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Heme-Copper Assembly Mediated Reductive Coupling of Nitrogen Monoxide ($\bullet\text{NO}$)

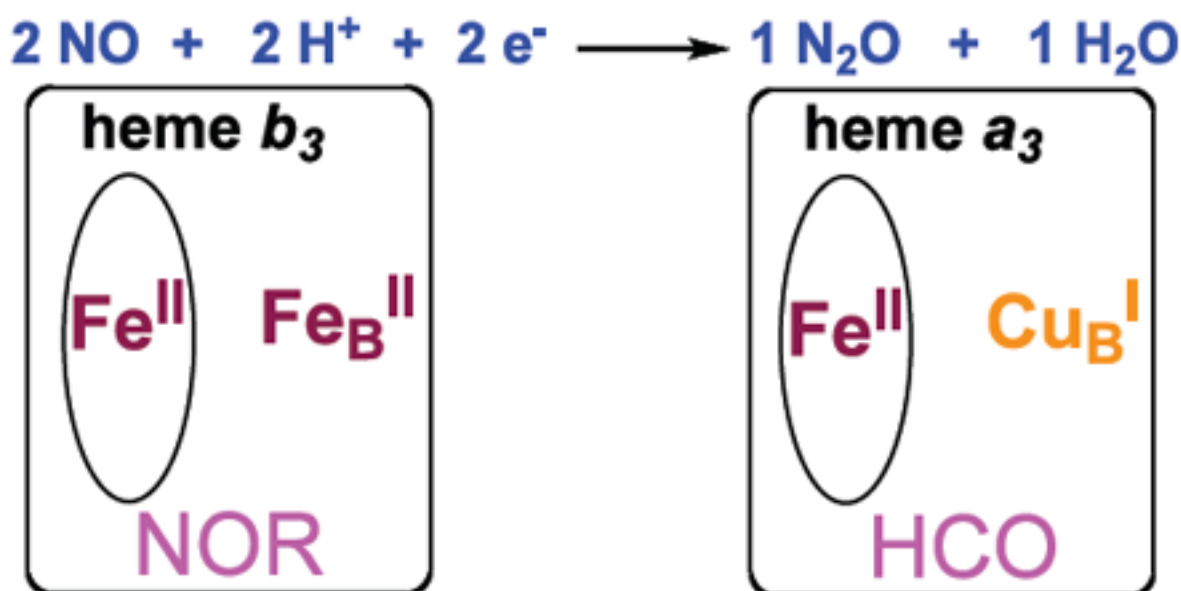
Jun Wang, Mark P. Schopfer, Amy A. N. Sarjeant, and Kenneth D. Karlin*

Department of Chemistry, The Johns Hopkins University, Baltimore, Maryland 21218 USA

Abstract

A iron-dinitrosyl species (${}^6\text{L}$)Fe(NO)₂ (**2**), generated from nitrogen monoxide ($\bullet\text{NO}$) binding to its related iron(II)-mononitrosyl complex (${}^6\text{L}$)Fe(NO) (**1**), efficiently effects reductive coupling of two $\bullet\text{NO}$ molecules to release nitrous oxide (N₂O), when Cu⁺ ion and two equiv acid are added; the heme/Cu product is [(${}^6\text{L}$)Fe^{III}...Cu^{II}(D)]³⁺ (D = H₂O or MeCN). In a control experiment where only (${}^6\text{L}$)Fe(NO)₂ (**2**) is exposed to two equiv acid, no UV-vis change is observed; $\bullet\text{NO}_{(\text{g})}$ is released and (${}^6\text{L}$)Fe(NO) is reformed. The copper ion complex within the ${}^6\text{L}$ ligand framework is required for the $\bullet\text{NO}$ coupling chemistry. In a further control experiment Cu⁺ ion is added to (${}^6\text{L}$)Fe(NO)₂ without acid present, [(${}^6\text{L}$)Fe(NO)...Cu^{II}(NO₂⁻)]⁺ is obtained, with the amount of N₂O_(\text{g}) released fitting with copper(I) ion promoted disproportionation chemistry, 3 $\bullet\text{NO}$ + ligand-Cu^I → N₂O + ligand-Cu^{II}(NO₂⁻). The chemical system described represents a (stoichiometric) functional model for heme/Cu protein nitric oxide reductase activity.

The subject of the interaction of nitrogen monoxide ($\bullet\text{NO}$) 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 $\bullet\text{NO}$.² In anaerobic denitrifiers with the biochemical pathway NO₃⁻ → NO₂⁻ → $\bullet\text{NO}$ → N₂O → N₂, $\bullet\text{NO}$ reductases (NORs) possess a heme/non-heme diiron active site which couples two $\bullet\text{NO}$ molecules giving nitrous oxide (N₂O).^{3,4} In addition to their role as a mediator for the reduction of O₂ to water accompanied by membrane proton translocation, heme-copper oxidases (HCOs) including cytochrome *c* oxidases (CcOs) 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.



