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First $\{Fe–NO\}^6$ Complex with an $N_2S_3Fe–NO$ Core as a Model of NO-Inactivated Iron-Containing Nitrile Hydratase. Are Thiolates and Thioethers Equivalent Donors in Low-Spin Iron Complexes?

Craig A. Grapperhaus,* Apurba K. Patra, and Mark S. Mashuta

Department of Chemistry, University of Louisville, Louisville, Kentucky 40292

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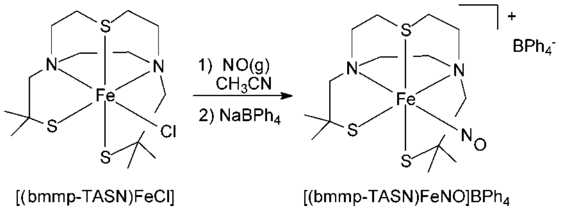
The spectroscopic and structural properties of [(bmmp-TASN)FeNO]BPh_4 (**1**) (bmmp-TASN = 4,7-bis(2′-methyl-2′-mercaptopropyl)-1-thia-4,7-diazacyclononane) have been determined and are compared with the nitric oxide inactivated form of iron-containing nitrile hydratase, $NHase_{dark}$. [(bmmp-TASN)FeNO]BPh_4 is prepared from the addition of NO to (bmmp-TASN)FeCl followed by addition of sodium tetraphenylborate. [(bmmp-TASN)FeNO]BPh_4 crystallizes from acetonitrile–methanol solutions upon ether vapor diffusion as dark blue plates in the monoclinic space group $P2_1/c$ with $a = 11.9521(14)$ Å, $b = 11.3238(13)$ Å, $c = 26.624(3)$ Å, $\beta = 98.280(2)^\circ$, and $Z = 4$. The ν_{NO} stretching frequency of 1856 cm^{-1} and the Mössbauer parameters, $\delta = 0.06$ mm/s and $\Delta E_q = 1.75$ mm/s, compare favorably with those of $NHase_{dark}$. The similarities of the iron–sulfur bond distances to the thiolate, 2.284(2) Å and 2.291(2) Å, versus thioether, 2.285(2) Å, are attributed to the low-spin configuration of the iron. The relationship between this structural observation and the spectroscopic properties of the complex are discussed.

The synthesis, isolation, and characterization of thiolato-metal complexes as models of cysteine-ligated metalloproteins are complicated by frequently undesirable sulfur-based reactivity. To avoid such spurious reactions, thioethers (RSR′) are sometimes employed in place of thiolates (RS^-) as the sulfur donor. The validity of substituting an anionic thiolate with a neutral thioether is questionable, although it is proposed that hydrogen-bonded thiolates in metalloenzymes possess thioether-like properties.[1]

Iron-containing nitrile hydratase (NHase) is a hydrolytic enzyme that catalyzes the conversion of nitriles to amides at a low-spin, non-heme iron(III) core.[2,3] Active $NHase_{light}$ reacts with endogenous nitric oxide to yield inactive $NHase_{dark}$, an $\{Fe-NO\}^6$ complex.[4-10] Activity is reinstated upon photolysis.[7,8] X-ray crystallographic studies of $NHase_{dark}$ reveal an unprecedented N_2S_3Fe-NO core with two amido nitrogen donors and three cysteine derived sulfurs.[4] Both of the cysteines *cis* to the NO binding site are posttranslationally modified yielding one sulfenato (RSO^-) and one sulfinato (RSO_2^-) donor, while the *trans* cysteine is unmodified.

Scheme 1



To mimic the reactive *cis*-dithiolato N_2S_3 donor environment at the active site of NHase, a dithiolato derivative of the facially coordinating N_2S ligand, TASN (1-thia-4,7-diazacyclononane), and its (chloro)iron(III) complex, (bmmp-TASN)FeCl, were prepared.[11] The synthetic protocol is similar to a trithiolato TACN iron(III) complex reported previously by Wieghardt *et al.*[12] In acetonitrile, (bmmp-TASN)FeCl reacts with NO, resulting in a rapid color change from blue to brown-violet, Scheme 1. Following ion ex-

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* To whom correspondence should be addressed. E-mail: grapperhaus@louisville.edu.

