ORGANIC LETTERS

2005
Vol. 7, No. 6
1193−1195

Formation of Compound I by Photo-Oxidation of Compound II

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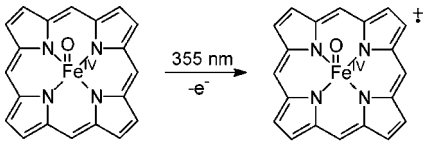
Received February 12, 2005

ABSTRACT



Compound I is the heme–iron(IV)–oxo porphyrin radical cation formed in peroxidase and catalase enzymes by reaction with hydrogen peroxide. As an alternative to chemical oxidations of porphyrin–iron(III) species, various compound I species were produced by 355 nm laser flash photolysis photooxidation of the corresponding compound II species, porphyrin–iron(IV)–oxo derivatives. The method is demonstrated by production and kinetic studies of the compound I species from 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin–iron, from horseradish peroxidase, and from wild-type horse skeletal myoglobin.

Compound I (cpd I), an iron(IV)−oxo porphyrin radical cation, is a central intermediate in the redox chemistry of porphyrin−iron compounds (Figure 1). The species is observed in reactions of heme-containing peroxidase and catalase enzymes with H_2O_2,[1] and cpd I models can be prepared by oxidations of porphyrin-iron(III) salts with, for example, *m*-chloroperoxybenzoic acid (mCPBA).[2] Cpd I also is the putative oxidant of the ubiquitous, heme-containing cytochromes P450, where it is often termed iron−oxo.[3] Cpd I has not been observed in P450 reactions, and cryo-reduction studies indicate that the P450 oxidant cannot be detected at −80 °C, reacting faster than it is formed in the chemical oxidation pathway.[4]

In this work, we report a new entry to cpd I in model compounds and proteins. Laser flash photolysis (LFP) oxidation of compound II (cpd II), a porphyrin−iron(IV)−oxo species, is a viable method for formation of this reactive transient. Cpd I is formed essentially instantly by photooxidation, permitting direct kinetic studies on time scales much shorter than those achieved in mixing experiments. Previously reported photochemical methods for production of high-valent metal-oxo species include photoinduced cleavage of ligands[5] and photochemical production of a diffusible oxidant.[6]

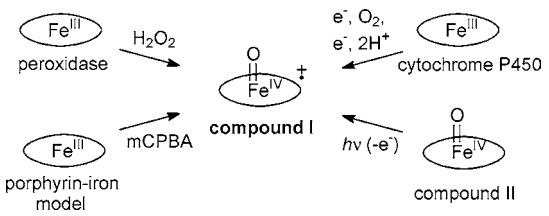


Figure 1. Compound I from various precursors. The ovals represent porphyrin rings.

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(2) Groves, J. T.; Haushalter, R. C.; Nakamura, M.; Nemo, T. E.; Evans, B. J. *J. Am. Chem. Soc.* **1981**, *103*, 2884−2886.

(3) *Cytochrome P450 Structure, Mechanism, and Biochemistry*, 2nd ed.; Ortiz de Montellano, P. R., Ed.; Plenum: New York, 1995. Sono, M.; Roach, M. P.; Coulter, E. D.; Dawson, J. H. *Chem. Rev.* **1996**, *96*, 2841−2887.

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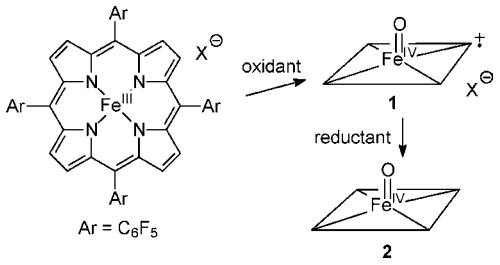
10.1021/ol050296j CCC: $30.25
Published on Web 02/24/2005

Oxidation of 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin−iron(III) salts by mCPBA or PhIO gives an analogue of cpd I, the iron(IV)−oxo porphyrin radical cation **1**, which can be observed spectroscopically in some cases (Scheme 1).[2,7] This transient is reduced to the neutral iron(IV)−oxo

Scheme 1



species **2**, which is relatively stable.

We produced species **2** in CH_3CN by reaction of the precursor iron(III) chloride with PhIO, conditions under which species **1** is not observed.[8] LFP irradiation of **2** (355 nm) gave species **1** (Figure 2A). The spectrum contained

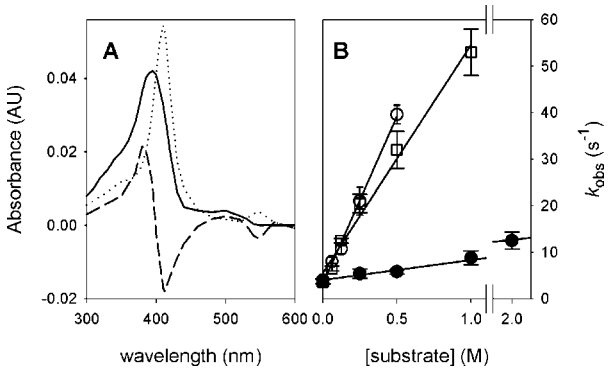


Figure 2. (A) Experimental spectrum from the LFP reaction of **2** (dashed line) was added to a scaled spectrum of **2** (dotted line) to give the spectrum of **1** (solid line). (B) Pseudo-first-order rate constants for reactions of **1** in CH_3CN with cyclohexene (○), *cis*-cyclooctene (□), and ethylbenzene (●).

