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Phenol Nitration Induced by a {Fe(NO)₂}¹⁰ Dinitrosyl Iron Complex

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Abstract

Cellular dinitrosyl iron complexes (DNICs) have long been considered NO carriers. Although other physiological roles of DNICs have been postulated, their chemical functionality outside of NO transfer has not been demonstrated thus far. Here we report unprecedented dioxygen reactivity of an N-bound {Fe(NO)₂}¹⁰ DNIC, [Fe(TMEDA)(NO)₂] (1). In the presence of O₂, 1 becomes a nitrating agent that converts 2,4,-di-*tert*-butylphenol to 2,4-di-*tert*-butyl-6-nitrophenol via formation of a putative iron-peroxynitrite [Fe(TMEDA)(NO)(ONOO)] (2) that is stable below –80 °C. Iron K-edge X-ray absorption spectroscopy on 2 supports a five-coordinated metal center with a bound peroxynitrite in a cyclic bidentate fashion. The peroxynitrite ligand of 2 readily decays at increased temperature or under illumination. These results suggest that DNICs could have multiple physiological or deleterious roles, including that of cellular nitrating agents.

Dinitrosyl iron complexes (DNICs), Chart 1, are naturally occurring iron species that are generated from the reactions of nitric oxide (NO) with cellular nonheme iron species such as iron-sulfur clusters. 1,2 A series of S- or N-bound DNICs have been reported $^{2-6}$ since the initial discovery 7 of a cysteine-bound DNIC formulated as $[Fe(NO)_2(SR)_2]^-$, an $\{Fe(NO)_2\}^9$ species in the Enemark-Feltham notation. 8 Although EPR-active S-bound $\{Fe(NO)_2\}^9$ DNICs are more common, N- or O- bound DNICs have been also observed. 9

Several physiological roles of DNICs have been suggested, including storage and transfer of NO. 1,10 However, the nature of the chemistry that allows DNICs to play these physiological roles is not well understood. A notable recent report from the Lippard group suggests that the NO-donating ability of an N-bound DNIC might be $\{Fe(NO)_2\}^9/\{Fe(NO)_2\}^{10}$ redox-dependent. While such a proposal offers important chemical insights, we were intrigued by the highly reducing nature of $\{Fe(NO)_2\}^{10}$ DNICs, which may suggest a rich chemistry between these motifs and oxidants such as O_2 . Such reactivity could shed light on undiscovered physiological or deleterious roles of DNICs. Herein, we describe unprecedented dioxygen reactivity of an N-bound $\{Fe(NO)_2\}^{10}$ DNIC, $[Fe(TMEDA)(NO)_2]$ (1), where TMEDA = N,N,N',N'-tetramethylethylenediamine (Chart 1), and we demonstrate the formation of an intermediate that leads to nitration of phenol.

Compound 1 was synthesized as previously reported by Hung et al.^{5d} Bubbling of O₂ through a solution of 1 in dichloromethane at -80 °C leads to the formation of an EPRsilent, dark purple complex with absorption bands at 460 ($\varepsilon = 420 \,\mathrm{M}^{-1} \mathrm{cm}^{-1}$) and 560 nm (ε = $580 \,\mathrm{M^{-1}cm^{-1}}$), Figure 1A. This purple complex is stable below $-80 \,\mathrm{^{\circ}C}$, but it decomposes to an orange, insoluble precipitate upon warming. 11,12 IR monitoring of this O₂-reaction also shows the generation of a new quasi-stable species with the appearance of two new v_{NO} peaks at 1589 and 1805 cm⁻¹ concomitant with the disappearance of the v_{NO} of 1 at 1630 and 1687 cm⁻¹ (Figure 1B).¹³ No other significant change occurs in the midfrequency region of the IR spectra. This transformation is very different from what was observed by Tonzetich et al. 6 in the case of another N-bound $\{Fe(NO)_2\}^{10}$ DNIC with a monoanionic bidentate β -diketiminate ligand. In that study, air exposure led to the oneelectron oxidation of the {Fe(NO)₂}¹⁰ DNIC to an EPR-active {Fe(NO)₂}⁹ DNIC, which was also signified by >130 cm⁻¹ upshifts of the two IR-active NO stretching frequencies. Here, the intermediate generated from 1/O₂ is EPR silent and it exhibits an up- (1805 cm⁻¹) and a downshifted (1589 cm⁻¹) NO band. These data are inconsistent with the simple oneelectron oxidation of 1.¹⁴ The possibility of generating a five-coordinate superoxide (O_2^-) adduct, [Fe(TMEDA)(NO)₂(O₂)], has been considered. Although such a species is not known, an iodide (I⁻) analogue, [Fe(TMEDA)(NO)₂(I)], has been reported to have two upshifted v_{NO} frequencies higher than 1700 cm^{-1,15} which is again different from what we observe in the $1/O_2$ reaction. ¹⁶ Interestingly, the v_{NO} at 1589 cm⁻¹ closely matches those from known trans-peroxynitrite species, O=NOOM, where M = Li, Na, K. 17 These IR and EPR characteristics, along with the EXAFS data (vide infra), led us to consider the intermediate to be a peroxynitrite bound iron mononitrosyl species, [Fe(TMEDA)(NO) $(ONOO)] (2).^{18}$

