Multiple Active Oxidants in Cytochrome P-450 Model Oxidations

James P. Collman,* Allis S. Chien, Todd A. Eberspacher, and John I. Brauman*

Contribution from the Department of Chemistry, Stanford University, Stanford, California 94305-5080

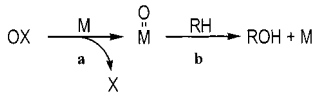
Received March 17, 2000

Abstract: We report evidence for the existence of distinct active oxidizing species in a cytochrome P-450 model system. Competitive oxidations of alkane substrates were carried out using [5,10,15,20-tetrakis-(pentafluorophenyl)porphyrinato]iron(III) chloride ($Fe(TP_{F5}P)Cl$) with various iodosylarenes as the oxygen source. The oxidation rate ratios for each pair of substrates were found to differ when different iodosylarenes were employed as terminal oxidants. These competitive oxidation results unambiguously demonstrate that the active oxidants generated from the various terminal oxidants are not the same. The influence of the oxygen donor on the selectivity requires that the donor is involved in the product-determining step. We propose that the active oxidant is a complex between the catalyst and the terminal oxidant.

Introduction

Recent developments in the elucidation of the mechanism of substrate oxidation mediated by cytochrome P-450 have revealed a system of far greater complexity than initially believed. Extensive background on the mechanism of substrate oxygenation by the cytochromes P-450 and metalloporphyrin models is available in numerous reviews.[1−11] Radical clock studies have shown that a mechanism involving a long-lived substrate radical is less likely than one with competing nonsynchronous concerted and cationic pathways.[12−15] Additionally, P-450 mutant studies have demonstrated the existence of multiple oxidized iron species which are capable of performing the oxidations characteristic of P-450.[16−21]

Scheme 1



These findings have prompted several new mechanistic proposals,[13,22−26] as well as a reexamination of the nature of the active oxidizing species in metalloporphyrin-based P-450 model systems. Previous studies have suggested that organometallic oxygenation catalysts, including non-heme complexes, do not proceed via the commonly proposed high-valent metal oxo intermediate.[27−31]

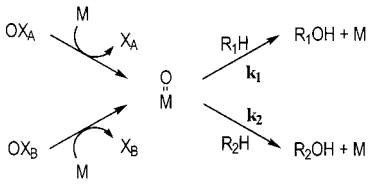
We report evidence for the existence of distinct active oxidizing species in an iron porphyrin alkane hydroxylation system employing iodosylarenes as oxygen donors. The overall reaction involves an alkane substrate (RH), which is oxidized by an iodosylarene (OX) in the presence of a metalloporphyrin catalyst (M) to give the product alcohol (ROH), with aryl iodide byproduct (X): $RH + OX \rightarrow ROH + X$.

The mechanism behind this transformation entails multiple steps. The current consensus mechanism[4,6,32,33] is shown in Scheme 1. The terminal oxidant reacts with the catalyst to produce the active oxidant (**a**), often proposed to be an Fe^{IV}-oxo-porphyrin radical cation.[34−39] The active oxidant then transfers the oxygen to the substrate (**b**), to give the oxidized product and regenerate the resting state catalyst.

