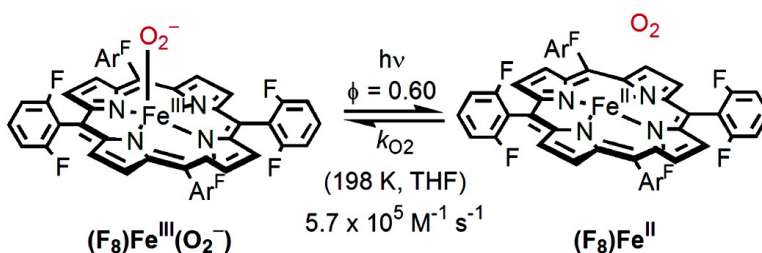


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Efficient Photodissociation of O₂ from Synthetic Heme and Heme/M (M = Fe, Cu) Complexes

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Hemes play a central role in the activation and transport of dioxygen (O₂) in living organisms.^{1–3} In this regard, study of the photodissociation of O₂ from oxy-hemes has been an important experimental tool for probing active site structure and reactivity.⁴ Pioneering investigations by Gibson showed that light excitation of oxy-myoglobin or hemoglobin resulted in the release of O₂ with low quantum yields.⁵ Traylor and co-workers later concluded that upon heme–O₂ photolysis the initial quantum yield is unity and that geminate recombination of O₂ with the ferrous heme accounted for the low yield measured on long time scales.⁶ However, recent ultrafast studies on oxy-myoglobin cast serious doubt on this conclusion^{7,8} and reveal a low intrinsic quantum yield of 0.3 at the earliest observation times.^{7,9} The elucidation of factors that control O₂ photodissociation and rebinding remains of great interest. Different O₂-binding modes^{10,11} and/or a distribution of protein conformations^{12,13} have been discussed in these regards.

In principle, spectroscopic studies of well-characterized synthetic oxy-hemes in fluid solution could provide valuable insight into the factors that govern O₂-binding and dissociation. In the past, photogeneration of O₂ from synthetic “picket-fence” oxy-hemes has focused upon axial ligand effects on the bimolecular O₂-binding step^{14,15} and geminate recombination of O₂.¹⁶ We find that photolysis of synthetic heme–O₂ adducts (formally Fe^{III}–superoxo (O₂[−]) complexes) in tetrahydrofuran (THF) at 198 K produces free O₂ with quantum yields comparable to that known for oxy-myoglobin. Furthermore, utilizing a simple, sterically unhindered tetraphenyl porphyrin leads us to a complex possessing the highest quantum yield measured (for complete O₂ dissociation), $\phi = 0.60$.

The compound (F₈)Fe^{II} (Chart 1), as a bis-THF adduct, (F₈)Fe^{II}-(thf)₂, (F₈ = tetrakis(2,6-difluorophenyl)-porphyrinate(2−)), has previously been synthesized and structurally characterized.^{17–19} It reacts with dioxygen below −40 °C in tetrahydrofuran (THF) to form an adduct, well described as a heme–superoxo compound, (F₈)Fe^{III}(O₂[−]), $\nu(\text{O}=\text{O}) = 1178 \text{ cm}^{-1}$ ($\Delta(^{18}\text{O}_2) = -64 \text{ cm}^{-1}$), having electronic absorptions at 416 nm (Soret band) and 536 nm (Q-band).^{18,20} Closely related heme–O₂ adducts can be generated in alternative environments. Thus, low-temperature oxygenation was also used to generate (⁶L)Fe^{III}(O₂[−]) where ⁶L is 5-(ortho-O-[N,N-bis(2-pyridylmethyl)-2-(6-methoxy)pyridinemethanamine]-phenyl)-10,15,20-tris(2-(2,6-di-fluorophenyl))porphine²¹ as well as bimetallic compounds with a superstructured neighboring iron(II)²² or copper(I)²³ ion coordinated to the tether arm of (⁶L)Fe^{II} (Chart 1).²⁴

Pulsed 355- or 532-nm light (fwhm 8–10 ns; 5 mJ/pulse) excitation of (F₈)Fe^{III}(O₂[−]) under 1 atm of O₂ in THF at 198 K resulted in the immediate appearance of the absorption difference spectrum shown in Figure 1.²⁵ This reveals a bleach of the absorption bands associated with the heme–superoxo compound and the appearance of new absorption bands with $\lambda_{\text{max}} = 427$ and 560 nm (Figure 1). This transient spectrum corresponds well with that calculated for (Abs[(F₈)Fe^{II}(thf)₂] − Abs[(F₈)Fe^{III}(O₂[−])]), indicating that the new intermediate formed is the ferrous solvento