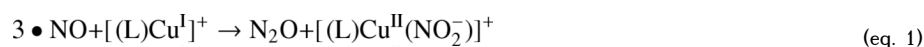
While there is extensive recent activity in the design and study of discrete heme/copper synthetic complexes which resemble HCO active sites and/or effect dioxygen binding and reduction chemistry,^{6,10} there are no cases where a synthetic small molecule heme/Cu complex reacts with $\bullet\text{NO}$ to effect reductive coupling leading to nitrous oxide. In this report, we describe such a system, employing the binucleating ligand ${}^6\text{L}$ 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 $({}^6\text{L})\text{Fe}^{\text{III}}(\text{OH})$ ¹¹ to straightforwardly give the iron(II)-nitrosyl compound $({}^6\text{L})\text{Fe}(\text{NO})$ (**1**) (Scheme 1), possessing the expected three-line hyperfine split EPR spectrum¹² (Fig. 1a).¹³ As has been reported previously,¹⁴ such complexes may react with additional $\bullet\text{NO}_{(\text{g})}$ to form a dinitrosyl species. This occurs here and evidence in support of this formulation, $({}^6\text{L})\text{Fe}(\text{NO})_2$ are as follows: (a) As monitored by UV-vis spectroscopy in acetone (-80°C), **1** reversibly binds $\bullet\text{NO}_{(\text{g})}$ to form $({}^6\text{L})\text{Fe}(\text{NO})_2$ (**2**); **2** is stable, but loses $\bullet\text{NO}_{(\text{g})}$ upon warming to RT. (b) With formation of **2** at -80°C , the EPR signal due to $({}^6\text{L})\text{Fe}(\text{NO})$ (**1**) disappears, as would be expected for **2**, having an even number of electrons. (c) Titration of a -80°C solution of $({}^6\text{L})\text{Fe}(\text{NO})_2$ (**2**) with one equiv $(\text{F}_8)\text{Fe}^{\text{II}}$ $\{\text{F}_8 = \text{tetrakis}(2,6\text{-difluorophenyl})\text{porphyrinate}(2-)\}$ leads to a clean conversion to $({}^6\text{L})\text{Fe}(\text{NO})$ (**1**) plus $(\text{F}_8)\text{Fe}(\text{NO})$ (X-Ray structure determined); **2** possesses two equiv of bound $\bullet\text{NO}$.¹³

The existence of $({}^6\text{L})\text{Fe}(\text{NO})_2$ (**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 $\bullet\text{NO}$ molecules are present for each heme or heme/Cu assembly. Thus, a copper ion source, $[\text{Cu}^{\text{I}}(\text{MeCN})_4]\text{B}(\text{C}_6\text{F}_5)_4$, was added to $({}^6\text{L})\text{Fe}(\text{NO})_2$ (**2**) at -80°C in acetone/MeCN (3:1) along with two equiv acid in the form of $\text{H}(\text{Et}_2\text{O})_2[\text{B}(\text{C}_6\text{F}_5)_4]$. Upon warming to RT, 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 $[({}^6\text{L})\text{Fe}^{\text{III}}\dots\text{Cu}^{\text{II}}(\text{D})]^{3+}$ (**3**) (D = MeCN or H_2O) (Scheme 1). This is based on the observed UV-vis spectrum, appearing as a single species with $\lambda_{\text{max}} = 396, 515 \text{ nm}$ in THF, matching that of a typical $(\text{porphyrinate})\text{Fe}^{\text{III}}\text{-X}$ high-spin complex (X = a non-coordinating anion like PF_6^- or here $\text{B}(\text{C}_6\text{F}_5)_4^-$). Further supporting this formulation, an EPR spectrum (Fig. 1b) reveals that a high-spin heme- Fe^{III} along with a Cu^{II} (tetragonal complex) are both present.^{13,16}

In order to rule out the possibility that only (⁶L)Fe(NO)₂ (**2**) itself mediates the •NO reductive coupling observed, its reaction with two equiv HBArF was investigated. No UV-Vis change was observed and even following warming from -80 °C, the iron(II) nitrosyl complex (⁶L)Fe(NO) (**1**) was produced; •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 (⁶L)Fe(NO)₂ (**2**) but in the absence of acid (Scheme 1). The product obtained is formulated as the heme-nitrosyl..Cu^{II}-nitrite complex [(⁶L)Fe(NO)...Cu^{II}(NO₂⁻)]⁺ (**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 ν_{NO} = 1687 cm⁻¹. An EPR spectrum of the product mixture (Fig. 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 N₂O, according to the well established chemistry:^{3c}