overlapping absorbances from the new transient and bleached regions from destroyed **2**. We used the bleaching in the Q-band region of **2** (546 nm) to calculate the intensities of the signals from destroyed **2** at other wavelengths and added a scaled spectrum of **2** to the LFP spectrum to determine the spectrum of the species produced in the LFP reaction. The Soret band from species **1** formed photochemically has a similar λ_{max} value as found in the chemical oxidation of an iron(III) precursor with *m*-CPBA.[8]

In a series of reactions, solutions of **2** in CH_3CN were irradiated with 355 nm light with varying laser power (44−118 mJ), and bleaching at $\lambda = 412$ nm was measured. When the data was plotted in log−log format,[8] the slope of the line was 1.03 ± 0.17 (error at 2σ), indicating a one-photon process. The quantum yield determined with benzophenone as the actinometric standard[9] was 1.1×10^{-3} mol/einstein of 355 nm light.[8]

Rate constants for oxidation reactions effected by **1** were determined. Species **2** was produced by PhIO oxidations of the porphyrin−iron(III) chloride in CH_3CN solutions containing substrate at varying concentrations, LFP irradiation gave **1**, and pseudo-first-order rate constants for decay of the Soret band of **1** were measured (Figure 2B). The kinetics are described by eq 1, where k_{obs} is the pseudo-first-order rate constant, k_0 is the background rate constant, k_{ox} is the second-order rate constant, and [Sub] is the concentration of substrate. The rate constants for reactions of **1** are $k_{ox} = 72 \pm 5$ M^{-1} s^{-1} (cyclohexene), $k_{ox} = 49 \pm 6$ M^{-1} s^{-1} (*cis*-cyclooctene), and $k_{ox} = 4.3 \pm 0.5$ M^{-1} s^{-1} (ethylbenzene).[10]

$$k_{obs} = k_0 + k_{ox}[\text{Sub}] \qquad (1)$$

Cpd I of horseradish peroxidase (HRP) is formed by reaction of the enzyme with H_2O_2 or PhIO.[1] We produced HRP cpd II by oxidation of the enzyme with PhIO in a pH 7.4 aqueous solution followed by a delay to permit decay of cpd I.[8] LFP irradiation of HRP cpd II with 355 nm light gave results such as shown in Figure 3A. Again, the Soret bands of cpd I and cpd II overlap, and the photolysis gave bleaching of the cpd II signal and formation of the cpd I signal. In the absence of a reductant, the spectrum was persistent for seconds.

When HRP cpd I was formed photochemically in the presence of ascorbate, the cpd I signal at 400 nm decayed, and the signal at 418 nm for cpd II grew at the same rate (Figure 3B). A series of LFP studies with varying concentrations of ascorbate gave the results shown in the inset in Figure 3B. The pseudo-first-order rate constant obtained in the LFP experiment with 1.0 mM ascorbate ($k_{obs} = 13.9 \pm 1.2$ s^{-1}) agreed with a value determined in a stopped-flow study for reaction of HRP cpd I generated by chemical oxidation ($k_{obs} = 12.9 \pm 0.3$ s^{-1}). The second-order rate constant for reaction of ascorbate with photogenerated HRP cpd I is $k_{ox} = (1.41 \pm 0.15) \times 10^4$ M^{-1} s^{-1}.[10]

Myoglobin (Mb) is an oxygen transport enzyme, but it can be oxidized by chemical oxidants such as H_2O_2. For wild-type Mb, the cpd I analogue cannot be detected[11] because it undergoes a rapid self-reaction to give a cpd II protein (or

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(8) Details are in the Supporting Information.
(9) Hoshino, M.; Arai, S.; Yamaji, M.; Hama, Y. *J. Phys. Chem.* **1986**, *90*, 2109−2111.
(10) Rate constants are for reactions at 20 ± 2 °C. Errors in pseudo-first-order rate constants are 1σ, and those in second-order rate constants are 2σ.
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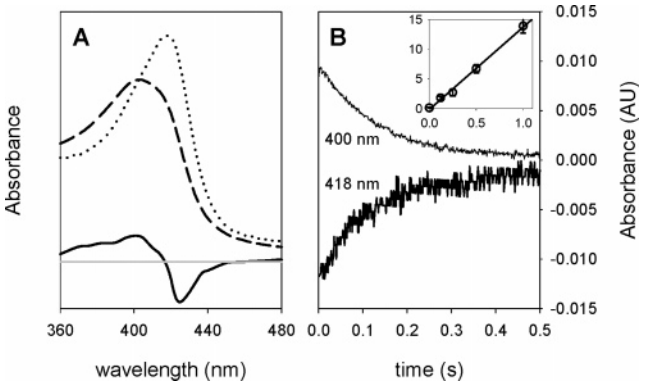

