Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 1731–1733, April 1987
Chemistry

Ferric chloride-catalyzed activation of hydrogen peroxide for the demethylation of *N,N*-dimethylaniline, the epoxidation of olefins, and the oxidative cleavage of vicinal diols in acetonitrile: A reaction mimic for cytochrome P-450

(Lewis acid)

HIROSHI SUGIMOTO, LEE SPENCER, AND DONALD T. SAWYER*

Department of Chemistry, Texas A&M University, College Station, TX 77843

Communicated by Thomas C. Bruice, November 12, 1986 (received for review February 5, 1986)

ABSTRACT **In dry acetonitrile, anhydrous $Fe^{III}Cl_3$ catalyzes the demethylation of *N,N*-dimethylaniline, the epoxidation of olefins, and the oxidative cleavage of 1-phenyl-1,2-ethanediol (and other 1,2-diols) by hydrogen peroxide. For each class of substrate the products closely parallel those that result from their enzymatic oxidation by cytochrome P-450. Because of (*i*) the close congruence of products, (*ii*) the catalytic nature of the $Fe^{III}Cl_3/H_2O_2$ reaction mimic, and (*iii*) the similarity of the dipolar aprotic solvent (acetonitrile) to the proteinaceous lipid matrix of the biomembrane, the form of the reactive intermediate may be the same in each case. A mechanism is proposed in which an initial Lewis acid–base interaction of $Fe^{III}Cl_3$ with H_2O_2 generates a highly electrophilic Fe^{III}-oxene species as the reactive intermediate. This is in contrast to the prevailing view that cytochrome P-450 acts as a redox catalyst to generate an Fe^{V}-oxo species or an Fe^{IV}-oxo cation radical as the reactive intermediate.**

The cytochrome P-450 enzymes catalyze a wide variety of substrate transformations (1), including monoxygenation via oxygen atom transfer (Eq. 1).

$$RH + O_2 + NAD(P)H + H^+ \xrightarrow{P\text{-}450} ROH + H_2O + NAD(P)^+$$

(RH = substrate) [1]

These proteins consist of a single polypeptide chain (45–50 kDa) that contains an iron(III) protoporphyrin IX prosthetic group. The resting state of the enzyme is low spin, with a thiolate of cysteine in one axial position and a water molecule in the other (2). The metal center is surrounded by hydrophobic protein residues, which provide an essentially nonaqueous matrix at the active site.

The exact sequence and nature of the electron-transfer steps that are involved in cytochrome P-450 and its facilitation of oxygen-atom transfer have not been elucidated. However, *in vivo* cytochrome P-450 utilizes molecular oxygen, which the system reduces by two equivalents to the peroxy level prior to transfer of an oxygen atom to substrate during the catalytic cycle. The fact that cytochrome P-450 can utilize hydrogen peroxide, alkyl hydroperoxides, or peroxy acids as a source of oxygen atoms for substrate monoxygenation (a process termed the "peroxide shunt") (3) supports the idea of a peroxide intermediate in the redox cycle for O_2 reduction in cytochrome P-450. This discovery provided the basis for a series of model studies with a variety of synthetic iron(III) porphyrins in conjunction with peroxides or peroxy acids (4, 5). Such systems are able to mimic the many substrate transformations that are catalyzed by cytochrome P-450 (including hydroxylation of aromatic and aliphatic hydrocarbons, epoxidation of olefins, dehydrogenation, and demethylation of amines). On the basis of such studies, mechanisms for the P-450-catalyzed oxygen-atom transfer have been proposed. These involve an active intermediate that usually is formulated as an iron(IV)-oxo porphyrin cation radical [+·$PorFe^{IV}$=O], which monoxygenates the substrate and dissociates to regenerate the iron(III) form of the enzyme.

The solution properties of synthetic metalloporphyrins have required the use of halogenated hydrocarbons or methanol for most model studies. However, the redox properties of complexed metal ions and the delicate balance of the ligand–metal interactions that are associated with metal ion catalyses are extremely sensitive to the immediate solution environment (6), and thus the choice of a model solvent is a critical consideration. We believe that the solution matrix of the active site of cytochrome P-450 is best modeled by dipolar aprotic solvents such as acetonitrile.