In fact, this seems to be the case. GC analysis reveals that N₂O 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 •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}(NO₂⁻)]⁺ (**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]⁺ + 2 NO_(g) + 2 H⁺ → [(⁶L)Fe^{III}..Cu^{II}]³⁺ + N₂O_(g) + H₂O. Collman et al.¹⁹ recently reported on stoichiometric functional model systems for heme/non-heme diiron NORs, and the continued interrogation of well-designed synthetic systems²⁰ 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 (N₂O₂²⁻) moiety²³ 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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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}-O-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 Et₃N/H₂O as indicated by the change to the known UV-vis spectrum with red-shifted Soret band, $\lambda_{\text{max}} = 440, 557 \text{ nm}$.
17. The same product **5** is observed if [(⁶L)Fe^{II}..Cu^I]⁺ is exposed to excess •NO(g), unpublished observations.
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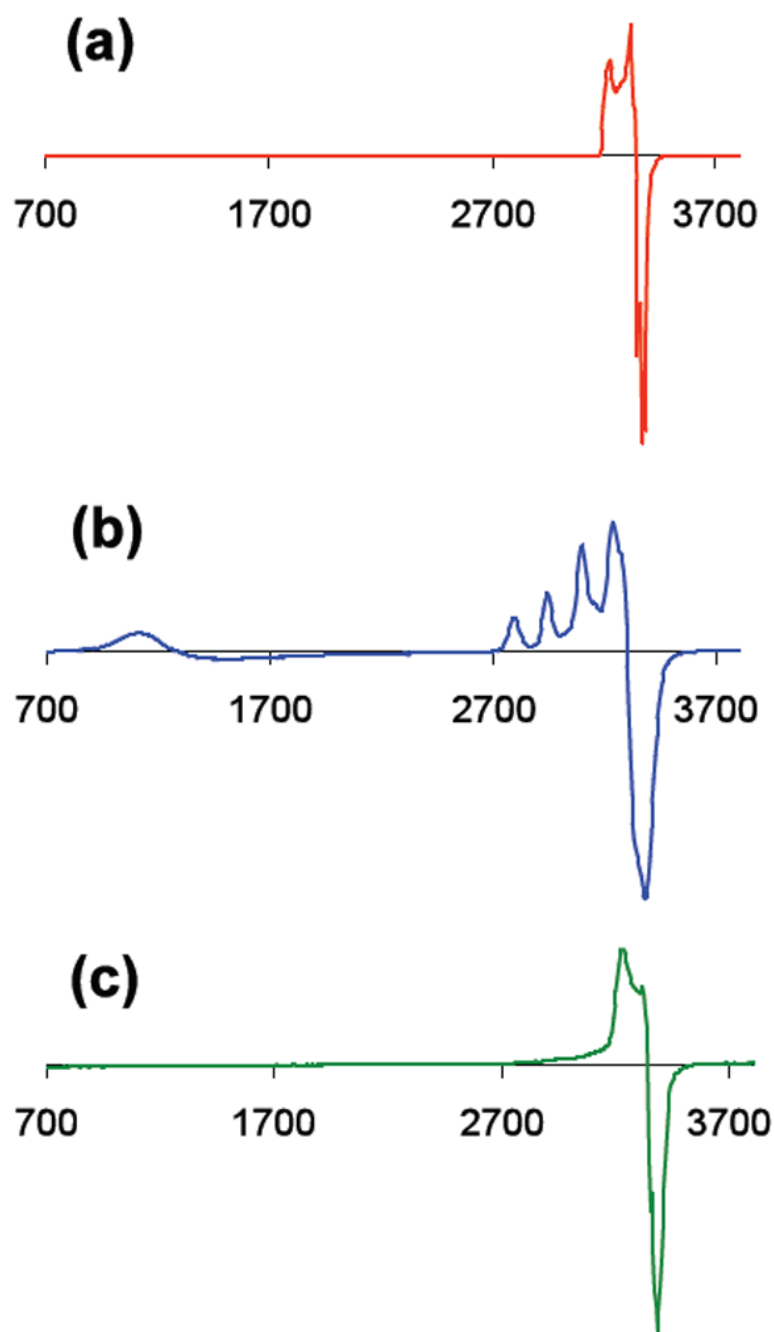
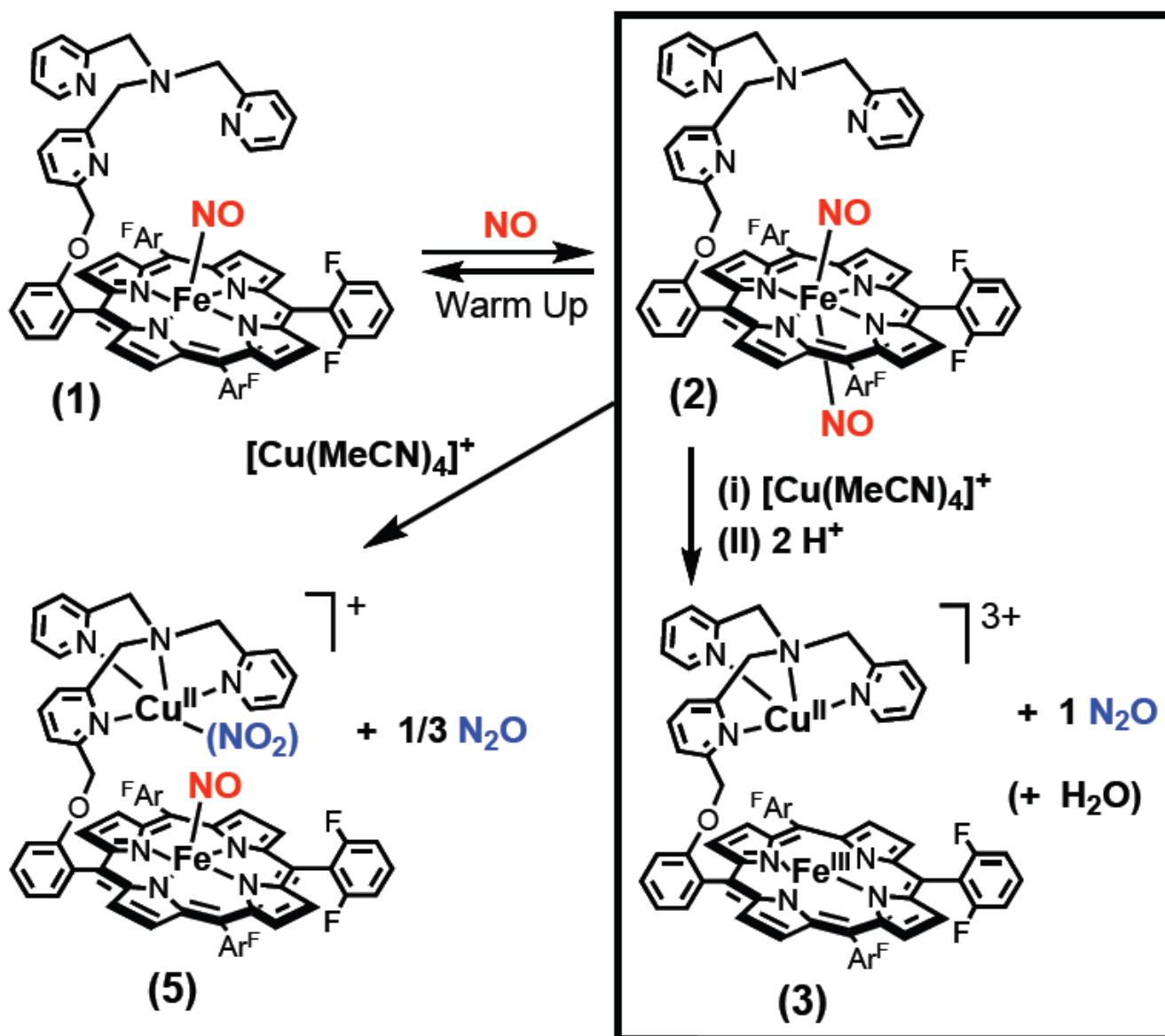


Figure 1.

EPR spectra of species formed in the chemistry described by Scheme 1: (a) $(^6\text{L})\text{Fe}(\text{NO})$ (**1**), (b) $[(^6\text{L})\text{Fe}^{\text{III}}\dots\text{Cu}^{\text{II}}(\text{D})]^{3+}$ (**3**), with high-spin Fe^{III} ($g \sim 6$) and Cu^{II} ($g_{\parallel} = 2.26$, $g_{\perp} = 2.05$, $A_{\parallel} = 150$ G, $A_{\perp} = 36$ G) centers; the spectrum matches a synthetically prepared 1:1 mixture of $[(\text{F}_8)\text{Fe}^{\text{III}}]\text{SbF}_6$ and Cu^{II} complex, (c) $[(^6\text{L})\text{Fe}(\text{NO})\dots\text{Cu}^{\text{II}}(\text{NO}_2^-)]^+$ (**5**); the spectrum matches a 1:1 mixture of $(\text{F}_8)\text{Fe}(\text{NO})$ and $[\text{Cu}^{\text{II}}(\text{NO}_2^-)]^+$ complex. See text.



Scheme 1.