(1) *Cytochrome P450: Structure, Mechanism, and Biochemistry*, 2nd ed.; Ortiz de Montellano, P. R., Ed.; Plenum Press: New York, 1995; p 652.
(2) Shilov, A. E.; Shteinman, A. A. *Acc. Chem. Res.* **1999**, *32*, 763−771.
(3) Woggon, W. D. *Top. Curr. Chem.* **1997**, *184*, 39−96.
(4) Sono, M.; Roach, M. P.; Coulter, E. D.; Dawson, J. H. *Chem. Rev.* **1996**, *96*, 2841−2887.
(5) Momenteau, M. *Perspect. Supramol. Chem.* **1996**, *3*, 155−223.
(6) Meunier, B. *Chem. Rev.* **1992**, *92*, 1411−1456.
(7) Ostovic, D.; Bruice, T. C. *Acc. Chem. Res.* **1992**, *25*, 314−320.
(8) Gunter, M. J.; Turner, P. *Coord. Chem. Rev.* **1991**, *108*, 115−161.
(9) White, P. W. *Bioorg. Chem.* **1990**, *18*, 440−456.
(10) Dawson, J. H.; Sono, M. *Chem. Rev.* **1987**, *87*, 1255−1276.
(11) Dolphin, D.; James, B. R. *ACS Symp. Ser.* **1983**, *211*, 99−115.
(12) Newcomb, M.; Shen, R.; Choi, S. Y.; Toy, P. H.; Hollenberg, P. F.; Vaz, A. D. N.; Coon, M. J. *J. Am. Chem. Soc.* **2000**, *122*, 2677−2686.
(13) Newcomb, M.; Letadicbiadatti, F. H.; Chestney, D. L.; Roberts, E. S.; Hollenberg, P. F. *J. Am. Chem. Soc.* **1995**, *117*, 12085−12091.
(14) Newcomb, M.; Letadic, M. H.; Putt, D. A.; Hollenberg, P. F. *J. Am. Chem. Soc.* **1995**, *117*, 3312−3313.
(15) Atkinson, J. K.; Hollenberg, P. F.; Ingold, K. U.; Johnson, C. C.; Letadic, M. H.; Newcomb, M.; Putt, D. A. *Biochemistry* **1994**, *33*, 10630−10637.
(16) Coon, M. J.; Vaz, A. D. N.; McGinnity, D. F.; Peng, H. M. *Drug Metab. Dispos.* **1998**, *26*, 1190−1193.
(17) Vaz, A. D. N.; McGinnity, D. F.; Coon, M. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3555−3560.
(18) Toy, P. H.; Newcomb, M.; Coon, M. J.; Vaz, A. D. N. *J. Am. Chem. Soc.* **1998**, *120*, 9718−9719.
(19) Vaz, A. D. N.; Pernecky, S. J.; Raner, G. M.; Coon, M. J. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 4644−4648.
(20) Toy, P. H.; Dhanabalasingam, B.; Newcomb, M.; Hanna, I. H.; Hollenberg, P. F. *J. Org. Chem.* **1997**, *62*, 9114−9122.
(21) Pratt, J. M.; Ridd, T. I.; King, L. J. *J. Chem. Soc., Chem. Commun.* **1995**, 2297−2298.
(22) Toy, P. H.; Newcomb, M.; Hollenberg, P. F. *J. Am. Chem. Soc.* **1998**, *120*, 7719−7729.
(23) Shaik, S.; Filatov, M.; Schroder, D.; Schwarz, H. *Chem. Eur. J.* **1998**, *4*, 193−199.
(24) Filatov, M.; Shaik, S. *J. Phys. Chem. A* **1998**, *102*, 3835−3846.
(25) Filatov, M.; Harris, N.; Shaik, S. *J. Chem. Soc., Perkin Trans. 2* **1999**, 399−410.
(26) Yoshizawa, K.; Shiota, Y.; Yamabe, T. *Organometallics* **1998**, *17*, 2825−2831.
(27) Nam, W.; Valentine, J. S. *J. Am. Chem. Soc.* **1990**, *112*, 4977−4979.
(28) Nishiyama, H.; Shimada, T.; Itoh, H.; Sugiyama, H.; Motoyama, Y. *Chem. Commun.* **1997**, 1863−1864.
(29) Nagata, T.; Imagawa, K.; Yamada, T.; Mukaiyama, T. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 1455−1465.
(30) Fontecave, M.; Mansuy, D. *Tetrahedron* **1984**, *40*, 4297−4311.
(31) Park, Y. C.; Na, H. G. *Main Group Metal Chem.* **1997**, *20*, 269−276.
(32) Gunter, M. J.; Turner, P. *Coord. Chem. Rev.* **1991**, *108*, 115−161.
(33) Groves, J. T. *J. Chem. Educ.* **1985**, *62*, 928−31.

