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# Carbon Monoxide and Nitrogen Monoxide Ligand Dynamics in Synthetic Heme and Heme-Copper Complex Systems

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#### **Abstract**

Intermolecular nitrogen monoxide ( $\bullet$ NO) and carbon monoxide (CO) transfer from iron-to-copper and back, a phenomenon not seen before, has been accomplished by employing transient absorbance laser flash photolytic methods. A 1:1 heme/copper component system was utilized that consisted of a six-coordinate ferrous species,  $F_8Fe^{II}(CO)(DCIM)$  or  $F_8Fe^{II}(NO)(thf)$  { $F_8$  = tetrakis(2,6-difluorophenyl)porphyrinate(2-); DCIM = 1,5-dicyclohexyl imidazole; thf = tetrahydrofuran}, and two ligand-copper(I)-complexes of tridentate { $^{Bz}L$ ; (benzyl)bis(2-pyridylmethyl)amine} and tetradentate coordination { $^{Py}L$ ; tris(2-pyridylmethyl)amine}. The results suggest a lower affinity for NO binding to copper(I) versus CO and a higher rate for NO binding to heme versus CO. In fact, the latter event has been observed in cytochrome c oxidase  $aa_3$ .

Photoinitiated ligand dissociation with spectroscopic monitoring (UV-visible, infrared, and resonance Raman) has typically been used to elucidate CO,  $O_2$ , and NO binding dynamics in heme proteins including heme-copper oxidases (HCOs). Through time-resolved spectroscopy, Woodruff and co-workers confirmed  $C_B$  as the site of  $O_2$ -entry to the HCO active-site, based on critical insights gained by monitoring CO-transfer between heme and  $C_B$  following photodissociation from heme  $C_B$ -CO (Scheme 1). However, the detailed role of  $C_B$  in HCO/ $O_B$ -NO( $O_B$ ) biochemistry is not well understood; in fact, copper-(NO) interactions are very difficult to study.

Nitrogen monoxide is a known respiratory inhibitor (like CO);  $^5$  it can also be a substrate undergoing (i) oxidation to nitrite  $^6$  or (ii) reductive coupling,  $2NO+2e^-+2H^+ \rightarrow N_2O+H_2O.^7$  Thus, the interaction of heme proteins, including HCOs, with  $^\bullet NO_{(g)}$  is a subject of considerable importance and broad current interest. Large differences in NO reduction capabilities amongst HCOs exist and are thought to be related to variations in active-site structure/chemistry. Despite this knowledge, little is known about the details of  $Cu_B/NO$  interactions, creating a large gap in our understanding of  $HCO/^\bullet NO_{(g)}$  chemistry. Here, it is shown how one can apply heme-NO/CO photoinitiated chemistry to gain insights into NO binding/dynamics via investigations employing synthetic heme/Cu assemblies.  $^3$ 

Previously, transient absorbance (TA) laser flash photolysis and time-resolved infrared spectroscopy were utilized to examine intramolecular CO migration from an *in situ* formed heme-CO species to copper(I) and back to the heme, by employing a heme/Cu complex of the ligand  $^6$ L (Chart 1). Attempts to extend this work to examine NO and/or O<sub>2</sub> heme/copper dynamics were ineffective due to complex instability. An advance here is to utilize synthetic heme/copper systems that consist of 1:1 components. More experiments become possible since

separate and stable heme-CO and heme-NO complexes can be employed. Specifically, we use here the heme  $F_8$ , the tetradentate chelate for copper  $^{Py}L$  (that found within  $^6L$ ), as well as a tridentate ligand  $^{Bz}L$  (Chart 1).  $^{8a}$  With these new systems, CO and NO transfer from iron(II)-to-copper(I) have been measured, Scheme 2.

Single wavelength excitation ( $\lambda_{ex} = 532$  nm; 298 K) of [(F<sub>8</sub>)Fe<sup>II</sup>(CO)(DCIM)] (Chart 1)<sup>8a</sup> results in CO photodissociation, formation of [(F<sub>8</sub>)Fe<sup>II</sup>(Solv)(DCIM)], and subsequent CO rebinding. In deoxygenated THF, CH<sub>3</sub>CN, and acetone solutions,  $k_{+FeCO}$  values were obtained in the presence and absence of exogenously added copper(I) complex species, <sup>Py</sup>LCu<sup>I</sup> or <sup>Bz</sup>LCu<sup>I</sup>. <sup>8b</sup> For the CO-migration experiments, samples were prepared in a ratio range of 1:1 to 1:20 (Fe<sup>II</sup>:Cu<sup>I</sup>; 1 eq.  $\approx$  70  $\mu$ M) equivalents under an inert atmosphere within the glovebox.

