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Heme-Copper Assembly Mediated Reductive Coupling of Nitrogen Monoxide (·NO)

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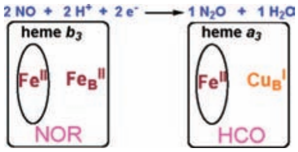
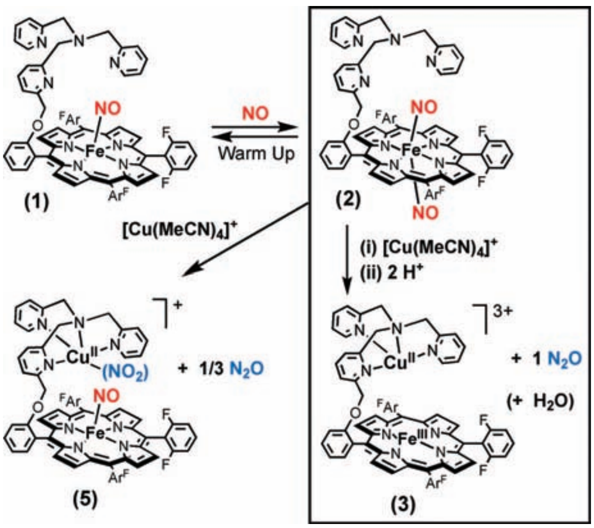
The subject of the interaction of nitrogen monoxide (·NO) with hemes is very important in the biology of this highly reactive diatomic molecule. As a signaling agent, heme proteins are involved in its biosynthesis and detection.[1] In aerobic microorganisms, heme cofactors are key in the oxidative response to toxic concentrations of ·NO.[2] In anaerobic denitrifiers with the biochemical pathway $NO_3^- \rightarrow NO_2^- \rightarrow \cdot NO \rightarrow N_2O \rightarrow N_2$, ·NO reductases (NORs) possess a heme/nonheme diiron active site which couples two ·NO molecules giving nitrous oxide (N_2O).[3,4] In addition to their role as a mediator for the reduction of O_2 to water accompanied by membrane proton translocation, heme-copper oxidases (HCOs) including cytochrome *c* oxidases (C*c*Os) effect this same reductive coupling reaction,[3a,b,5] (diagram). HCO active sites contain a high-spin heme/Cu_B dinuclear center;[6] HCOs and NORs are evolutionarily related.[5,7] The NO coupling mechanism in HCOs has received considerable recent attention via biophysical and computational studies,[3a,8] and in a designed small protein model study.[9] Yet, we are far from having a sufficient understanding of the underlying metal/NO and metal ion mediated NO−NO coupling chemistry.



While there is extensive recent activity in the design and study of discrete heme/copper synthetic complexes which resemble HCO active sites and/or effect dioxygen binding and reduction chemistry,[6,10] there are no cases where a synthetic small molecule heme/Cu complex reacts with ·NO to effect reductive coupling leading to nitrous oxide. In this report, we describe such a system, employing the binucleating ligand ^{6}L and its iron and heme-copper derivatives which have been previously used in our investigations involving O_2-chemistry.[10a] The starting point is the reductive nitrosylation of (^{6}L)Fe^{III}(OH)[11] to straightforwardly give the iron(II)−nitrosyl compound (^{6}L)Fe(NO) (**1**) (Scheme 1), possessing the expected three-line hyperfine split EPR spectrum[12] (Figure 1a).[13] As has been reported previously,[14] such complexes may react with additional $\cdot NO_{(g)}$ to form a dinitrosyl species. This occurs here and evidence in support of this formulation, (^{6}L)$Fe(NO)_2$, are as follows: (a) As monitored by UV−vis spectroscopy in acetone (−80 °C), **1** reversibly binds $\cdot NO_{(g)}$ to form (^{6}L)$Fe(NO)_2$ (**2**); **2** is stable, but loses $\cdot NO_{(g)}$ upon warming to room temperature. (b) With formation of **2** at −80 °C, the EPR signal due to (^{6}L)Fe-(NO) (**1**) disappears, as would be expected for **2**, having an even number of electrons. (c) Titration of a −80 °C solution of (^{6}L)$Fe(NO)_2$ (**2**) with 1 equiv (F_8)Fe^{II} {F_8 = tetrakis(2,6-difluorophenyl)porphyrinate(2-)} leads to a clean conversion to (^{6}L)Fe(NO) (**1**) plus (F_8)Fe(NO) (X-ray structure determined); **2** possesses 2 equiv of bound ·NO.[13]