Iron K-edge X-ray absorption spectroscopy was used to further probe complex 2. Comparison of the edge energies of 1 and 2 shows a shift by +1.8(4) eV upon O_2 exposure to cold solutions of 1 (Figure 2A), which is consistent with the formal oxidation of 1 by 1 electron. The Fe $1(s) \rightarrow 3(d)$ transition found in the pre-edge region of 2 has a peak area slightly larger than that corresponding to 1 (35(1) vs. 36(1) eV% relative to the edge height). This is most consistent with 2 having an Fe-center contained in a non-centrosymmetric coordination environment. The EXAFS region for 2 is best modeled as a five coordinate Fespecies with a coordinated bidentate O_2NO^- moiety (Figure 2B). We find strong multiple-scattering (MS) pathways originating from this cyclic O_2NO^- ligand (average N/O distance 1.91(1) Å; see inset Figure 2B). In addition, strong MS pathways are also found originating from the NO ligand (1.67(1) Å). Two additional N-scatters derived from the TMEDA ligand are also observed. The thermal decomposition product resulting from warming of 2 to room temperature yields a further shift in the edge energy of +2.6(3) eV, consistent with the production of an Fe $^{2+/3+}$ species (Figure 2A).

Attempts at characterization of **2** by resonance Raman spectroscopy were hampered by photosensitivity. The decomposition of **2** with white light was monitored at -90 °C by UV-Vis spectroscopy but it failed to reveal new absorption features (Figure S5). In the IR, continuous white-light illumination of **2** leads to the progressive and irreversible bleaching of the 1581 and 1807 cm⁻¹ bands with concomitant appearance of a single band at 1744 cm⁻¹ (Figure 3). The loss of the IR band at 1581 cm⁻¹ and the appearance of a new v_{NO} at 1744 cm⁻¹ suggest that illumination of **2** might lead to the formation of a new ironmononitrosyl species after photochemistry at the peroxynitrite ligand. Like **2**, the photoproduct is EPR silent at 10 K (data not shown). While the nature of the photoproduct remains in doubt, ¹⁹ the photoactive behavior of **2** is reminiscent of $[Co(CN)_5(ONOO)]^{3-}$, where photolysis destroys the coordinated $ONOO^{-}$.