In THF solvent and following CO photoejection from  $[F_8Fe^{II}(CO)(DCIM)]$ , the heme-CO rebinding process followed  $1^{st}$ -order kinetics and decreased from  $65 \, s^{-1} \, (k_{+FeCO})$  to  $10 \, s^{-1} \, (k_{-CuCO/+FeCO})$  in the presence of  $^{Py}LCu^I$  and to  $7 \, s^{-1} \, (k_{-CuCO/+FeCO})$  in the presence of  $^{Bz}LCu^I$ . The  $1^{st}$ -order rate constants result from solvent coordination to the metal ion after CO photodissociation. The smaller k values measured in the presence of  $^{R}LCu^I$  are ascribed to processes where photo-ejected CO first reacts with  $^{R}LCu^I$  giving  $^{R}LCu^I$ -CO (which are separately isolable) and returns to  $[F_8Fe^{II}(solv)(DCIM)]$  following CO de-ligation (Scheme 2). A similar result was obtained with an HCO; when site-directed mutagenesis led to the absence of active-site  $Cu_B$ , a greater rate of CO rebinding to heme<sub>a3</sub> resulted. In CH<sub>3</sub>CN solvent, a change in the rate for CO rebinding to  $[F_8Fe^{II}(CO)(DCIM)]$  was not observed in the presence of either  $^{R}LCu^{I}$  species; instead,  $k_{+FeCO} = 16 \, s^{-1}$  was consistently measured. The results suggest that  $CH_3CN$  hinders CO binding to  $^{R}LCu^{I}$  since nitriles are strong Lewis basic ligands for copper(I) ions.

Photoinitiated CO transfer from [F<sub>8</sub>Fe<sup>II</sup>(CO)(DCIM)] to <sup>Py</sup>LCu<sup>I</sup> also occurred in acetone based on the observation of a decrease in  $k_{+FeCO}$  from 77 s<sup>-1</sup> to 12 s<sup>-1</sup> ( $k_{-CuCO/+FeCO}$ ). However, addition of <sup>Bz</sup>LCu<sup>I</sup> to [F<sub>8</sub>Fe<sup>II</sup>(CO)(DCIM)] in acetone results in a thermal CO transfer reaction and a disproportionation reaction, leading to <sup>Bz</sup>LCu<sup>I</sup>-CO, [F<sub>8</sub>Fe<sup>II</sup>(DCIM)<sub>2</sub>], and [F<sub>8</sub>Fe<sup>II</sup>(Solv)<sub>2</sub>] based on benchtop UV-visible absorption changes and infrared analysis of the product mixture (Scheme 3). These observations indicate that the CO equilibrium binding constant for <sup>Bz</sup>LCu<sup>I</sup> is higher than for [(F<sub>8</sub>)Fe<sup>II</sup>(Solv)(DCIM)], i.e.,  $K_{CuCO} > K_{FeCO}$ .

Single wavelength excitation ( $\lambda_{ex} = 532$  nm; 298 K) of  $[F_8Fe^{II}(NO)(solv)]^{10}$  results in •NO photodissociation, formation of  $[F_8Fe^{II}(solv)_2]$ , and subsequent •NO rebinding. An absorption difference spectrum, Abs $\{[F_8Fe^{II}(thf)_2] - [F_8Fe^{II}(NO)(thf)]\}$ , of this •NO rebinding process in THF is shown in Figure 1. The calculated  $\Delta A$  spectrum obtained through bench-top UV-visible spectroscopy overlays perfectly, confirming the assigned process. Bimolecular rate constants ( $k_{NO}$ ) could not be determined due to difficulties purifying NO while passing through the gas mixer; this situation will be addressed in future studies.