Scheme 1



The existence of (^{6}L)$Fe(NO)_2$ (**2**) is very useful for the present case.[15] The system is simplified and a stoichiometric reaction (e.g., one enzyme turnover, by analogy) can now be studied; *exactly* two ·NO molecules are present for each heme or heme/Cu assembly. Thus, a copper ion source, $[Cu^I(MeCN)_4]B(C_6F_5)_4$, was added to (^{6}L)$Fe(NO)_2$ (**2**) at −80 °C in acetone/MeCN (3:1) along with 2 equiv acid in the form of $H(Et_2O)_2[B(C_6F_5)_4]$. Upon warming to room temperature, identification of the products reveals that an NOR-type reaction has taken place: (i) Nitrous oxide is produced in very good yield (80% ave; best run gave 87%), as determined by GC analysis of the reaction vessel headspace. (ii) The product heme complex is formulated as $[(^6L)Fe^{III}...Cu^{II}(D)]^{3+}$ (**3**) (D = MeCN or H_2O) (Scheme 1). This is based on the observed UV−vis spectrum, appearing as a single species with λ_{max} = 396, 515 nm in THF, matching that of a typical (porphyrinate)Fe^{III}−X high-spin complex (X = a noncoordinating anion like PF_6^- or here $B(C_6F_5)_4^-$). Further supporting this formulation, an EPR spectrum (Figure 1b) reveals that a high-spin heme-Fe^{III} along with a Cu^{II} (tetragonal complex) are both present.[13,16]

To rule out the possibility that only (^{6}L)$Fe(NO)_2$ (**2**) itself mediates the ·NO reductive coupling observed, its reaction with 2 equiv HBArF was investigated. No UV−vis change was observed and even following warming from −80 °C, the iron(II) nitrosyl complex (^{6}L)Fe(NO) (**1**) was produced; $\cdot NO_{(g)}$ was released as determined by GC analysis.[13] Copper ion has a critical role in the NOR chemistry described here.

The acid (proton) source present is key to the chemistry observed, as is also known for ·NO reduction catalyzed by HCOs.[3a,8b] In a further control experiment, copper(I) ion was added to (^{6}L)$Fe(NO)_2$ (**2**) but in the absence of acid (Scheme 1). The product obtained is formulated as the heme-nitrosyl...Cu−nitrite complex [(^{6}L)Fe-

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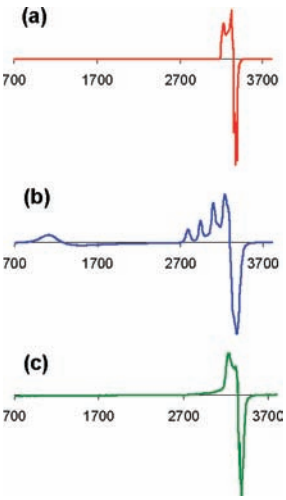

Figure 1. EPR spectra of species formed in the chemistry described by Scheme 1: (a) $(^6L)Fe(NO)$ (**1**), (b) $[(^6L)Fe^{III}...Cu^{II}(D)]^{3+}$ (**3**), with high-spin Fe^{III} ($g \approx 6$) and Cu^{II} ($g_{\|} = 2.26$, $g_{\perp} = 2.05$, $A_{\|} = 150$ G, $A_{\perp} = 36$ G) centers; the spectrum matches a synthetically prepared 1:1 mixture of $[(F_8)Fe^{III}]SbF_6$ and Cu^{II} complex, (c) $[(^6L)Fe(NO)...Cu^{II}(NO_2^-)]^+$ (**5**); the spectrum matches a 1:1 mixture of $(F_8)Fe(NO)$ and $[Cu^{II}(NO_2^-)]^+$ complex (see text).