This prompted an earlier study (7), in which we demonstrated that the combination of $Fe^{III}Cl_3$ and H_2O_2 in dry acetonitrile (MeCN) efficiently epoxidizes olefins and monoxygenates alkanes, alcohols, ethers, aldehydes, thioethers, and sulfoxides. We now report that $Fe^{III}Cl_3$ *catalyzes* H_2O_2 to epoxidize olefins, to demethylate *N,N*-dimethylaniline ($PhNMe_2$), and to cleave α-diols to give aldehydes. These substrate conversions are analogous to those catalyzed by cytochrome P-450. A mechanism is proposed in which an initial Lewis-acid-induced activation of H_2O_2 by $Fe^{III}Cl_3$ occurs to form an electrophilic Fe(III)-oxene reactive intermediate.

MATERIALS AND METHODS

Burdick and Jackson "distilled in glass" acetonitrile (0.004% H_2O) was dried by passage through a column of Woelm N Super I alumina. Anhydrous $Fe^{III}Cl_3$ (Aldrich) was used as received. Pure H_2O_2 (assay 98%) was prepared from 50% (wt/wt) H_2O_2 (J. T. Baker Chemical, Phillipsburg, NJ). Careful removal of water from 10 ml of 50% H_2O_2 at 0°C under high vacuum gave 2.5–3 ml of pure hydrogen peroxide. This was quickly dissolved in dry acetonitrile (25 ml) to make an approximately 1 M solution, which was assayed by permanganate titration (the presence of acetonitrile did not interfere). The acetonitrile solution of H_2O_2 was stable at 0°C; its assay did not change over a period of 1 month. Other reagents and substrates were analytical grade or highest purity available and were used without further purification.

Hazard Warning! Pure H_2O_2 is an exceptional oxidant. Trace quantities of reduced transition metals can initiate its

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Por, porphyrin; NHE, normal hydrogen electrode.
*To whom reprint requests should be addressed.

violent decomposition and oxidation of organic materials. Exercise extreme care, use adequate safety protection, and work with small quantities during the course of its purification, storage, and use.

In a typical experiment, 0.1 mmol of $Fe^{III}Cl_3$ (1 M in CH_3CN) was added slowly over 1 min to a mixture of 1.0 mmol of substrate and 3.0 mmol of H_2O_2 [1 M H_2O_2 (98%) in MeCN]. The reaction mixture was held at −10°C with stirring for various reaction times, after which the product solution was added to 50 ml of NaCl-saturated H_2O. This was then extracted with 20 ml of diethyl ether and the latter was analyzed by use of capillary gas chromatography, GC–MS, and HPLC.

RESULTS

Table 1 summarizes the reactivities and products from the slow addition of $Fe^{III}Cl_3$ to a binary mixture of H_2O_2 and *N,N*-dimethylaniline, an olefin, or a 1,2-diol in dry acetonitrile. (Under the reaction conditions all substrates were unreactive in the presence of either $Fe^{III}Cl_3$ or H_2O_2 alone.) The oxidation state of the $Fe^{III}Cl_3$ catalyst is conserved in all the experiments on the basis of electrochemical measurements [in dry acetonitrile the characteristic reduction potential for the $Fe^{III}Cl_3/Fe^{II}Cl_3^-$ couple is +0.46 V vs. the normal hydrogen electrode (NHE)] and redox titrations, prior to and after each experiment. In the absence of substrate, the combination of $Fe^{III}Cl_3$ with H_2O_2 results in its rapid conversion to O_2 and H_2O.

As with cytochrome P-450, the $Fe^{III}Cl_3$ system catalyzes H_2O_2 to epoxidize olefins.

$$Fe^{III}Cl_3 + RCH{=}CHR' + H_2O_2 \xrightarrow{MeCN} \underset{\diagdown\,O\,\diagup}{RCH{-}CHR'} + Fe^{III}Cl_3(OH_2) \qquad [2]$$