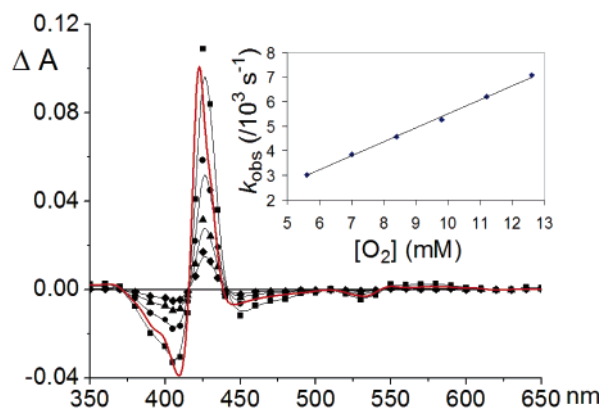
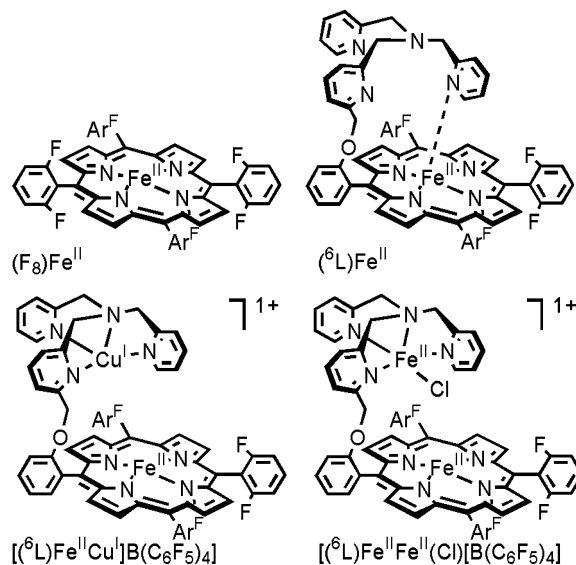


Figure 1. Time-resolved absorption spectra recorded after pulsed 532-nm light excitation of (F₈)Fe^{III}(O₂[−]) in THF at 198 K. The data are shown at delay times of 10 ns (■), 100 μs (●), 200 μs (▲), and 300 μs (◆). A spectrum simulated as (Abs[(F₈)Fe^{II}(thf)₂] − Abs[(F₈)Fe^{III}(O₂[−])] is overlaid in red. The inset shows the observed rate constant as a function of the dioxygen concentration leading to $k_{\text{O}_2} = 5.7 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$.

Chart 1



compound, (F₈)Fe^{II}(thf)₂, and O₂ has been released (Scheme 1). If superoxide (O₂[−]) had been photoreleased, the absorption difference spectra of the resulting ferric heme would be present, contrary to what was observed.²⁶

The coordination of O₂ to the reduced heme takes place on a time scale amenable to nanosecond flash photolysis measurements. Observed rate constants for O₂ rebinding (Scheme 1), k_{obs} , under pseudo first-order conditions (excess O₂), were independent of the monitoring wavelength, the [(F₈)Fe^{III}(O₂[−])] concentration (0.2–6.0 μM), and the laser excitation energy (5–10 mJ cm^{−2}) under

Scheme 1

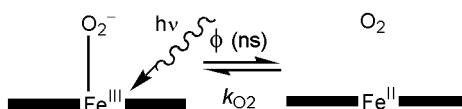


Table 1. O₂-Rebinding Rate Constants (*k*_{O₂} and Corresponding Quantum Yields (ϕ) for the Various Synthetic Heme Complexes in THF at 198 K^a

complex	<i>k</i> _{O₂} (M ⁻¹ s ⁻¹)	ϕ
(F ₈)Fe ^{III} (O ₂ ⁻)	5.7×10^5	0.60 ± 0.02
(⁶ L)Fe ^{III} (O ₂ ⁻)	6.4×10^5	0.22 ± 0.03
[(⁶ L)Fe ^{III} (O ₂ ⁻)Cu ^I] ⁺	6.8×10^5	0.34 ± 0.04
[(⁶ L)Fe ^{III} (O ₂ ⁻)Fe ^{II} (Cl)] ⁺	9.0×10^5	0.18 ± 0.02

^a The viscosity of THF at 198 K is 2.1 centipoise.³²

all conditions studied (Figure S1).²⁷ At low [O₂] concentrations (1–5 mM), the process was also shown to be first-order, and a plot of *k*_{obs} as a function of the dioxygen concentration (Figure 1 inset) is linear and yields a second-order rate constant for O₂-rebinding to (F₈)Fe^{II}(thf)₂ of *k*_{O₂} = $5.7 \pm 0.5 \times 10^5$ M⁻¹ s⁻¹ at 198 K.²⁸

