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Mononitrosyl Non-Heme Diiron Complexes: Importance of Iron Oxidation State in Controlling the Nature of the Nitrosylated Products

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Abstract

Mononitrosyl diiron complexes having either an $[\text{Fe}^{\text{II}}\{\text{FeNO}\}^7]$ or an $[\text{Fe}^{\text{III}}\{\text{FeNO}\}^7]$ core formulation have been synthesized by methods that rely on redox state-induced differentiation of the diiron starting materials in an otherwise symmetrical dinucleating ligand environment. The synthesis, X-ray structures, Mössbauer spectroscopy, cyclic voltammetry, and dioxygen reactivity of the $[\text{Fe}^{\text{III}}\{\text{FeNO}\}^7]$ is described.

Nitric oxide is a major component of the immune defense system in macrophages.¹ Although the immune system can generate micromolar concentrations of NO, many pathogenic microbes such as *T. vaginalis*,² *E. coli*,^{3,4} and *N. meningitidis*^{5,6} reduce NO to N_2O ⁷ by expressing flavodiiron nitric oxide reductases (FNORs).⁸ Crystallographic studies^{9–11} of these enzymes reveal active sites (*D. gigas*)⁹ comprising two non-heme iron centers in an unsymmetrical coordination environment provided by histidine, glutamate, aspartate, and water-based ligands. The active site binds NO to generate a high-spin $\{\text{FeNO}\}^7$ species with total spin $S = 3/2$.^{8,11,12} Although two conflicting mechanisms involving both mono and dinitrosyl diiron intermediate species have been proposed for this chemistry,^{8,13,14} recent results favor the mononitrosyl.^{11,15} These reports include formation of a stable mononitrosyl adduct, formed by addition of one equiv of NO per diiron(II) site of both FMN-free¹⁰ and FMN-containing flavin diiron proteins.¹⁵

Reactions of NO with synthetic non-heme diiron complexes have been known for over 15 years.^{13,16} The first report of a dinitrosyl compound, $[\text{Fe}_2(\text{N-Et-HPTB})(\text{PhCOO})(\text{NO})_2](\text{BF}_4)_2$ (**5a**),¹⁷ where *N-Et-HPTB* is the anion of *N,N,N',N''*-tetrakis(2-(1-ethylbenzimidazolyl))-2-hydroxy-1,3-diaminopropane, was followed by the study of an analogous compound, $[\text{Fe}_2(\text{BPMP})(\text{OPr})(\text{NO})_2](\text{BPh}_4)_2$ (**5b**).¹⁸ Compounds **5a**¹⁷ and **5b**¹⁸ represent $\{\text{Fe}(\text{NO})^7\}_2$ species ($S = 3/2$) and **5b** could be reduced by two electrons, leading to formation of N_2O .¹⁸ Although formation of an EPR silent $[\text{Fe}^{\text{III}}\{\text{FeNO}\}^7]$ species was postulated during oxidation of deoxyhemerythrin to the semi-met form by nitrite ion,¹⁹ there is no report to date of any synthetic mononitrosyl diiron compound coordinated by an O/N-donor dinucleating ligand system. Here we describe the synthesis and characterization of the two such compounds in this class and the importance of the iron oxidation state in controlling the nature of the resulting nitrosylated diiron products.

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Supporting Information

X-ray crystallographic data for **3**, **4a–4d** in CIF format, structural tables, ORTEP diagrams, cyclic voltammetry, Mössbauer, UV-Vis and IR spectroscopic data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Compounds **5a** and **5b** were stable in solution only in the presence of excess NO gas, and formation of mononitrosyl diiron species was never observed.^{17,18} We discovered, however, that with use of a mixed valent diiron(II,III) compound to be reported in detail else-where, $[\text{Fe}_2(\text{N-Et-HPTB})(\text{PhCOO})(\text{DMF})_2](\text{BF}_4)_3$ (**2**),²⁰ addition of one equiv of trityl nitrosothiol (Ph_3CSNO) yielded a mixture of two of four crystalline forms of the compound $[\text{Fe}_2(\text{N-Et-HPTB})(\text{NO})(\text{OH})(\text{DMF})_2](\text{BF}_4)_3$, **4c** and **4d** (Scheme 1). There are slight differences in the core structures of **4c** and **4d** (Table 1, Fig. S1), but they are otherwise chemically identical. The product was con-taminated with $[\text{Fe}_2(\text{N-Et-HPTB})(\text{PhCOO})](\text{BF}_4)_2$ (**6**),²¹ as evidenced by Mössbauer spectroscopy (Fig. S2) and by an X-ray structure determination of light yellow crystals (**6**) found in the product. To avoid these complications, alternative synthetic routes were explored.