The conversion of norbornene is stereospecific to the *exo*-epoxide. For *cis*-stilbene, the *cis*-to-*trans* epoxide ratio is 2:1, but when the H_2O_2-to-substrate ratio is increased to 6:1, the *cis*-to-*trans* epoxide ratio increases to 4:1. Substitution of $[Fe^{III}(H_2O)_6](ClO_4)_3$ for $Fe^{III}Cl_3$ decreases the overall conversion efficiency for *cis*-stilbene by a factor of 9 and yields no epoxide (PhCHO is the major product). Addition of one equivalent of tetrabutylammonium chloride (Bu_4NCl) to the *cis*-stilbene/H_2O_2 mixture prior to $Fe^{III}Cl_3$ addition results in a decrease in conversion efficiency by a factor of 3. [Addition of one equivalent of Bu_4NCl to a solution of $Fe^{III}Cl_3$ causes its reduction potential to shift from +0.46 V ($Fe^{III}Cl_3/Fe^{II}Cl_3^-$) to +0.34 V vs. NHE (characteristic of the $Fe^{III}Cl_4^-/Fe^{II}Cl_3^- + Cl^-$ couple).]

Substitution of *m*-chloroperbenzoic acid in place of H_2O_2 results in the analogous epoxide products. Careful competition experiments in the presence and absence of $Fe^{III}Cl_3$ have demonstrated that it accelerates the epoxidation process. Attempts to utilize iodosylbenzene and pentafluoroiodosylbenzene with $Fe^{III}Cl_3$ produced a wide variety of products. Many of these derived from the attack on substrate by chlorine atoms, presumably from the direct oxidation of chloride ion by iodosylbenzene. However, catalytic olefin epoxidations have been observed in acetonitrile through the use of iodosylbenzene in conjunction with simple non-chloride-ligated iron(III) salts (8).

In a manner that parallels cytochrome P-450, the $Fe^{III}Cl_3/H_2O_2$/MeCN system demethylates *N,N*-dimethylaniline to form *N*-methylaniline and formaldehyde (Eq. 3).

$$Fe^{III}Cl_3 + PhNMe_2 + H_2O_2 \rightarrow PhNHMe + H_2C{=}O + Fe^{III}Cl_3(OH_2) \qquad [3]$$

The same reactivity has been observed by use of an Fe(III) porphyrin/iodosylbenzene system and has been rationalized in terms of an "oxygen rebound" mechanism (9). For the latter an initial formation of an Fe^{IV}-oxo porphyrin cation radical has been proposed, which abstracts a hydrogen atom from the methyl group of *N,N*-dimethylaniline to generate the *N*-methylene radical and a "crypto-hydroxyl" metal center. These recombine to form the observed products and regenerate the Fe(III) porphyrin center.

Finally, the $Fe^{III}Cl_3/H_2O_2$/MeCN system also oxidatively cleaves 1,2-diols.

$$\begin{matrix} OH & OH \\ | & | \\ RCH{-}CHR' \end{matrix} + Fe^{III}Cl_3 + H_2O_2 \rightarrow RCHO + R'CHO + Fe^{III}Cl_3(OH_2)_2 \qquad [4]$$

The efficient oxidative cleavage of both $PhCH(OH)CH_2OH$ and $PhCMe(OH)CMe(OH)Ph$ by the $Fe^{III}Cl_3/H_2O_2$/MeCN system (Table 1) closely parallels the results from a recent study (10) in which $Cr^{III}Ph_4PorCl$ was used as the cytochrome-P-450 model, 4-$CNPhNMe_2O$ as the O atom transfer agent, and $PhCH(OH)CH_2OH$ as the model substrate for cholesterol side-chain cleavage.

DISCUSSION

Preliminary kinetic studies have demonstrated that the reaction of the $Fe^{III}Cl_3/H_2O_2$ system with substrate is first order

Table 1. Products and conversion efficiencies for the $Fe^{III}Cl_3$-catalyzed epoxidation of olefins, demethylation of $PhNMe_2$, and oxidative cleavage of 1,2-diols by H_2O_2 in dry acetonitrile