The presence of peroxynitrite is often indicated by oxidation and/or nitration chemistry especially with phenolic substrates. ²¹ When one equiv. of 2,4-di-tert-butylphenol (DBP) is added to 2 and the reaction mixture is subsequently warmed to room temperature, 2,4-ditert-butyl-6-nitrophenol (NO₂-DBP) is observed along with the oxidative coupling product 2,2'-dihydroxy-3,3',5,5'-tetra-t-butyl-1,1'biphenyl (Scheme 1).²² These reaction products do not form when DBP is added after warming the solution of 2 to room temperature, signifying that intermediate 2 is a crucial species in phenol nitration and oxidation. At room temperature, 2 is too unstable to be monitored by regular UV-Vis spectroscopy, although a glimpse of purple color can be seen momentarily. Despite the short lifetime of 2 at room temperature, when O₂ is added to a mixture of 1 and DBP (1 equiv) at room temperature nitration chemistry still occurs, yielding NO₂-DBP, while only a small amount (2%) of the oxidative coupling product is generated. This indicates that 1/O2 induces nitration much more efficiently than oxidation at room temperature. ²³ When ¹⁸O₂ is used, approximately 50 % of 18 O atom incorporates into the substrate. GC-MS analysis of NO₂-DBP displays a distribution of 18 O₂N-DBP, $^{18/16}$ O₂N-DBP, and 16 O₂N-DBP in a ratio of 30(2)%, 48(1)%, and 22(2)%. ¹² Although the mechanistic investigation of phenol nitration by 2 is beyond the scope of this report, the O atom isotope distribution in NO₂-BDP warrants future mechanistic studies and likely involves the cleavage of peroxynitrite and participation of the other NO.24

In summary, we have described unprecedented O_2 reactivity of an $\{Fe(NO)_2\}^{10}$ irondinitrosyl complex $[Fe(TMEDA)(NO)_2]$ (1). In the presence of O_2 , 1 becomes a potent nitrating agent via formation of a putative iron-peroxynitrite species. The O_2 reactivity of 1 demonstrated here suggests that the physiological functions of DNICs are not limited to NO storage and transfer and deserve further studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

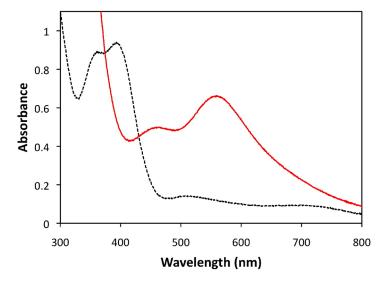
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- 11. The presence of nitrite (NO₂⁻), Fe, and TMEDA in the precipitate has been qualitatively confirmed. ¹² Further speculation concerning the nature of decomposition product(s) is not warranted.
- 12. See Supporting Information.
- 13. Synthesis of the intermediate using 15 NO shows clear IR shift to lower energy supporting the v_{NO} assignments and formation of putative [Fe(TMEDA)(NO)(ONOO)] (2) (vide infra). 12
- 14. Reversible $\{Fe(NO)_2\}9/10$ redox behavior of $\bf 1$ is observed in cyclic voltammetry, with $E_{1/2}=-0.527$ V (vs. ferrocene/ferrocenium). Chemical oxidation of $\bf 1$ by I_2 has been shown to yield a five-coordiate compound $[Fe(TMEDA)(NO)_2I].15$ Attempts to isolate a four-coordinate counterpart, $[Fe(TMEDA)(NO)_2]+$, were not successful probably due to its strong preference to be five-coordinate, as was previously discussed. 15
- 15. v_{NO} = 1773 and 1719 cm⁻¹ in diethyl ether. Chen CH, Ho YC, Lee GH. J Organomet Chem. 2009; 694:3395–3400.
- 16. Even more evidence to disfavor a superoxide adduct formation includes the lack of an isotope sensitive v_{O-O} in the region of ~1100 cm⁻¹ (Figure 1b) and the inconsistent EXAFS data for such a model. See Supporting Information for alternative EXAFS fitting results.
- 17. $v_{NO} = 1528-1581$ cm⁻¹ for trans-MOONO, where M = K, Na, and Li. Lo WJ, Lee YP, Tsai JHM, Tsai HH, Hamilton TP, Harrison JG, Beckman JS. J Chem Phys. 1995; 103:4026–4034.

18. The two v_{NO} of **2** at 1589 and 1805 cm⁻¹ remain unchanged upon $^{18}O_2$ substitution. Characterization of **2** by ESI-MS was attempted without success due to the insufficient stability of **2**.

