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

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Photoinitiated ligand dissociation with spectroscopic monitoring (UV−vis, IR, and resonance Raman) has typically been used to elucidate CO, O_2, and NO binding dynamics in heme proteins, including heme−copper oxidases (HCOs).[1−3] Through time-resolved spectroscopy, Woodruff and co-workers confirmed Cu_B as the site of O_2 entry into the HCO active site on the basis of critical insights gained by monitoring CO transfer between $heme_{a3}$ and Cu_B following photodissociation from $heme_{a3}$−CO (Scheme 1).[1b,c] However, the detailed role of Cu_B in HCO/•NO(g) biochemistry is not well-understood; in fact, copper−NO interactions are very difficult to study.[4]

Nitrogen monoxide is a known respiratory inhibitor (as is 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 •NO(g) is a subject of considerable importance and broad current interest. Large differences in NO reduction capabilities among 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/•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 IR 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).[3b] Attempts to extend this work to examine NO and/or O_2 heme/copper dynamics were ineffective because of complex instability.[4] An advance here is the utilization of synthetic heme/copper systems that consist of 1:1 components. More experiments become possible because separate and stable heme−CO and heme−NO complexes can be employed. Specifically, here we used the heme F_8, the tetradentate chelate for copper ^{Py}L (that found within ^{6}L), 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 (λ_{ex} = 532 nm; 298 K) of [$(F_8)Fe^{II}$(CO)(DCIM)] (Chart 1)[8a] resulted in CO photodissociation, formation of [$(F_8)Fe^{II}$(Solv)(DCIM)], and subsequent CO rebinding. In deoxygenated THF, CH_3CN, and acetone solutions, k_{+FeCO} values were obtained in the presence and absence of exogenously added copper(I) complex species $^{Py}LCu^I$ or $^{Bz}LCu^I$.[8b] For the CO-migration experiments, samples were prepared in a Fe^{II}/Cu^I ratio range of 1:1 to 1:20 equiv (1 equiv ≈ 70 μM) under an inert atmosphere within a glovebox.

In THF solvent and following CO photoejection from [F_8Fe^{II}(CO)(DCIM)], the heme−CO rebinding process followed first-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 first-order rate constants result from solvent coordination to the metal ion after CO photodissociation.[2] The smaller k values measured in the presence of $^RLCu^I$ are ascribed to processes in which photoejected CO first reacts with $^RLCu^I$ to give $^RLCu^I$−CO (which is separately isolable) and returns to [F_8Fe^{II}(solv)(DCIM)] following CO deligation (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 $heme_{a3}$ resulted.[9] In CH_3CN solvent, a change in the rate for CO rebinding to [F_8Fe^{II}(CO)(DCIM)] was not observed in the presence of either $^RLCu^I$ species; instead, k_{+FeCO} = 16 s^{-1} was consistently measured. The results suggest that CH_3CN hinders CO binding to $^RLCu^I$, since nitriles are strong Lewis basic ligands for copper(I) ions.

Photoinitiated CO transfer from [F_8Fe^{II}(CO)(DCIM)] to $^{Py}LCu^I$ also occurred in acetone, as evidenced by the observation of a decrease in k_{+FeCO} from 77 to 12 s^{-1} ($k_{-CuCO/+FeCO}$). However, addition of $^{Bz}LCu^I$ to [F_8Fe^{II}(CO)(DCIM)] in acetone resulted in a

Scheme 1

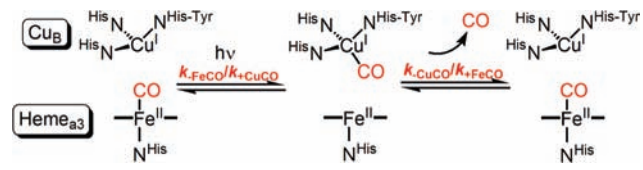
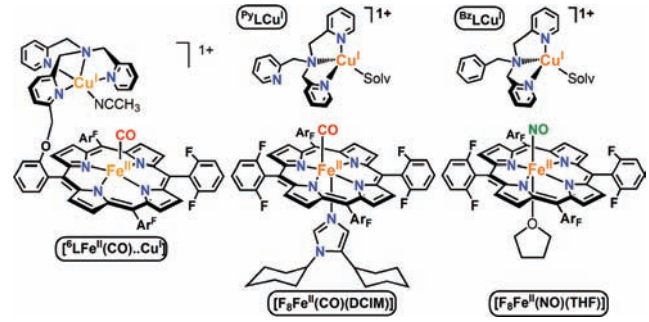