Substrate (RH)	Reaction time, min	Reaction conversion efficiency, %*	Catalyst turnover number†	Products (yield)
Olefins				
Norbornene	10	52	5	*exo*-Epoxide (80%), other nonepoxide products (20%)
Cyclohexene	30	37	4	Epoxide (64%), dicyclohexyldioxane (13%)
1,4-Cyclohexadiene	20	39	4	Benzene (76%), epoxide (17%)
cis-Stilbene	20	63	6	PhCHO (50%), epoxides (50%) (*cis*-to-*trans* epoxide ratio, 2.5:1)
Dimethylaniline				
$PhNMe_2$	20	39	4	PhNHMe (95%), PhN(CHO)Me (5%)
1,2-Diols				
$PhCH(OH)CH_2OH$	30	43	4	[PhCHO + CH_2O] (66%),‡ PhC(O)CHO (19%), [PhCH(OH)CHO + $PhC(O)CH_2OH$] (15%)
$PhCMe(OH)CMe(OH)Ph$	30	30	3	PhC(O)Me (100%)

*Percentage of substrate converted to products.
†Millimoles of RH converted per mmol of $Fe^{III}Cl_3$ added.
‡For the reaction conditions PhCHO was inert.

in both $Fe^{III}Cl_3$ and H_2O_2 concentrations but independent of substrate concentration. Such behavior is consistent with the rate-determining formation of a reactive intermediate from $Fe^{III}Cl_3/H_2O_2$. In the absence of direct structural evidence, conclusions as to the form of the intermediate are speculative. However, without electron transfer between the Fe(III) center and H_2O_2, the initial interaction between $Fe^{III}Cl_3$ and H_2O_2 is that of a Lewis acid with a base, a process facilitated by the base-free solvent system. The deactivation of the $Fe^{III}Cl_3/H_2O_2$ system by donor ligands such as water or excess chloride ($Fe^{III}Cl_4^-$ is inert with respect to H_2O_2 disproportionation or activation) also is consistent with a neutralization of the Lewis acidity of the Fe(III) center.

The ability of $Fe^{III}Cl_3$ to activate peracids in a manner that parallels its activation of H_2O_2 [coupled with the similar reactivity of iodosylbenzene induced by simple iron(III) salts in acetonitrile (8)] indicates that a common reactive iron–oxygen atom intermediate is formed. Such a species can be written in several formulations (Eq. 5).

$$Cl_3Fe^{V}(O^{2-}) \leftrightarrow (\cdot Cl)Cl_2Fe^{IV}(O^{2-}) \leftrightarrow Cl_3Fe^{IV}(O^{-}\cdot) \leftrightarrow Cl_3Fe^{III}(\dot{Q}) \quad [5]$$

For the $Fe^{III}Cl_3/H_2O_2$ system, formation of an Fe^{V}-oxo species is much less likely, given the electronegative chloro ligands and their inability to stabilize a high-valent metal center. Furthermore, electron donation from coordinated chloride to the metal center to generate an Fe^{IV}-oxo chloro radical is precluded. Thus, we favor a mechanism in which the reactive intermediate is formulated as an Fe^{III}-oxene [$Fe^{III}(\dot{Q})$] species, which results from a Lewis acid–base interaction between $Fe^{III}Cl_3$ and H_2O_2 (Eq. 6).

$$Fe^{III}Cl_3 + H_2O_2 \rightarrow [Cl_3Fe^{III}(OH)_2] \rightarrow Cl_3Fe^{III}(\dot{Q})(OH_2) \quad [6]$$

In the absence of substrate the $Fe^{III}(\dot{Q})$ intermediate dehydrogenates H_2O_2 to give dioxygen (Eq. 7).

$$Fe^{III}Cl_3 + H_2O_2 \rightarrow Cl_3Fe^{III}(\dot{Q})(OH_2) \xrightarrow{H_2O_2} Cl_3Fe^{III}(OH_2)_2 + O_2 \quad [7]$$

The high degree of electrophilicity and radical character of the oxene intermediate would facilitate hydrogen atom abstraction from substrates such as the methyl group of *N,N*-dimethylaniline to generate a "crypto-hydroxyl" metal center able to undergo the well-known "oxygen rebound" mechanism (4).

$$Cl_3Fe^{III}(\dot{Q}) + (CH_3)_2NPh \rightarrow \left[Cl_3Fe^{III}(\cdot OH) + \cdot CH_2(CH_3)NPh\right] \xrightarrow{H_2O} Cl_3Fe^{III}(H_2O) + HOCH_2(CH_3)N{-}Ph \rightarrow CH_3NHPh + CH_2O \quad [8]$$