- 19. The EPR silent character of the photoproduct suggests a diamagnetic or integer spin species. This electronic structure may reflect the iron-mononitrosyl complex or the overall spin of this complex coupled with the nearby product of the photolyzed peroxynitrite. For an analogous situation in a metalloprotein, see the EPR studies {FeNO}⁶ myoglobin complexes, where the photolyzed NO (S =1/2) remains spin-coupled with the high-spin iron(III) (S=5/2). Hori H, Ikeda-Saito M, Lang G, Yonetani T. J Biol Chem. 1990; 265:15028–15033. [PubMed: 2168399] Hori H, Masuya F, Dou Y, Ikeda-Saito M. J Inorg Biochem. 2000; 82:181–187. [PubMed: 11132625]
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- 22. Future investigations include the reaction with Tyr as the substrate using water-soluble analogs of 1.
- 23. This may also indicate that the oxidative coupling product can be generated from other low temperature stable species besides 2.
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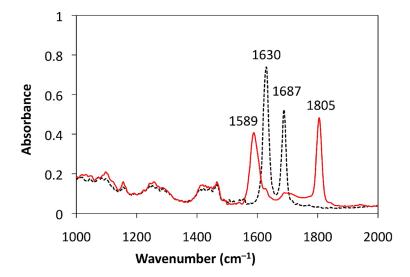


Figure 1. UV-Vis (A) and IR (B) spectra of [Fe(TMEDA)(NO)₂] (1) (black dashed line), and of the putative intermediate [Fe(TMEDA)(NO)(ONOO)] (2) (red) at -80 °C in dichloromethane.

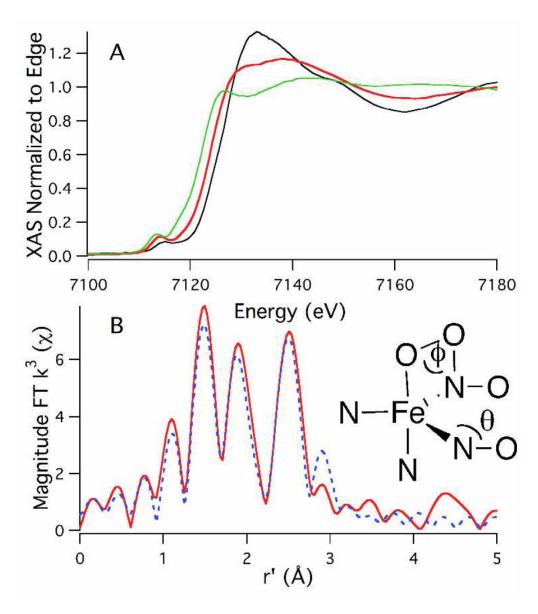


Figure 2. A) XANES region of the XAS for 1 (green), 2 (red) and the thermal decomposition product (black). B) Experimental (red solid line) and simulated (blue dashed line) magnitude FT k^3 EXAFS data for 2. Best fit includes: shell #1: 2 N scatterers, r=2.14(1) Å, $\sigma^2=0.003(2)$ Ų; O2NO shell: 1 O2NO scatterer, $r_1=1.91(1)$ Å, $r_2=1.91$ Å (restrained), $\sigma^2=0.002(1)$ Ų, $\phi=98(7)^\circ;$ NO shell: 1 NO scatterer, r=1.67(1) Å; $\sigma^2=0.008(2)$ Ų; $\theta=192(2)^\circ.$ $E_0=7120.4$ eV. $\epsilon^2=0.57.$

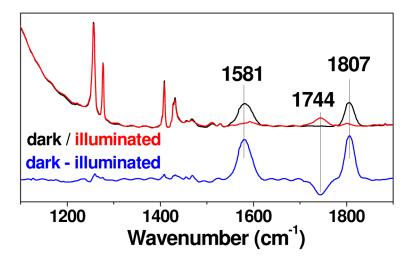


Figure 3. IR spectra of a [Fe(TMEDA)(NO)(ONOO)] (2) in CH₂Cl₂ at 11 K before (black) and after illumination (red). The dark minus illuminated difference spectrum is also shown (blue). Sharp IR bands are from dichloromethane.

Scheme 1.

Chart 1. Dinitrosyl iron complexes (DNICs)