The other heme dioxygen complexes, (⁶L)Fe^{III}(O₂⁻),²¹ [(⁶L)Fe^{III}(O₂⁻)Fe^{II}(Cl)]⁺,²² and [(⁶L)Fe^{III}(O₂⁻)Cu^I]⁺,²³ were also studied to examine whether the presence of a Lewis base or a second metal center affects the O₂-photodissociation quantum yield or rebinding kinetics.²⁹ The photogenerated transient spectra were within experimental error, the same as that observed after excitation of (F₈)Fe^{III}(O₂⁻) (Figure S2).²⁷ Small, but measurable, changes in the observed rate constant for dioxygen coordination were observed (Table 1).

The most striking data were the differences in the quantum yield (ϕ) for photorelease of O₂, determined by comparative actinometry (Table 1).²⁷ In all cases, the quantum yields were measured on a 10 ns, μ s, and ms (10⁻⁹ – 10⁻³ s) time scale. To our knowledge, the $\phi = 0.60 \pm 0.02$ after light absorption by (F₈)Fe^{III}(O₂⁻) is the highest ever measured by these methods. Such a high yield can be attributed to the sterically unhindered nature of the porphyrin allowing solvent to easily coordinate to the heme and thus efficiently displace O₂. The $\phi = 0.22 \pm 0.03$ for (⁶L)Fe^{III}(O₂⁻) is remarkably similar to that measured for oxy-myoglobin, which also has a ligated strong (i.e., pyridine or imidazole) base axial base.⁷ The O₂ quantum yield of the heme/non-heme compound was not dramatically different (Table 1), perhaps indicating that O₂ was released from the porphyrin face opposite that of the non-heme Fe^{II} moiety (Chart 1).³⁰ The Cu^I containing analogue [(⁶L)Fe^{III}(O₂⁻)Cu^I]⁺ is of direct relevance to cytochrome *c* oxidase model chemistry where the role of the copper center in dioxygen activation is of considerable interest.^{3,31} It is tempting to suggest that the improved quantum yield for photodissociation in [(⁶L)Fe^{III}(O₂⁻)Cu^I]⁺ compared to (⁶L)Fe^{III}(O₂⁻) stems from O₂-coordination to copper; however, additional spectroscopic studies on shorter time scales are necessary to fully elucidate the mechanism(s).

In conclusion, we have shown that synthetic heme–O₂ adducts photorelease O₂ with quantum yields very close to the value known for oxy-myoglobin.⁷ At a minimum, this indicates that the protein matrix and structure of the natural heme pocket are not strict requirements for modeling oxy-myoglobin photoreactivity. In addition, we report the highest quantum yield for complete dioxygen release for any oxy-heme ever measured. This finding opens the door toward the use of other synthetic hemes, with tailored electronic and steric properties, for further fundamental studies and for possible applications, e.g., in therapeutic controlled photorelease of dioxygen.^{33,34}