A reaction system containing $[\text{Fe}_2(\text{N-Et-HPTB})(\text{PhCOO})](\text{BF}_4)_2$ (**6**)²¹ and one or two equiv of Ph_3CSNO generated a green solution which, upon work-up, afforded only the starting material **6**, as determined by IR spectroscopy and X-ray crystallography. This result is consistent with the chemistry of **5a** and **5b**, from which NO slowly dissociates.^{17,18} A different diiron(II) starting compound, $[\text{Fe}_2(\text{N-Et-HPTB})(\text{DMF})_4](\text{BF}_4)_3$ (**1**),²⁰ was therefore employed. Reaction of one equiv of Ph_3CSNO with **1** yielded $[\text{Fe}_2(\text{N-Et-HPTB})(\text{NO})_{0.6}(\text{DMF})_{3.4}](\text{BF}_4)_3$ (**3**) in 66% yield, in which one of the two iron centers contains NO (59.7%) or DMF (40.3%), disordered in the crystal lattice. Compound **3** contains a $[\text{Fe}^{\text{II}}\{\text{FeNO}\}^7]$ site and should be regarded as a mixture of ~60% $[\text{Fe}^{\text{II}}\{\text{FeNO}\}^7]$ species and ~40% Fe^{II}_2 species. Reaction of **1** with Ph_3CSNO in the presence of the one electron oxidant, $(\text{Cp}_2\text{Fe})(\text{BF}_4)$, however, afforded the different mononitrosyl compound, **4a**, as greenish-brown crystals in 45% yield. An alternative method, reaction of **1** with $\text{NO}(\text{BF}_4)$, gave **4b** in 90% yield. This reaction may proceed by formal one-electron oxidation of **1** to yield a diiron(II,III) species that is rapidly quenched by NO generated in situ, which adds to the Fe(II) center. The results of these reactions, taken together (Scheme 1), along with the previous report of dinitrosylation that occurs during reaction of diiron(II) compounds with excess NO gas,^{17,18} clearly establish the crucial role of iron oxidation states within these diiron compounds in determining the nature of the nitrosylated products.

Compounds **4a–4d** contain the same $[\text{Fe}^{\text{III}}\{\text{FeNO}\}^7]$ center, with identical chemical formulas, $\text{Fe}_2(\text{N-Et-HPTB})(\text{NO})(\text{OH})(\text{DMF})_2](\text{BF}_4)_3$, and identical electronic absorption spectra (Fig. S3). They display small to moderately different structural features that most likely arise from the variable solvent molecules trapped in the crystal lattice. Selected bond lengths, angles, and NO stretching frequencies for these $[\text{Fe}^{\text{III}}\{\text{FeNO}\}^7]$ complexes (**4a–4d**) and the $[\text{Fe}^{\text{II}}\{\text{FeNO}\}^7]$ complex, **3** are collected in Table 1 (see Fig. S4 for IR spectra). Relevant structural parameters and ν_{NO} for the complexes **4a–4d** are quite similar to that reported for **5a** and **5b** (Table 1, footnote). In the preparation of **4c** and **4d**, the bridging benzoate of **2** is lost upon reaction with Ph_3CSNO , and a hydroxide ion, generated from water in the starting materials, binds terminally to the non-nitrosylated iron center. As a result of the benzoate loss, the Fe–Fe separations increase to 3.622 Å (**4c**) and 3.615 Å (**4d**), compared with 3.575 Å in **2**. The Fe–O_{N-Et-HPTB} distances are distinctly different (Table 1) for the two iron centers in **4a–4d**, reflecting the different oxidation states of the metal and in a manner similar, but not identical, to that observed for the diiron(II,III) compound, **2**.²⁰ The presence of short and long Fe–O_{N-Et-HPTB} bond distances that are not as extreme as those in **2** (Table 1) is consistent with the $[\text{Fe}^{\text{III}}\{\text{FeNO}\}^7]$ formulation of the iron centers in **4a–4d**. Mössbauer parameters obtained for **3** (Fig. 2), **4a** (Fig. 2), and **4b–4d** (Fig. S2 and S5) are very similar to that observed previously for **5a**¹⁷ ($\delta = 0.67$ mm/s, $\Delta E_{\text{Q}} = 1.44$ mm/s), indicating presence of a $\{\text{Fe}(\text{NO})\}^7$ species in both **3** and **4a–4d**. Compound **3** should give rise to a doublet for Fe (II) site with 70% relative area and a doublet for

{FeNO}⁷ having 30% relative area. The observed data (Fig. 2) reveal 64% Fe(II) site and 36% {FeNO}⁷ site, is in close agreement with this prediction.