Although a similar rebound process may occur in the cleavage of 1,2-diols [via the sequential abstraction of hydrogen atoms from carbon centers by the $Fe^{III}(\dot{Q})$ reactive intermediate], a more plausible process is the concerted removal of the two diol hydrogen atoms to generate a dioxetane intermediate followed by facile homolytic cleavage to the observed products (Eq. 9).

$$Fe^{III}Cl_3 + H_2O_2 + RCH(OH)CH(OH)R' \xrightarrow{MeCN} Cl_3Fe^{III}(OH_2)_2 + [RCHCHR'](O{-}O) \rightarrow RCHO + R'CHO \quad [9]$$

A heterolytic two-electron process has been postulated (11) for the cleavage of 1,2-bis(4-methoxyphenyl)ethane-1,2-diol.

The inability of the iodosylbenzene/$Fe^{III}Cl_3$ system to epoxidize olefins can be related to the ease of oxidation of coordinated chloride ion by iodosylbenzene. In view of the ubiquity of chloride ion in natural systems this result must raise questions as to whether iodosylbenzene is an appropriate oxygenating agent to utilize in model studies of cytochrome P-450.

In summary, the $Fe^{III}Cl_3/H_2O_2$/MeCN system is able to catalyze a variety of substrate transformations analogous to those of cytochrome P-450. The simplicity of the system allows the mechanism of the various reactions to be examined in the absence of the complications that are introduced by the electronic effects of the porphyrin ring and the axial thiolate group. The resulting data prompt us to suggest that the enzyme acts as a Lewis acid to activate reduced dioxygen to an equivalent metal-oxene electrophilic center rather than an iron(V)-oxo center or an iron(IV)-oxo porphyrin cation radical. The iron(III)-oxene formulation is preferred because of the electrophilic and radical character of the oxygen atom. The postulate of a hypervalent iron-oxo intermediate in the cytochrome P-450 redox cycle [with an estimated (1) redox potential of +1.5–2.0 V vs. NHE] is incompatible with an axial thiolate moiety and the one-electron oxidation potential of O_2, $O_2-\cdot$, or H_2O_2 (−0.16 V, +0.89, and +0.5 V vs. NHE, respectively). Thus, we propose electrophilic oxygen atom transfer chemistry for the activated center of cytochrome P-450, $(RS\cdot)PorFe^{II}(\dot{Q})$.

This work was supported by the National Science Foundation under Grant CHE-8516247 and the National Institutes of Health under Grant GM-36289. Most of the experiments were conducted at the University of California, Riverside.

1. Guengerich, P. F. & MacDonald, T. L. (1984) *Acc. Chem. Res.* **17**, 9–16.
2. White, R. E. & Coon, M. J. (1980) *Annu. Rev. Biochem.* **49**, 315–356.
3. Hrycay, E. G. & O'Brien, P. J. (1973) *Arch. Biochem. Biophys.* **157**, 7–22.
4. Groves, J. T. & McClusky, G. A. (1976) *J. Am. Chem. Soc.* **98**, 859–861.
5. Groves, J. T., Haushalter, R. C., Nakamura, M., Nemo, T. E. & Evans, B. J. (1981) *J. Am. Chem. Soc.* **103**, 2884–2886.
6. Burgess, J. (1978) in *Metal Ions in Solution* (Wiley, New York), pp. 229–258.
7. Sugimoto, H. & Sawyer, D. T. (1985) *J. Org. Chem.* **50**, 1784–1786.
8. VanAtta, R. B., Franklin, C. C. & Valentine, J. S. (1984) *Inorg. Chem.* **23**, 4121–4123.
9. Shannon, M. F., Pai, E. F. & Bruice, T. C. (1981) *J. Am. Chem. Soc.* **103**, 4580–4583.
10. Murray, R. I. & Sliger, S. G. (1985) *J. Am. Chem. Soc.* **107**, 2186–2187.
11. Okamoto, T., Sasaki, K. & Shimada, M. (1985) *J. Chem. Soc. Chem. Commun.* **7**, 381–383.