$(NO)...Cu^{II}(NO_2^-)]^+$ (**5**), Scheme 1. A UV−vis spectrum reveals bands associated with $(^6L)Fe(NO)$ (**1**) {λ_{max} (THF) = 411, 547 nm} and IR spectroscopy gives $\nu_{NO} = 1687$ cm^{-1}. An EPR spectrum of the product mixture (Figure 1c) matches to a mixture of typical (porphyrinate)Fe^{II}−NO and Cu^{II} species, consistent with our proposed course of reaction.[13]

Thus, without added acid, one of the two nitrogen monoxide molecules (per heme/Cu assembly) remains in the product. Our expectation was that any remaining •NO released could be disproportionated to nitrite and N_2O, according to the well-established chemistry:[3c]

$$3{\cdot}NO + [(L)Cu^I]^+ \rightarrow N_2O + [(L)Cu^{II}(NO_2^-)]^+ \qquad (1)$$

In fact, this seems to be the case. GC analysis reveals that N_2O is produced in 90% yield according to eq 1. This is far less than should be or is produced by our NOR chemistry with acid present, Scheme 1. In further support of the chemistry outlined here, ion chromatography analysis of an aqueous solution derived from a reaction mixture indicates nitrite is present.[13] In separate experiments, we have observed that a copper(I) complex of the tris(2-pyridylmethyl)-amine moiety found in ^6L does react with $\cdot NO_{(g)}$ according to eq 1. To reiterate, without added acid, heme-copper mediated NOR chemistry does not occur and $[(^6L)Fe(NO)...Cu^{II}(NO_2^-)]^+$ (**5**) is instead produced, Scheme 1.[17]

In summary, we have shown that the heme-copper assembly described here efficiently effects reductive coupling of nitrogen monoxide molecules in the same stoichiometry as is known for HCOs. Two equiv •NO,[18] the heme, the juxtaposed copper ion, and protons are all required, $[(^6L)Fe^{II}...Cu^I]^+ + 2NO_{(g)} + 2H^+ \rightarrow [(^6L)Fe^{III}...Cu^{II}]^{3+} + N_2O_{(g)} + H_2O$. Collman et al.[19] recently reported on stoichiometric functional model systems for heme/nonheme diiron NORs, and the continued interrogation of well-designed synthetic systems[20] will be important in the elucidation of fundamental aspects of heme/NO_x[12,21] and heme/M/•NO (M = Cu, Fe))[7] interactions and their reductive (or oxidative)[2,22] chemistries. We note that a heme and/or copper coordinated hyponitrite ($N_2O_2^{2-}$) moiety[23] has been postulated (or even detected)[8a] in protein chemistry.[8] For this present system, further studies will be directed to mechanistic analyses and attempts to generate or find this or other intermediates that may form.

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Supporting Information Available: Details concerning synthesis, spectroscopy, and reactivity and CIF file. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) The dinitrosyl complex **2** is inherently interesting and will be the subject of separate future reports.
(16) ESI-MS analysis of the reaction mixture gives only the well-known structurally characterized μ-oxo complex $[(^6L)Fe^{III}\text{-}O\text{-}Cu^{II}]^+$ (**4**) (m/z = 1173, matching a spectrum calculated for the structure which includes the distinctive pattern due to the $^{63,65}Cu$ isotopes). In fact, solutions of **3** are cleanly converted to **4** with addition of Et_3N/H_2O as indicated by the change to the known UV−vis spectrum with red-shifted Soret band, λ_{max} = 440, 557 nm.
(17) The same product **5** is observed if $[(^6L)Fe^{II}.\ .Cu^I]^+$ is exposed to excess $\cdot NO_{(g)}$, unpublished observations.
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