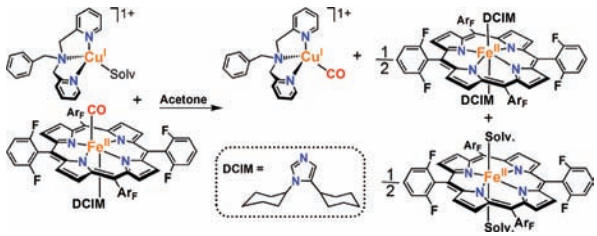
Chart 1



Scheme 2

10.1021/ja906172c CCC: $40.75

Scheme 3



thermal CO transfer reaction and a disproportionation reaction, leading to ^{Bz}LCu^{I}−CO, [F_8Fe^{II}(DCIM)$_2$], and [F_8Fe^{II}(Solv)$_2$], as evidenced by benchtop UV−vis absorption changes and IR analysis of the product mixture (Scheme 3). These observations indicate that the CO equilibrium binding constant for ^{Bz}LCu^{I} is higher than that for [(F_8)Fe^{II}(Solv)(DCIM)] (i.e., $K_{CuCO} > K_{FeCO}$).

Single-wavelength excitation (λ_{ex} = 532 nm; 298 K) of [F_8Fe^{II}(NO)(solv)][10] resulted 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 ΔA spectrum obtained through benchtop UV−vis spectroscopy overlays perfectly, confirming the assigned process. Bimolecular rate constants (k_{NO}) could not be determined because of difficulties in purifying NO during its passage through the gas mixer; this situation will be addressed in future studies.

In the presence of 1:1 and 1:20 (Fe^{II}/Cu^{I}) equiv of ^{Py}LCu^{I} (Scheme 2), biexponential ·NO rebinding kinetics (k_1, k_2) were observed upon photoejection of NO from [(F_8)Fe^{II}(NO)(thf)] in THF. The first, faster process (k_1) involves direct rebinding of the free ·NO molecule to the heme without transfer to ^{Py}LCu^{I}; this occurs with the same rate as measured independently ($k_1 \approx k_{+FeNO}$ = 432 s^{-1}). The second, slower process (k_2) involves ·NO binding to [(F_8)Fe^{II}(thf)$_2$] following initial coordination to ^{Py}LCu^{I}; a decreased rebinding rate of $k_2 \approx k_{-CuNO/+FeNO}$ = 64 s^{-1} was observed. Notably, unlike this present case of ·NO(g), direct rebinding of CO to the heme in the presence of ^{Py}LCu^{I} (k_1) was not observed (see above). This finding of "inefficient" NO iron-to-copper migration (i.e., some ·NO rebinds to the heme) may suggest a lower affinity of ^{Py}LCu^{I} for ·NO than CO, i.e. $K_{CuCO} > K_{CuNO}$. In fact, exactly such conclusions concerning Cu_B were drawn by Vos et al.[11] from NO dynamics experiments for cytochrome *c* oxidase *aa*$_3$. Further, since the NO and CO migration experiments were conducted with the same component concentration range, the results suggest that binding of ·NO to the reduced heme is faster than that for CO ($k_{+FeNO} > k_{+FeCO}$), as is known from heme−protein studies.[2a,5]

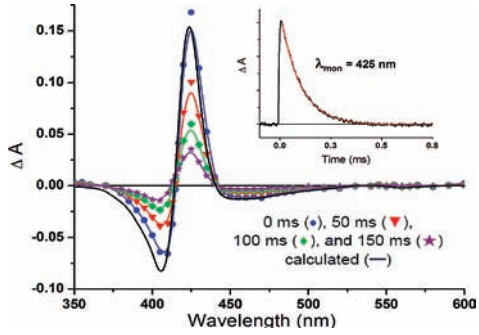


Figure 1. Absorption difference spectra (λ_{ex} = 532 nm; 298 K) representing NO rebinding to [F_8Fe^{II}(thf)$_2$] following photoejection from [F_8Fe^{II}(NO)(thf)]; the inset is a kinetic trace with a first-order fit.

While the study of the binding of small gaseous ligands (·NO, CO, O_2) 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 intramolecularly 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^{I}/·NO reaction chemistries.[12] Since ·NO migration was observed, we can conclude that like CO, ·NO kinetically favors binding to copper but thermodynamically favors coordination to iron. Future experiments will be directed toward obtaining complementary thermodynamic data while employing 1:1 Fe/Cu component systems and elucidating trends with systematic variation of Cu chelate (i.e., with different denticity, donor-atom type, or Cu$^{II/I}$ $E_{1/2}$ value) and/or heme system.

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Supporting Information Available: Experimental details, Xray crystallographic data for {F_8Fe^{II}(DCIM)$_2$} (CIF), ΔA spectra for CO rebinding to F_8Fe^{II}(DCIM)(Solv), and plots for 2nd order CO binding rates.[8b] This material is available free of charge via the Internet at http://pubs.acs.org.

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