH groups. Using sulfur K-edge X-ray absorption spectroscopy (XAS) and DFT calculations, Dey et al. showed that hydrogen bonding reduces the Fe−S bond covalency and the electron-donating power of the S atom.[12] This is in agreement with the shortening of the Fe−S bond and the increase of the *f*(Fe−S) force constant upon stepwise removal of the hydrogen bonds in the series **2** → **3** → **4** (cf. Table 1). Interestingly, this stepwise strengthening of the Fe−S bond goes along with a simultaneous weakening of the Fe−N(O) bond, giving the first evidence for a σ-trans effect of the coordinated thiolate on the Fe−N−O moiety.

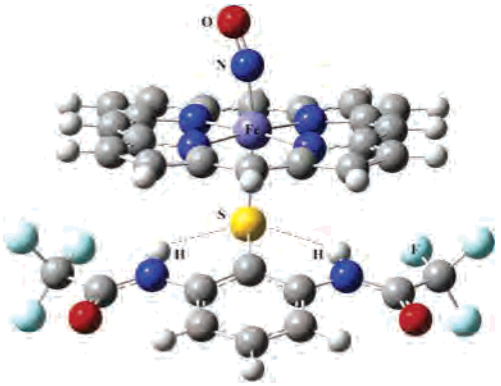


Figure 1. DFT (BP86/TZVP)-optimized structure of [Fe(P)(NO){S-2,6-$(CF_3CONH)_2C_6H_3$}] (**2**) showing two NH−S hydrogen bonds.

Table 1. Comparison of Calculated Geometries and Force Constants for Ferric [Fe(P)(SR)(NO)] Using BP86/TZVP

	geometric parameters (Å)			force constants[a]		
molecule	Δ(Fe−N)	Δ(Fe−S)	∠(Fe−N−O)	Fe−N	Fe−S	N−O
1 exp.[7]	1.672	2.356	159.7			
2 (2 HB)[b]	1.668	2.434	167	4.31	0.94	14.43
3 (1 HB)[b]	1.679	2.377	164	4.10	1.03	14.21
4 (0 HB)[b]	1.685	2.343	164	3.99	1.16	14.03
5[b]	1.691	2.300	164	3.87	1.40	13.96

[a] Force constants (in mdyn/Å) in internal coordinates were extracted from the Gaussian output using a modified version of the program Redong[9,10] (QCPE 628). [b] HB = hydrogen bond(s); calculated.

To understand the σ-trans effect of the thiolate ligand in detail, the electronic structure of model complex **4** is analyzed. Figure S1 in the Supporting Information shows the obtained molecular orbital (MO) diagram for complex **4**, and charge contributions are given in Table S1 in the Supporting Information. In general, iron(III) heme nitrosyl complexes are known to have an Fe^{II}NO$^+$ electronic structure, and this also applies to complex **4**.[6,13] This electronic structure corresponds to an oxidation of NO upon binding to Fe^{III}. This is also observed for ruthenium(III) nitrosyls and has been extensively studied for these systems.[14] Therefore, in the case of **4**, Fe has a $[d_{xz}, d_{yz}, d_{x^2-y^2}]^6 \approx [t_2]^6$ electronic configuration using the coordinate system shown in Chart 2. Both π* orbitals of NO$^+$ are empty and undergo π-back-bonding interactions with d_{xz} and d_{yz} of Fe. The strength of this interaction is evident from the corresponding antibonding combinations $\pi_h^*\_d_{yz}\_p_z(S)$ ⟨129⟩ and $\pi_v^*\_d_{z^2}/d_{xz}$ ⟨130⟩. MO ⟨130⟩ has 67% π_v^* and 26% d orbital character, which corresponds to a very strong interaction. These very strong π back-bonds lead to a transfer of a significant amount of electron density from the d_{xz} and d_{yz} orbitals of Fe to the π* orbitals of NO. Contour plots of these orbitals are shown in Figure S2 in the Supporting Information. The third t_2-type orbital of Fe, $d_{x^2-y^2}$ ⟨125⟩, is practically nonbonding (cf. Table S1 in the Supporting Information). This bonding situation is schematically shown in Chart 2. A weak σ interaction between σ_{nb} of NO and d_{z^2}/d_{xz} is also observed. The bonding combination $\sigma_{nb}\_d_{z^2}/d_{xz}$ ⟨76⟩ has 48% σ_{nb} and 9% d character. This electronic structure description is comparable to iron(III) heme nitrosyl complexes with trans N donors.[15] But, then why does the Fe−N−O unit bend in the presence of an axial thiolate? This is due to an additional Fe−N(O) σ interaction that is

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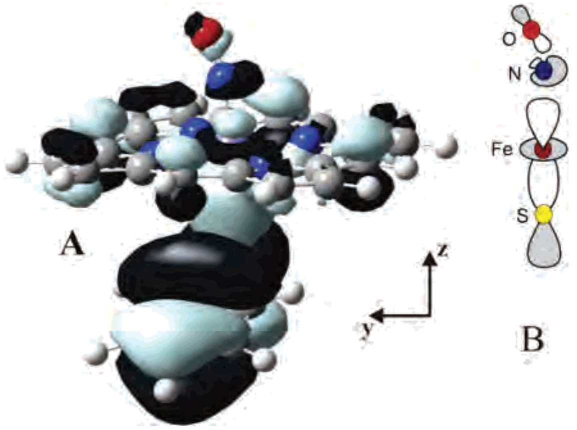

Figure 2. Contour plot of $A_{2u} + p_z(\mathrm{S})\_d_{z^2}/d_{xz}\_\sigma^*$ ⟨124⟩ of compound **4** (A). Illustration of the contributions from the Fe−N−O moiety (B).