Figure 3. (A) Soret bands from HRP cpd I (dashed line) and HRP cpd II (dotted line) formed by PhIO oxidation of the enzyme and observed LFP spectrum (solid line) upon photolysis of cpd II; the absorbance scale is arbitrary, and the gray line is zero absorbance for the LFP results. (B) Kinetic traces for decay of cpd I (400 nm) and growth of cpd II (418 nm) from reaction of LFP-generated cpd I with 0.5 mM ascorbate; the inset shows pseudo-first-order k_{obs} values (in units of s^{-1}) for decay of cpd I as a function of ascorbate concentration (in mM).

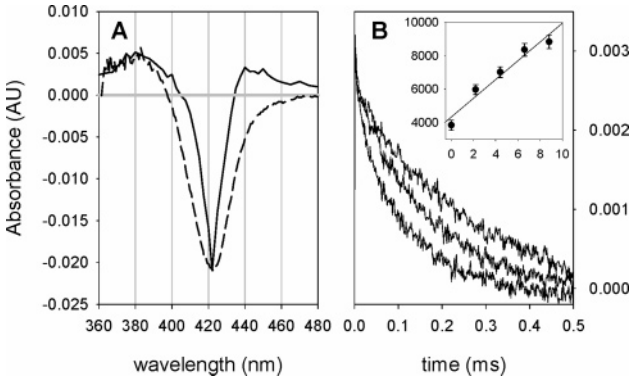

Figure 4. (A) Spectrum observed by 355 nm photolysis of horse skeletal Mb cpd II analogue (solid line) and spectrum produced by subtraction of the sperm whale Mb H64D cpd II spectrum from the corresponding cpd I spectrum using data from ref 13a (dashed line).[14] (B) Kinetic traces at 385 nm for decay in the presence of (from the top) 0.0, 2.2, and 8.8 mM $PhCH_2N(CH_3)_2$; the inset shows k_{obs} values (in units of s^{-1}) as a function of amine concentration (in mM).

protein peroxyl) radical.[12] In sperm whale Mb, substitution for His64, which is located distal to the heme iron, results in mutants that do not react rapidly with H_2O_2 but are oxidized by mCPBA to give relatively long-lived cpd I analogues.[13]

When horse skeletal Mb was oxidized with H_2O_2 at pH 8.5, formation of a cpd II analogue with $\lambda_{max} = 422$ nm for the Soret band was apparent.[8] LFP irradiation with 355 nm light gave the results shown in Figure 4A. The maximum for the bleached signal is at λ_{max} for the Soret band of the cpd II analogue, but increased absorbances are seen on both sides of the bleached signal, indicating that the product of the photolysis has a broad absorbance and a molar extinction coefficient at λ_{max} that is smaller than that of the precursor. These features are present in spectra of the cpd I analogues in Mb H64X mutants,[13] and the LFP result is similar to a difference spectrum produced by subtracting the spectrum of the sperm whale Mb H64D cpd II analogue from that of the cpd I analogue (Figure 4A).[13−15]

The Mb cpd I analogue formed in the LFP experiment was highly reactive as expected and decayed in the absence of substrate with a lifetime ($\tau = 1/k$) of only 260 μs. Nonetheless, kinetic studies could be performed. The decay was accelerated in the presence of *N*,*N*-dimethylbenzylamine (Figure 4B), which apparently reacted as a one-electron reductant. The second-order rate constant for reaction of the Mb cpd I analogue with the amine is $k_{ox} = (5.6 \pm 1.4) \times 10^5$ M^{-1} s^{-1}.[10]

The production of various cpd I species by photooxidation of cpd II species suggests broad generality. Unlike chemical oxidations, the method produces cpd I essentially instantly. We demonstrated sub-millisecond kinetic studies with the Mb cpd I analogue, and the limit for LFP methods is even shorter. One can envision a variety of studies of high-valent porphyrin−iron−oxo species formed by this method. An obvious objective is production of the elusive cytochrome P450 iron-oxo species, and although preparation of the requisite P450 cpd II analogues will be challenging,[16] the photooxidation approach appears to be promising.

Acknowledgment. This work was supported by a grant from the National Institutes of Health (GM-48722).

Supporting Information Available: Experimental details and spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

OL050296J

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(14) The sperm whale Mb H64D mutant cpd I and cpd II difference spectrum was generated from the original data in ref 13a. We thank Dr. T. Matsui and Prof. Y. Watanabe for providing these data.

(15) Irradiation of various oxidation states of Mb with 248 nm light was previously reported. At this wavelength, both the heme-iron and amino acids of the protein are photoionized. Following irradiation of the Mb cpd II analogue, signals ascribed to a cpd I analogue did not decay in 1 ms. See: Candeias, L. P.; Steenken, S. *Photochem. Photobiol.* **1998**, *68*, 39−43.

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