In the presence of 1:1 and 1:20 (Fe<sup>II</sup>:Cu<sup>I</sup>) equivalents of <sup>Py</sup>LCu<sup>I</sup> (Scheme 2), biexponential •NO rebinding kinetics ( $k_1$ ,  $k_2$ ) are observed upon photoejection of NO from [(F<sub>8</sub>)Fe<sup>II</sup>(NO) (thf)] in THF. The first, faster process ( $k_1$ ) involves the free •NO molecule directly rebinding to the heme without transferring to <sup>Py</sup>LCu<sup>I</sup>; this occurs with the same rate as measured independently,  $k_1 \approx k_{+\text{FeNO}} = 432 \text{ s}^{-1}$ . The second, slower process ( $k_2$ ) involves •NO binding to [(F<sub>8</sub>)Fe<sup>II</sup>(thf)<sub>2</sub>], however following initial coordination to <sup>Py</sup>LCu<sup>I</sup>; a decreased rebinding rate of  $k_2 \approx k_{-\text{CuNO/+FeNO}} = 64 \text{ s}^{-1}$  was observed. Note that unlike this present case with •NO(g), direct rebinding of CO to the heme in the presence of <sup>Py</sup>LCu<sup>I</sup> ( $k_1$ ) was not observed (*vide supra*). This finding of "inefficient" NO iron-to-copper migration (i.e., some •NO rebinds to the heme) may suggest a lower affinity of <sup>Py</sup>LCu<sup>I</sup> for •NO versus CO, i.e.  $K_{\text{CuCO}} > K_{\text{CuNO}}$ . In fact, exactly such conclusions concerning Cu<sub>B</sub> were drawn by Vos *et. al.* on NO

dynamics experiments for cytochrome c oxidase  $aa_3$ . Further, since the NO and CO migration experiments were conducted at the same component concentration range, the results suggest that binding of •NO to the reduced heme is faster than that for CO ( $k_{+\rm FeNO} > k_{+\rm FeCO}$ ), as is known from heme-protein studies.  $^{2a,5}$ 

While the study of small gaseous ligand (•NO, CO, O<sub>2</sub>) binding to hemes or heme-copper proteins is a mature field, research activity in the area is still vigorous as kinetic spectroscopic interrogations continue to yield new insights into dynamics, structure, and even mechanism of reaction. In this report, we have shown the first example of reversible 1:1 intermolecular small molecule transfer, from a heme to copper(I), for both CO and •NO; kinetic parameters were obtained. The rates of reaction are less than those observed in intramolecular-preorganized systems such as in some HCOs or even the  $^6$ L heme/Cu framework, yet fast enough to prevent or overcome other irreversible Cu<sup>I</sup>/•NO reaction chemistries.  $^{12}$  Since •NO migration was observed, we can conclude that similar to CO, •NO kinetically favors binding to copper but thermodynamically favors coordination to iron. Future experiments will be directed to obtaining complementary thermodynamic data while employing Fe/Cu = 1:1 component systems and elucidating trends with systematically varied Cu-chelates (i.e., with different denticity, donor-atom type or Cu<sup>II/I</sup> E<sub>1/2</sub> value) and/or heme system.

## **Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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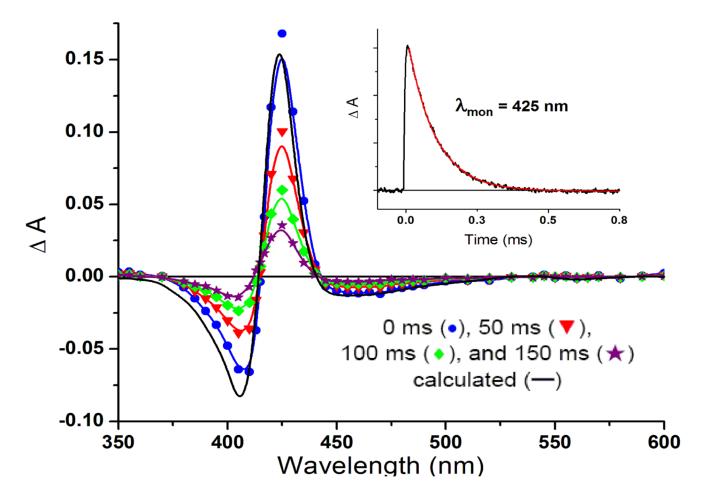


Figure 1. Absorption difference spectra ( $\lambda_{ex} = 532$  nm; 298 K) representing NO rebinding to  $[F_8Fe^{II}(thf)_2]$  following photoejection from  $[F_8Fe^{II}(NO)(thf)]$ ; the inset is a kinetic trace with a  $1^{st}$  order fit.

Scheme 1.

Scheme 2.

Scheme 3.

Chart 1.