In DMF solution, compound **4a** displays two irreversible reductions (Fig. 3, see Fig. S7 for reproducibility in repeated scans); identical results were obtained for **4b**. By comparison to the presence of the irreversible, two-electron reduction in the dinitrosyl diiron compound, **5b**, at -1.1 V,¹⁸ the reduction of **4a** at -1.14 V can be assigned to the {Fe(NO)}⁷ center. The reduction at -0.64 V therefore corresponds to the Fe^{III}(OH) center. Reduction of the {Fe(NO)}⁷ unit in **4a** might lead to partial release of NO⁻ and generation of an additional Fe(II) center in solution. Such behavior would possibly account for the presence of two oxidative waves on the reverse sweep (Fig. 3), one at -0.59 V and the other at -0.85 V, corresponding to oxidation of {Fe(NO)}⁷ and denitrosylated material. One additional feature in the CV is a small oxidation at -0.04 V, which we tentatively assign to restoration of an Fe^{III}(OH) unit.

In DMF solution, **4a** exhibits a broad electronic absorption spectroscopic feature centered at 600 nm ($\epsilon = 225 \pm 6$), a shoulder at 490 nm ($\epsilon = 377 \pm 9$), another weak shoulder at 420 nm ($\epsilon = 1424 \pm 42$), and a strong UV band at 302 nm ($\epsilon = 5910 \pm 57$) (Fig. 4, Fig. S3). This solution reacts rapidly with O₂ as revealed by a change in color from light brownish-green to dark bluish-green ($\lambda_{\text{max}} = 600$ nm, $\epsilon = 725 \pm 17$; Fig. 4a). The green solution is unstable at room temperature and full bleaching of the 600 nm band occurs within 5 h (Fig. 4b). A similar situation occurs for solutions of **4b–4d** (Fig. S6), consistent with their identical chemical nature. The identity of the green species is currently under investigation.

In conclusion, mononitrosyl diiron compounds having an [Fe^{III}.{FeNO}⁷] formulation and with slightly varying Fe—N—O bond angles have been synthesized and characterized. The synthetic strategy used to achieve such mononitrosyl complexes relied on the presence of mixed valent character in the initial diiron core. Diiron(II) starting materials under identical conditions yielded either no or only partial nitrosylation to form products with a formal[Fe^{II}.{FeNO}⁷] core. The work described here reveals the important role of iron oxidation states for controlling the nature of the nitrosylation in diiron complexes in a symmetrical ligand environment. It is possible that FNORs might adopt a similar strategy to generate mononitrosyl adducts required for N₂O formation. Further experiments, including studies of the photoreactivity of the mononitrosyl diiron complexes and their reaction with NO gas are currently in progress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- (1). Missall TA, Lodge JK, McEwen JE. Eukaryot. Cell. 2004; 3:835–846.
- (2). Sarti P, Fiori PL, Forte E, Rappelli P, Teixeira M, Mastronicola D, Sanciu G, Giuffrè A, Brunori M. Cell. Mol. Life Sci. 2004; 61:618–623. [PubMed: 15004700]
- (3). Gardner AM, Helmick RA, Gardner PR. J. Biol. Chem. 2002; 277:8172–8177. [PubMed: 11751865]
- (4). Gomes CM, Giuffrè A, Forte E, Vicente JB, Saraiva LM, Brunori M, Teixeira M. J. Biol. Chem. 2002; 277:25273–25276. [PubMed: 12101220]