Table 2. Charge Contributions of the $A_{2u} + p_z(\mathrm{S})\_d_{z^2}/d_{xz}\_\sigma^*$ MOs (⟨124⟩ and ⟨127⟩) Calculated with BP86/TZVP (in %)

	Fe	N		O		S
molecule	d	s	p	s	p	Σs
4[a] (optimized)	14.6	1.5	1.3	0	1.1	26.6
4a[a] (linearized **4**)	16.1	1.5	0.9	0	0.8	28.5
[Fe(P)(MI)(NO)]$^+$ (**6a**)[b]	1	0.2	0.1	0	0	2

[a] The values for compounds **4** and **4a** are the sums over the contributions from orbitals ⟨124⟩ and ⟨127⟩. [b] Reference 15 (MI = 1-methylimidazole); model complex **6a**, vide infra.

mediated by the d_{z^2}/d_{xz} orbital of Fe and a σ*-type orbital of NO (MOs ⟨124⟩ and ⟨127⟩; cf. Figure S1 in the Supporting Information). The contour plot of MO ⟨124⟩ is shown in Figure 2A. Importantly, this orbital is *antibonding* with respect to both the Fe−N(O) and N−O bonds!

Charge contributions are 15% d and 4% σ* (cf. Table 2), which corresponds to a weak antibonding interaction. This orbital is Fe−S σ bonding and also has porphyrin A_{2u} character.

Figure 2B illustrates schematically the orbital interactions for the Fe−N−O unit. In the case of complex **4a**, which is obtained by linearization of the Fe−N−O unit in **4**, no change to the composition of this orbital is observed. However, because of the increased overlap of d_{z^2} and σ* in this case, it is effectively stronger Fe−N(O) antibonding than in the bent case. Hence, the bending of the Fe−N−O moiety slightly decreases the unfavorable Fe−N antibonding interaction and, hence, the bent structure **4** is about 1.6 kcal/mol lower in energy than the corresponding linear structure **4a**. Further evidence that this unfavorable orbital interaction is, in fact, responsible for the bending comes from a comparison with [Fe(P)(MI)(NO)]$^+$ [**6a**; MI = 1-methylimidazole; *f*(Fe−N) = 4.82 mdyn/Å],[15] which is the corresponding complex with *trans*-MI coordination. In this case, no such antibonding Fe−N(O) interaction is found, as shown in Table 2, and the complex is, in fact, linear (both experimentally and theoretically).

Table 3. Experimental and Calculated Vibrational Properties of Ferric Heme Nitrosyl Adducts in Proteins and Model Complexes[a]

molecule	ν(N−O), cm^{-1}	ν(Fe−N), cm^{-1}	ref
1	1850, 1839	n.o.	7
2 (calcd)	1859	604	this work
SR(III)[b]	1828	510	16a
P450nor(III)−NO	1853	530	16b
N-donor trans ligand:			
Mb(III)−NO[b]	1927	595	16c,d
[Fe(TPP)(MI)(NO)]BF_4 (**6**)	1911		15
[Fe(OEP)(MI)(NO)]ClO_4 (**7**)	1921		17

[a] Complete table: Table S2 in the Supporting Information. [b] SR = picket fence porphyrin model complex with an axial thiolate ligand; Mb = myoglobin.

Another consequence of the σ-trans effect of the thiolate ligand is the low ν(N−O) stretching frequency, as was already mentioned above (cf. Table 3). The ν(N−O) mode of complex **1** is ~1850 cm^{-1} in solution (CH_2Cl_2) and 1839 cm^{-1} in solid state (reaction of [Fe(OEP)(S3)] powder with NO).[7] The calculated frequency for compound **2** is 1859 cm^{-1}, which is in excellent agreement with the experiment (calculated frequencies and force constants for N−O in models **2**−**5** are listed in Tables 1 and S2 in the Supporting Information). For P450nor, ν(N−O) is observed at 1853 cm^{-1} and ν(Fe−N) at 530 cm^{-1}. In comparison, ferric heme nitrosyls with trans N-donor coordination show ν(N−O) and ν(Fe−N) at 1927 cm^{-1} and 595 cm^{-1} ($Mb^{III}NO$), respectively. Model complex data are in agreement with this (cf. Table 3). Importantly, this shows that upon exchange of the trans N donor in ferric heme nitrosyls against a thiolate *both the Fe−N(O) and N−O vibrations decrease, implying that both bonds become weaker*. This direct correlation of Fe−N(O) and N−O bond strengths again shows that this must be related to a weakening of a σ bond[10] (a change in π backbonding would lead to an inverse correlation). This is another direct experimental proof that thiolate mediates a σ-trans effect on the coordinated NO. The reason for the lower ν(N−O) and ν(Fe−N) frequencies again relates to the interaction of d_{z^2} with σ* of NO shown in Figure 2, which weakens *both* the Fe−N and N−O bonds relative to the ferric heme nitrosyls with trans N-donor coordination.

The difference between axial N- and S-donor-coordinated ferric heme nitrosyls is due to a σ-trans effect of the coordinated S on the bound NO mediated by an NO σ* orbital. In this way, the Fe−N and N−O bonds are weakened, and Fe−N−O is bent in the S-coordinated case. This is reflected by lower ν(N−O) and ν(Fe−N) stretching frequencies in the presence of thiolate coordination.

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Supporting Information Available: MO diagram for compound **4**, complete table of vibrational properties of ferric heme NO complexes, figures and tables of Cartesian coordinates of the optimized structures of **2**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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