10.1021/ja000961d CCC: $19.00
Published on Web 10/27/2000

Scheme 2



The active oxidant in the consensus mechanism is a simple metal-oxo; this species should mediate reactions with identical selectivity regardless of its origin, whether from iodosylbenzene itself, an iodosylarene derivative, or any other single oxygen atom donor. As illustrated in Scheme 2 for a two-substrate competition experiment, the active oxidant, M═O, is completely dissociated from the terminal oxidant; therefore k_1/k_2 should be independent of the identity of OX.

Results and Discussion

Competitive Oxidations. Competitive hydroxylations of alkane substrates have been carried out with [5,10,15,20-tetrakis-(pentafluorophenyl)porphyrinato]iron(III) chloride (Fe($TP_{F5}P$)-Cl) as the catalyst, and iodosylbenzene (PhIO), pentafluoroiodosylbenzene (F_5-PhIO), or iodobenzene diacetate (IBDA) as the oxygen source. Reactions were performed competitively, as opposed to comparing rates of single substrate oxidations in separate reactions. Competitive rates of product appearance were determined for assorted pairs of alkane substrates as listed in Table 1. The ratio of substrate oxidation rates was taken as an indication of the inherent preference of the active oxidant for oxygenating one substrate over the other.

Contrary to the prediction of Scheme 2, the competitive oxidation results in Table 1 clearly show small but statistically significant changes in the ratio of substrate oxidation rates when different terminal oxidants are employed. In the competitive oxidation of cyclohexane and d_{12}-cyclohexane, $k_1/k_2 = 5.8$ with PhIO as the terminal oxidant, $k_1/k_2 = 4.3$ with F_5-PhIO, and $k_1/k_2 = 6.3$ with IBDA as the terminal oxidant. Similarly, a cyclohexane/cyclopentane mix gives $k_1/k_2 = 2.5$ with PhIO, compared to $k_1/k_2 = 3.1$ with F_5-PhIO and $k_1/k_2 = 2.2$ with IBDA. The competitive oxidation ratios are internally consistent, within experimental error. For a given set of substrates, the third ratio can be derived from two others, *i.e.* $(k_a/k_b)\cdot(k_b/k_c) = k_a/k_c$.

Each substrate was oxidized to the corresponding alcohol in nearly quantitative yield based on oxidant consumed, with small amounts of ketone over-oxidation product. The yield of ketone in comparison to its corresponding alcohol was also found to be oxidant-dependent: [RO]/[ROH] was less than 1% in PhIO oxidations, but up to 4% and 10% in F_5-PhIO and IBDA oxidations, respectively.

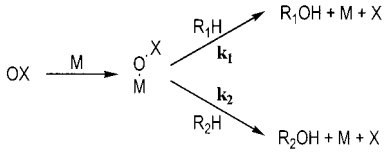
Nature of the Active Oxidants. These competitive oxidation results unambiguously show that the active oxidants generated from these oxygen donors are not the same. The substrate selectivities are different with different terminal oxidants, therefore the active oxidants must not be identical, *i.e.* they cannot all be pure high-valent metal-oxo species. The differences in reactivity with different iodosylarenes requires the presence of the aryl iodide during the product-determining step (Scheme 3, k_1/k_2 is dependent on the identity of X).