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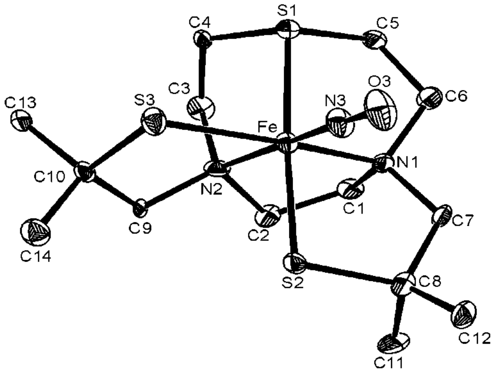

Figure 1. ORTEP[13e] view **1** showing 50% displacement ellipsoids. H atoms and the BPh_4^- anion have been omitted. Selected bond lengths (Å): Fe−N(1), 2.024(5); Fe−N(2), 2.063(5); Fe−N(3), 1.609(6); Fe−S(1), 2.285(2); Fe−S(2), 2.284(2); Fe−S(3), 2.291(2); N(3)−O(3), 1.167(6). Selected bond angles (deg): Fe−N(3)−O(3), 177.2(6); N(1)−Fe-N(2), 85.4(2); N(1)−Fe-S(1), 88.22(15); N(2)−Fe−S(1), 87.63(15); N(2)−Fe−N(3), 175.4(3).

change, [(bmmp-TASN)FeNO]BPh_4, **1**, is obtained as an air-stable, crystalline solid (64% yield). Recrystallization from acetonitrile/ether yields X-ray quality crystals.[13]

Refinement of the X-ray crystal structure of **1** confirms a N_2S_3Fe−NO central core, Figure 1. The iron ion sits in a pseudo-octahedral environment with the TASN backbone (N1, N2, S1) occupying one face. This positions the two thiolate sulfurs, S2 and S3, *cis* to one another and *cis* to the NO mimicking the positions of the modified cysteine sulfurs in $NHase_{dark}$. The thioether of the TASN ring, intended to mimic the unmodified cysteine thiolate, is positioned *trans* to the thiolate S2 and not the NO as in $NHase_{dark}$. It has been proposed that the thiolate *trans* to the NO site, the presumed substrate binding site, is crucial to the function of NHase.[14]

A comparison of the metric data from **1** and $NHase_{dark}$ reveals that the former reproduces accurately the metal−ligand bonds in the first coordination sphere in the enzyme. Table 1 lists comparable Fe−NO and average Fe−N and Fe−S bond distances. Selected bond distances and bond angles for **1** are provided in the caption of Figure 1. The tertiary amine donors in the model effectively reproduce the Fe−N distances in $NHase_{dark}$, 2.043(7) versus 2.07 Å, respectively. In general, the Fe−N bond distances in NHase are better modeled by tertiary amines than deprotonated amides or imines.[2,15] The average Fe−S distance in **1**, 2.287(3) Å, reproduces well the average Fe−S bond distance to

Table 1. Structural and Spectroscopic Comparisons of $NHase_{dark}$ (S = 0) and [(bmmp-TASN)FeNO]BPh_4 (**1**) (S = 0)