- (5). Anjum MF, Stevanin TM, Read RC, Moir JWB. *J. Bacteriol.* 2002; 184:2987–2993. [PubMed: 12003939]
- (6). Stevanin TM, Moir JWB, Read RC. *Infect. Immun.* 2005; 73:3322–3329. [PubMed: 15908358]
- (7). Moënné-Loccoz P, Fee JA. *Science*. 2010; 330:1632–1633. [PubMed: 21164002]
- (8). Kurtz DM Jr. *Dalton Trans.* 2007:4115–4121.
- (9). Frazao C, Silva G, Gomes CM, Matias P, Coelho R, Sieker L, Macedo S, Liu MY, Oliveira S, Teixeira M, Xavier AV, Rodrigues-Pousada C, Carrondo MA, Le Gall J. *Nat. Struct. Mol. Biol.* 2000; 7:1041–1045.
- (10). Silaghi-Dumitrescu R, Kurtz DM Jr, Ljungdahl LG, Lanzilotta WN. *Biochemistry*. 2005; 44:6492–6501. [PubMed: 15850383]
- (11). Hayashi T, Caranto JD, Wampler DA, Kurtz DM Jr, Moënné-Loccoz P. *Biochemistry*. 2010; 49:7040–7049. [PubMed: 20669924]
- (12). Brown CA, Pavlosky MA, Westre TE, Zhang Y, Hedman B, Hodgson KO, Solomon EI. *J. Am. Chem. Soc.* 1995; 117:715–732.
- (13). Berto TC, Speelman AL, Zheng S, Lehnert N. *Coord. Chem. Rev.* 2013; 257:244–259.K.
- (14). Blomberg LM, Blomberg MA, Siegbahn PM. *J. Biol. Inorg. Chem.* 2007; 12:79–89. [PubMed: 16957917]
- (15). Hayashi T, Caranto JD, Matsumura H, Kurtz DM Jr, Moënné-Loccoz P. *J. Am. Chem. Soc.* 2012; 134:6878–6884. [PubMed: 22449095]
- (16). Wasser IM, de Vries S, Moënné-Loccoz P, Schröder I, Karlin KD. *Chem. Rev.* 2002; 102:1201–1234. [PubMed: 11942794]
- (17). Feig AL, Bautista MT, Lippard S. *J. Inorg. Chem.* 1996; 35:6892–6898.
- (18). Zheng S, Berto TC, Dahl EW, Hoffman MB, Speelman AL, Lehnert N. *J. Am. Chem. Soc.* 2013; 135:4902–4905.
- (19). Nocek JM, Kurtz DM, Pickering RA, Doyle MP. *J. Biol. Chem.* 1984; 259:12334–12338. [PubMed: 6092330]
- (20). Majumdar A, Apfel U-P, Jiang Y, Moënné-Loccoz P, Lippard SJ. Submitted for Publication. 2013
- (21). Dong Y, Menage S, Brennan BA, Elgren TE, Jang HG, Pearce LL, Que L Jr. *J. Am. Chem. Soc.* 1993; 115:1851–1859.

Scheme 1.

Schematic depiction for the synthesis of mononitrosyl complexes. Complexes **4a-4d** are chemically equivalent but possess different Fe—N—O angles (Table 1). Also shown are the ligand and immediate coordination environment of **3**, **4a** and **5a**.

Figure 1. Molecular structures of **3** and **4a** showing 50% probability thermal ellipsoids with partial atom labeling schemes. Hydrogen atoms are omitted (except for OH group) for clarity. Note that NO is in partial occupancy disorder with DMF at Fe1 of **3**. See Fig. S1 for **4b-4d**.

Figure 2.
⁵⁷Fe Mössbauer spectra for solid samples of **3** and **4a** at 77K.

Figure 3.

Cyclic voltammetric trace for **4a** in DMF. Conditions: GC working, Pt auxiliary, and Ag pseudo electrode; scan rate = 100 mV/s; [**4a**] = 1 mM; $[(\text{Bu}_4\text{N})(\text{PF}_6)] = 0.1 \text{ M}$.

Figure 4. Electronic absorption spectroscopic monitoring for the reaction of **4a** (0.2 mM in DMF, brown line) with oxygen at r.t.: (a) Generation of a green solution, 1 min/scan, 18 min; (b) decomposition of the green solution, 15 min/scan, 5h.

Table 1

Selected bond distances, angles, and NO stretching frequencies

compounds	3	4a	4b	4c	4d
Fe—N (Å)	1.762(1)	1.787(2)	1.764(4)	1.794(4)	1.790(4)
N—O (Å)	1.118(2)	1.110(4)	1.109(6)	1.100(6)	1.147(6)
Fe—O _L (Å)	2.032(3)	1.952(2)	1.951(3)	1.958(3)	1.965(3)
	2.020(3)	2.044(2)	2.048(3)	2.041(3)	2.044(3)
Fe—OH (Å)	-----	1.832(2)	1.828(3)	1.837(3)	1.832(3)
Fe—Fe (Å)	3.678	3.621	3.630	3.622	3.615
Fe—O _L —Fe (°)	130.4(2)	130.0(1)	130.4(2)	129.8(2)	128.7(2)
Fe—N—O (°)	157.8(1)	171.0(3)	168.5(4)	166.9(5)	174.4(5)
ν_{NO} (cm ⁻¹)	1772	1792	1792	1792	1804

1²⁰: Fe—O_L (Å) = 2.012(2), 2.063(2); Fe—Fe (Å) = 3.716; Fe—O—Fe (°) = 131.56(10). **2**²⁰: Fe—Oligand (Å) = 1.929(2), 2.047(2); Fe—Fe (Å) = 3.575; Fe—O—Fe (°) = 128.06(10). **5a**¹⁷: Fe—N_{av} (Å) = 1.750(1); N—O_{av} (Å) = 1.154(4); Fe—N—O (°) = 166.6(7), 168.3 (7); ν_{NO} (cm⁻¹) = 1785. **5b**¹⁸: Fe—N(Å) = 1.774(2), 1.796(3); N—O (Å) = 1.156(3), 1.172(3); Fe—N—O (°) = 155.4 (2), 144.7(2); ν_{NO} (cm⁻¹) = 1760.