Table 1. Competitive Oxidation Results

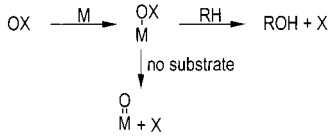
		k_1/k_2[a]		
substrate 1	substrate 2	PhIO	F5−PhIO	IBDA
cycloheptane	cyclohexane	2.4(2)	1.6(2)	
cycloheptane	cyclopentane	5.9(2)	4.9(2)	
cycloheptane	d_{12}−cyclohexane	13(1)		10(1)
cyclohexane	cyclopentane	2.5(1)	3.1(1)	2.2(1)
cyclohexane	d_{12}−cyclohexane	5.8(2)	4.3(2)	6.3(2)
cyclopentane	d_{12}−cyclohexane	2.3(1)		2.8(2)
cyclohexane	*n*-pentane	5.2(3)	4.1(3)	

[a] Numbers in parentheses indicate the error (95% confidence level) in the final digit.

Scheme 3



Scheme 4



While these results cannot rigorously exclude the possibility that a simple metal-oxo is formed with one of the terminal oxidants, there is no obvious reason a metal-oxo would be formed in one case while the other oxidants generate catalyst-oxidant complexes. The simplest explanation for the influence of the terminal oxidant is that the species which transfer oxygen to the substrates are complexes between the catalyst and the terminal oxidant. Reports of μ-oxo manganese porphyrin complexes which contain one iodosylbenzene per manganese ($[XMn^{IV}TPP(OIPh)]_2O$) have been published.[40−42]

In prior studies, a reasonably long-lived oxidized iron intermediate has been observed which is competent in olefin epoxidation.[39] This intermediate has been generated independently from the reaction of mCPBA or PhIO with various iron porphyrins, and is spectroscopically consistent with an Fe^{IV} oxo-porphyrin radical cation.[34−36,38,39,43] It should be noted that this iron-oxo species has been observed only by stoichiometric generation, in the absence of substrate. The catalytic reaction could be quite different.

A logical explanation is that there are two available pathways for the catalyst−oxidant complex: oxidation of substrate, or formation of a metal-oxo species (Scheme 4). When substrate is present, the catalyst−oxidant complex is consumed in the oxidation of substrate, but if there is no substrate to oxidize, the catalyst−oxidant complex goes on to form the metal-oxo which is also competent to oxidize certain substrates.[39]

Conclusion

On the basis of the studies presented above, we conclude that the active oxidizing species generated from the reactions

(34) Gold, A.; Jayaraj, K.; Doppelt, P.; Weiss, R.; Chottard, G.; Bill, E.; Ding, X.; Trautwein, A. X. *J. Am. Chem. Soc.* **1988**, *110*, 5756−5761.
(35) Fujii, H. *Chem. Lett.* **1994**, 1491−1494.
(36) Balch, A. L.; Latosgrazynski, L.; Renner, M. W. *J. Am. Chem. Soc.* **1985**, *107*, 2983−2985.
(37) Sugimoto, H.; Tung, H. C.; Sawyer, D. T. *J. Am. Chem. Soc.* **1988**, *110*, 2465−2470.
(38) Fujii, H.; Ichikawa, K. *Inorg. Chem.* **1992**, *31*, 1110−1112.
(39) Groves, J. T.; Haushalter, R. C.; Nakamura, M.; Nemo, T. E.; Evans, B. J. *J. Am. Chem. Soc.* **1981**, *103*, 2884−2886.
(40) Smegal, J. A.; Schardt, B. C.; Hill, C. L. *J. Am. Chem. Soc.* **1983**, *105*, 3510−3515.
(41) Smegal, J. A.; Hill, C. L. *J. Am. Chem. Soc.* **1983**, *105*, 3515−3521.
(42) Birchall, T.; Smegal, J. A.; Hill, C. L. *Inorg. Chem.* **1984**, *23*, 1910−1913.

of Fe($TP_{F5}P$)Cl with PhIO, F_5-PhIO, and IBDA are different from one another. Although the possibility that a pure metal-oxo is formed in one case cannot be ruled out, the most reasonable postulate is that the active oxidants in all three cases are the respective catalyst−oxidant complexes.