	$NHase_{dark}$	[(bmmp-TASN)FeNO]BPh_4 (**1**)
	Bond Length (Å)	
Fe−NO	1.65[4]	1.609(6)
Fe−Navg	2.07[4]	2.043(7)
Fe−Savg	2.30[4]	2.287(3)
	Bond Angle (°)	
Fe−N−O	158.6[4]	177.2(6)
	Infrared and Mössbauer Data	
ν_{NO} (cm^{-1})	1852[6]	1856
δ (mm/s)	0.03[9]	0.06
ΔE_q (mm/s)	1.47[9]	1.75

the three cysteine-derived sulfurs in $NHase_{dark}$, 2.30 Å. The high degree of structural similarity in the N_2S_3 core between the model and $NHase_{dark}$ is further reflected in the Fe−NO bond distance, 1.609(6) and 1.65 Å, respectively. While in **1** this bond is linear, 177.2(6)°, $NHase_{dark}$ contains a notably bent Fe−N−O bond, 158.6°, which is attributable to interactions with the so-called "claw" composed of serine112 and oxygen from the post-translationally modified cysteines.[4] The model lacks such second coordination sphere influences.

Of particular interest is the high degree to which **1** mimics the iron−sulfur bonds in $NHase_{dark}$. The Fe−$S_{thiolato}$ bond distances of 2.291(2) and 2.284(2) Å in **1** are statistically equivalent with the Fe−$S_{thioether}$ bond length of 2.285(2) Å. In the enzyme, there is no significant differentiation between the Fe−S bond distances for the post-translationally modified and unmodified cysteines consistent with similar observations by Darensbourg *et al.* in synthetic nickel complexes.[16,17] Similar results with iron have also been observed.[18] This is attributable to compensation of the decreased σ-donor ability of sulfur upon addition of electron-withdrawing oxygens by the elimination of a 4π-electron repulsion between the sulfur lone pair of the thiolate and the filled metal d-orbitals (t_{2g} in an O_h molecule) as first described by Lichtenberger *et al.*[16,19]

For octahedral, low-spin iron(II)-thiolates, t_{2g}^6, a π-repulsion between the filled ligand p-orbitals (lone pairs) and the metal d-orbitals lengthens the Fe−S bond distance. The effect would be similar, but somewhat lessened, for low-spin iron(III), t_{2g}^5. Upon alkylation, the sulfur lone pair is contained in the sulfur−carbon σ-bond, thus eliminating the π^*-interaction. Coincidentally, this is equally compensated by the decrease in σ-donor ability of the sulfur upon charge

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(13) (a) Crystal data for **1**: dark blue plate, monoclinic, space group $P2_1/c$, a = 11.9521(14) Å, b = 11.3238(13) Å, c = 26.624(3) Å, β = 98.280(2)°, V = 3565.9(7) $Å^3$, D_{calcd} = 1.352 g cm^{-3}, Z = 4. Data were collected on a Bruker Smart APEX CCD using Mo Kα radiation. For all 4034 unique reflections (R(int) = 0.062), the final anisotropic full-matrix least-squares refinement on F^2 for 429 variables data converged at R1 = 0.084 and wR2 = 0.13 with a GOF of 1.015. (b) Sheldrick, G. M. SHELXS-90. *Acta Crystallogr.* **1990**, *A46*, 467. (c) Sheldrick, G. M. *SHELXL-97, Program for the Refinement of Crystal Structures*; University of Göttingen: Göttingen, Germany, 1997. (d) *SHELXTL 6.12*, *Program Library for Structure Solution and Molecular Graphics*; Bruker Advanced X-ray Solutions: Madison, WI, 2001. (e) Farrugia, L. J. *ORTEP-3 for Windows*, *J. Appl. Crystallogr.* **1997**, *30*, 565.

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neutralization yielding the similarity in Fe−S bond distances for coordinated thiolates and thioethers for low-spin complexes. In the case of octahedral, high-spin iron (II), $t_{2g}^4e_g^2$, the presence of two holes in the t_{2g} metal orbitals significantly reduces the π*-interaction in the thiolato form. Upon alkylation, the charge neutralization effect dominates, and the thiolate and thioether to iron bond lengths are significantly different. In the high-spin complex [{(bmmp-TASN)-Fe}$_2$(μ-O)], the average Fe−$S_{thiolate}$ distance is 2.354(2) Å while the Fe−$S_{thioether}$ distance is 2.605(1) Å.[11]

The high degree to which **1** reproduces structural features of the first coordination sphere of $NHase_{dark}$ suggests that, for low-spin iron complexes, thioethers may serve as effective *structural* mimics of thiolates. It is not implicit, however, that this legitimacy applies to electronic properties as well. That is, the Fe−S bond distance reports the strength of the metal−ligand bond but does not explicitly detail the nature of the bond. This is addressed by spectroscopic, not structural, investigations.