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Supporting Information Available: Figures of wavelength-independent O₂-rebinding and difference spectra. Description of comparative actinometry calculations. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- James, B. R. *The Porphyrins*; Academic Press: New York, 1978; Vol. V.
- Momenteau, M.; Reed, C. A. *Chem. Rev.* **1994**, *94*, 659–698.
- Collman, J. P.; Boulatov, R.; Sunderland, C. J.; Fu, L. *Chem. Rev.* **2004**, *104*, 561–588.
- Antonini, E.; Brunori, M. *Hemoglobin and Myoglobin in Their Reactions with Ligands*; North-Holland Publishing: Amsterdam, 1971.
- Gibson, Q. H.; Ainsworth, S. *Nature* **1957**, *180*, 156–157.
- Jongeward, K. A.; Magde, D.; Taube, D. J.; Marsters, J. C.; Traylor, T. G.; Sharma, V. S. *J. Am. Chem. Soc.* **1988**, *110*, 380–387.
- Ye, X.; Demidov, A.; Champion, P. M. *J. Am. Chem. Soc.* **2002**, *124*, 5914–5924.
- Walda, K. N.; Liu, X. Y.; Sharma, V. S.; Magde, D. *Biochemistry* **1994**, *33*, 2198–2209.
- Related femtosecond analysis of MbNO photolytic processes affords a greater yield (0.8–1.0) than previously measured on a picosecond time scale (0.5); ref 7. Zemojtel, T.; Rini, M.; Heyne, K.; Dandekar, T.; Nibbering, E. T. J.; Kozlowski, P. M. *J. Am. Chem. Soc.* **2004**, *126*, 1930–1931.
- Watanabe, T.; Ama, T.; Nakamoto, K. *J. Phys. Chem.* **1984**, *88*, 440–445.
- De Angelis, F.; Car, R.; Spiro, T. G. *J. Am. Chem. Soc.* **2003**, *125*, 15710–15711.
- Austin, R. H.; Beeson, K. W.; Eisenstein, L.; Frauenfelder, H.; Gunsalus, I. C. *Biochemistry* **1975**, *14*, 5355–5373.
- Agmon, N.; Hopfield, J. J. *J. Chem. Phys.* **1983**, *79*, 2042–2053.
- Collman, J. P.; Brauman, J. I.; Doxsee, K. M.; Sessler, J. L.; Morris, R. M.; Gibson, Q. H. *Inorg. Chem.* **1983**, *22*, 1427–1432.
- Collman, J. P.; Brauman, J. I.; Iverson, B. L.; Sessler, J.; Morris, R. M.; Gibson, Q. H. *J. Am. Chem. Soc.* **1983**, *105*, 3052–3064.
- Grogan, T. G.; Bag, N.; Traylor, T. G.; Magde, D. *J. Phys. Chem.* **1994**, *98*, 13791–13796.
- Obias, H. V.; van Strijdonck, G. P. F.; Lee, D.-H.; Ralle, M.; Blackburn, N. J.; Karlin, K. D. *J. Am. Chem. Soc.* **1998**, *120*, 9696–9697.
- Ghiladi, R. A.; Kretzer, R. M.; Guzei, I.; Rheingold, A. L.; Neuhold, Y.-M.; Hatwell, K. R.; Zuberbühler, A. D.; Karlin, K. D. *Inorg. Chem.* **2001**, *40*, 5754–5767.
- Thompson, D. W.; Kretzer, R. M.; Lebeau, E. L.; Scaltrito, D. V.; Ghiladi, R. A.; Lam, K.-C.; Rheingold, A. L.; Karlin, K. D.; Meyer, G. J. *Inorg. Chem.* **2003**, *42*, 5211–5218.
- Kim, E.; Helton, M. E.; Wasser, I. M.; Karlin, K. D.; Lu, S.; Huang, H. W.; Moenne-Loccoz, P.; Incarvito, C. D.; Rheingold, A. L.; Honecker, M.; Kaderli, S.; Zuberbühler, A. D. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3623–3628.
- Ghiladi, R. A.; Karlin, K. D. *Inorg. Chem.* **2002**, *41*, 2400–2407.
- Wasser, I. M.; Huang, H.-w.; Moenne-Loccoz, P.; Karlin, K. D. Submitted for publication, 2004.
- Ghiladi, R. A.; Ju, T. D.; Lee, D.-H.; Moenne-Loccoz, P.; Kaderli, S.; Neuhold, Y.-M.; Zuberbühler, A. D.; Woods, A. S.; Cotter, R. J.; Karlin, K. D. *J. Am. Chem. Soc.* **1999**, *121*, 9885–9886.
- An alternative photochemical method was sometimes used for in situ generation of the heme–superoxo/non-heme compound. Prolonged photolysis of the heme/non-heme oxo-bridged compound under 1 atm of dioxygen in THF at 198 K produced UV–visible absorption spectra consistent with complete generation of the heme–superoxo/non-heme compound.
- Steady-state UV–vis absorption measurements before and after laser excitation showed no evidence for permanent photochemistry.
- Hoshino, M.; Baba, T. *J. Am. Chem. Soc.* **1998**, *120*, 6820–6821.
- See Supporting Information.
- At 298 K other synthetic hemes have been shown to bind dioxygen with rate constants of 10⁸ M⁻¹ s⁻¹; see refs 14 and 15.
- The superoxide species, [(⁶L)Fe^{III}(O₂⁻)Cu^I]⁺, is stable at 198 K but converts to the peroxo species, [(⁶L)Fe^{III}(O₂²⁻)Cu^{II}]⁺, upon warming.
- Dioxygen is not known to bind to the non-heme portion of this complex.
- Kim, E.; Chufan, E. E.; Kamaraj, K.; Karlin, K. D. *Chem. Rev.* **2004**, *104*, 1077–1133.
- Yaws, C. L. *Handbook of Transport Property Data*; Gulf Publishing: Houston, TX, 1995.
- MacArthur, R.; Sucheta, A.; Chong, F. F. S.; Einarsdóttir, O. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8105–8109.
- Van Eps, N.; Szundi, I.; Einarsdóttir, O. *Biochemistry* **2000**, *39*, 14576–14582.

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