Experimental Section

Materials. [5,10,15,20-Tetrakis(pentafluorophenyl)porphyrinato]-iron(III) chloride (Fe($TP_{F5}P$)Cl) was synthesized and metalated by literature procedures.[44] Iodosylbenzene (PhIO)[45] was converted from the diacetate, while pentafluoroiodosylbenzene (PFIB)[46] (**Caution**: PFIB has been known to explode,[47] even at ambient temperature and pressure[48]) was prepared in two steps from pentafluoroiodobenzene. Pentane, cyclopentane, cyclohexane, and cycloheptane were purified by standard procedures, which included the removal of alkene contaminants,[49] and stored over 4 Å molecular sieves under argon. Methylene chloride was dried and distilled from P_2O_5 under N_2, then stored over 4 Å molecular sieves under argon. d_{12}-Cyclohexane and iodobenzene diacetate were purchased from Aldrich. Other materials were purchased in the highest possible purity and used as received, after confirming purity by GC and/or GC-MS.

Product Analysis. Reaction products were detected and quantified using a Hewlett-Packard 6950 gas chromatograph fitted with a (5% phenyl)-methyl siloxane capillary column (HP5-MS, Agilent Technologies, 0.25 mm i.d. × 30 m), and flame-ionization detector. All peaks were identified by co-injection with the known compounds. Oxidation products were quantified by comparison with an internal standard (chlorobenzene): multilevel calibration plots for detector response were prepared for iodobenzene, pentafluoroiodobenzene, and the alcohol and ketone products versus the internal standard, using known stock solutions which approximated experimental concentrations.

Competitive Oxidation Studies. For each pair of substrates, a 1:1 (m/m) mixed substrate stock solution was prepared, which was used for the entire series of reactions. A stock solution containing the catalyst and GC internal standard (PhCl) in CH_2Cl_2 was prepared under inert atmosphere conditions, such that 100 μL of the stock solution contained 0.05 μmol of catalyst and 5 μmol of PhCl. PhIO (50 μmol, 11.1 mg), PFIB (50 μmol, 15.6 mg), or IBDA (50 μmol, 16.1 mg) and a stir bar were placed in a 10 mL round-bottomed flask, which was sealed with a SubaSeal septum. The flask was placed in an ice bath, and the atmosphere in the flask was replaced with Ar. The appropriate amounts of substrates (500 μmol each, 1 mmol total) and solvent, totaling 900 μL, were syringed into the flask.

To initiate the reaction, 100 μL of the catalyst stock solution was injected. Final reaction concentrations were as follows: catalyst, 0.05 mM; substrates, 500 mM each; PhCl, 5 mM in 1 mL total reaction volume. At appropriate intervals, 5 μL aliquots were removed, diluted with 15 μL of CH_2Cl_2, and filtered through a microfiber glass plug. Samples were analyzed immediately by GC.

Acknowledgment. We thank Dr. Lee Slaughter for helpful discussions and the NSF (Grant No. CHE9123187-A4) for financial support.

JA000961D

(43) Bill, E.; Ding, X. Q.; Bominaar, E. L.; Trautwein, A. X.; Winkler, H.; Mandon, D.; Weiss, R.; Gold, A.; Jayaraj, K.; Hatfield, W. E.; Kirk, M. L. *Eur. J. Biochem.* **1990**, *188*, 665−672.

(44) Collman, J. P.; Tyvoll, D. A.; Chng, L. L.; Fish, H. T. *J. Org. Chem.* **1995**, *60*, 1926−1931.

(45) Saltzman, H.; Sharefkin, J. G. *Org. Synth.* **1963**, *43*, 60−61.

(46) Schmeisser, M.; Dahmen, K.; Sartori, P. *Chem. Ber.* **1967**, *100*, 1633−1637.

(47) Collman, J. P. *Chem. Eng. News* **1985**, *63*, 2−2.

(48) Groves, J. T. Personal communication

(49) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*, 2nd ed.; Pergamon Press: Oxford, England, 1980.