For **1**, a ν_{NO} stretching frequency of 1856 cm^{-1} is observed. This provides the best spectroscopic model to date of $NHase_{dark}$, 1852 cm^{-1}.[2,6,10,15b] These values are in the range typical for $\{MNO\}^6$ complexes which may be regarded as $Fe^{IV}-NO^-$, $Fe^{III}-NO^\bullet$, or $Fe^{II}-NO^+$. As the latter is analogous to a metal carbonyl, ν_{NO} is a function of the back-donating ability of the metal. This suggests the electronic properties of the iron in **1** are similar to those in $NHase_{dark}$.

Mössbauer spectroscopy effectively probes the electronic properties of the iron ion. Compound **1** displays an isomer shift, 0.06 mm/s, and quadrupole splitting, 1.75 mm/s, which reproduce well those recently reported for $NHase_{dark}$, 0.03 and 1.47 mm/s, respectively.[9] The quadrupole splitting value confirms a low-spin configuration. The small value of the isomer shift in $NHase_{dark}$ has been attributed to a high formal oxidation state, iron(IV), on the basis of comparisons to a series of iron−nitrosyl complexes reported by Wieghardt *et al*.[20]

While unequivocal oxidation state assignments of iron−nitrosyl complexes are beyond the scope of the current study, the similarity of the thiolate and thioether donors in **1** suggests high occupancy of the metal t_{2g} orbitals, iron(II). A low-spin, iron(II), $N_2S_3Fe^{II}-CO$ complex with two thiolate and one thioether donor has recently been reported by Sellman *et al*. with Fe−N and Fe−S bond distances nearly identical to those of **1**.[21] However, no Mössbauer spectrum of the carbonyl complex was reported.[21] While it is tempting to assign the oxidation state of iron in **1** on the basis of structural similarities to $N_2S_3Fe^{II}-CO$, the small variations of the ionic radius of low-spin iron II, III, and IV ions preclude any convincing assignment of formal oxidation states on the basis of M−L bond distances.[22]

In summary, thiolates may be substituted by thioethers in low-spin iron complexes without compromising the integrity of *structural* models of thiolato-ligated metalloproteins. In the case of **1**, the dithiolato−thioether donor set also reproduces, to a first approximation, the donor ability of the thiolato, sulfenato, sulfinato donor set in $NHase_{dark}$. This net electronic similarity likely results from replacing two *S*-oxygenates in $NHase_{dark}$ with one thioether (*S*-alkylate) in **1**. This is consistent with reports that *S*-alkylation of *nickel−dithiolates* results in no large *structural* changes, although significant electronic effects, shifts in redox potentials as large as 1.4 V, are observed.[23] While **1** is the best mimic of $NHase_{dark}$ to date, inclusion of *S*-oxygenation, and possible second coordination sphere effects that may prove important to photoreversibility of NO-binding, remains a challenge in modeling $NHase_{dark}$. The photostability and solution air sensitivity of **1** are under investigation.

Whether such a tempting structural substitution of thioether for thiolate is applicable to other metal ions with fully occupied t_{2g} orbitals remains under investigation. However, caution should be applied when substituting a thioether for a thiolate with appropriate considerations of the influence on the electronic structure and the ramifications of loss of ligand-based reactivity that may be crucial for function.

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Supporting Information Available: Experimental procedures for the synthesis of **1**, Mössbauer and infrared spectra, X-ray structural data, and tables of atomic coordinates, bond lengths and angles, anisotropic displacements, and hydrogen coordinates. This material is available free of charge via the Internet at http://pubs.acs.org.

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