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Dioxygen activation by iron complexes. The search for reactive intermediates

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Abstract

Three different modes of oxygen activation by iron complexes are described: (1) Oxygen atom transfer by ferric porphyrin peroxo complexes to electron-deficient olefins via a nucleophilic mechanism, (2) oxidative dehydrogenation of a cyclam complex, probably involving a radical intermediate, and (3) stereospecific, non-radical hydroxylation of alkanes by organic peracids catalyzed by iron complexes by an unknown mechanism. These three reactions are presented to remind us that the possible modes of interaction between iron and oxygen are remarkably diverse and that a variety of mechanisms should thus be considered in assessing possible reaction pathways of heme and non-heme iron-containing enzymes.

1. Introduction

The mechanism of oxygen atom transfer from dioxygen to substrates catalyzed by iron-containing monooxygenase enzymes has been investigated for many decades. Studies of the enzyme cytochrome P-450 itself as well as of iron porphyrin complexes reacted with oxygen atom donors led to development of the first detailed mechanistic description of the mechanism by which one class of such oxygen atom transfers may occur [1]. But current research into mechanisms of other iron-containing monooxygenase enzymes, both heme and non-heme, suggest that other classes of mechanisms are possible [2], and it now appears that the modes of interaction between iron and oxygen may be quite diverse. In this paper, we describe results that we have obtained in three types of systems, each of which involves different types of iron complexes and some form of peroxide. The first example provides evidence for oxygen atom transfer by direct nucleophilic attack on electron-deficient olefins by ferric porphyrin peroxo complexes, the same type of peroxo complex that undergoes O–O bond cleavage leading to the electrophilic reactions characteristic of cytochrome P-450. The second example describes the oxidative dehydrogenation of a cyclam complex that seems to involve a radical intermediate, possibly formed during the decomposition of a ferrous hydroperoxide complex. Yet under different conditions, this ferrous hydroperoxide is believed to be the intermediate in the non-radical epoxidation of olefins by cyclam complexes and H_2O_2 [3]. This reaction may be

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related to that which occurs with iron bleomycin, an anti-tumor drug believed to function by selectively cleaving DNA with dioxygen and ascorbic acid [4]. The third example first reviews work carried out previously by other investigators that demonstrates electron-deficient organic peracids may react directly with alkanes to give stereospecific hydroxylation and then reports our observation that iron complexes can catalyze this reaction. Thus far, there is no direct evidence for a non-radical enzymatic pathway for the hydroxylation of alkanes, but it seems possible that such a reaction may one day be identified. Like all the other reactive intermediates discussed in this report, however, it might well only perform such reactivity in a very specific biological environment. Determining and fine-tuning the behavior of reactive intermediates in a specific biological environment remains a major challenge to chemists and biologists alike.

2. Results

2.1. Nucleophilic reactivity of ferric heme peroxo complexes

Cytochrome P-450 is one of the most thoroughly studied monooxygenase enzymes, and yet several early steps in the mechanism of oxygen transfer are not yet clearly understood [1,2,5–10]. The initial steps of the mechanism involve binding of dioxygen by a heme-iron center followed by one-electron reduction to form a ferric peroxo or hydroperoxo complex, $Fe^{III}(O_2^{2-})$ or $Fe^{III}(OOH^-)$. This peroxo complex may then react by three different pathways: (a) heterolytic O–O cleavage to generate a (porphyrin)$Fe^V=O$ or (oxidized porphyrin)$Fe^{IV}=O$ high valent oxo species, (b) homolytic O–O cleavage to generate a (porphyrin)$Fe^{IV}=O$ [11–13] or (c) direct nucleophilic attack on enzyme-bound substrate [7,14–17]. There are several non-heme oxygenase enzymes that might react by a similar mechanism as cytochrome P-450, but even less is known about the actual pathway in those reactions [18].

Model studies have been very helpful in elucidating the reactivities and other properties of reactive complexes analogous to proposed intermediates in the cytochrome P-450 mechanism; much of the evidence for the intermediacy of the (porphyrin)$Fe^V=O$ high valent oxo species as well as for the homolytic bond cleavage to generate (porphyrin)$Fe^{IV}=O$ has been obtained from careful studies of reaction pathways of ferric porphyrin complexes with single oxygen atom donors [1,5,8,9,18]. However, studies using single oxygen atom donor cannot address the first part of the catalytic cycle of the cytochrome P-450 family, i.e., the binding and reduction of dioxygen followed by the O–O bond cleavage of the resulting ferric porphyrin peroxo (or hydroperoxo) complex.

Our laboratory had been studying synthetic analogues of the peroxy ferric heme intermediate for some time [19–22]. These complexes are prepared by oxidative addition of superoxide to synthetic ferrous porphyrin complexes, which produces high-spin ferric porphyrin peroxo complexes, $[(porphyrin)Fe^{III}(O_2)]^-$; the structures of these complexes are almost certainly analogous to that of the corresponding manganic porphyrin peroxo complex, i.e., the peroxo ligand is bonded to Mn^{III} in a bidentate, triangular fashion [22]. The reactivity of these ferric porphyrin peroxo complexes is of great interest to us in our ongoing attempts to mimic the three different modes of reaction described above for different members of the cytochrome P-450 family [23–26].

In order to have a good model of the ferric peroxo heme intermediate, we have synthesized and characterized $[Fe^{III}(PPIXDME)(O_2)]^-$, the peroxo complex of the dimethylester derivative of protoporphyrin IX, the naturally occurring porphyrin in the cytochrome P-450 family. In addition, we have prepared the peroxo complex of another electron-rich porphyrin ligand, tetramestitylporphyrin (TMP), and, for comparative

Table 1
Spectral properties of mononuclear iron(III) porphyrin peroxo complexes

Compound	λ_{max} (Soret region) [a] (nm)	λ_{max} (α,β-region [a] (nm)	EPR [b]	ν_{O-O} (cm^{-1})
Fe(III)(TMP)O_2^-, **2a** [c]	434	549 (sh), 568, 595 (sh), 612	4.24	
Fe(III)(F_{20}TPP)O_2^-, **2b** [c]	431	538, 559, 588 (sh), 610 (sh)	4.27	802 [d]
Fe(III)(PPIXDME)O_2^-, **2c** [c]	429	536 (sh), 553, 583, 592 (sh)	4.26	
Fe(III)(OEP)O_2 – [e]	423	530 (sh), 543, 569, 582 (sh)	4.23	806
Fe(III)(TPP)O_2^- [e]	432	545 (sh), 563, 595 (sh), 606	4.24	

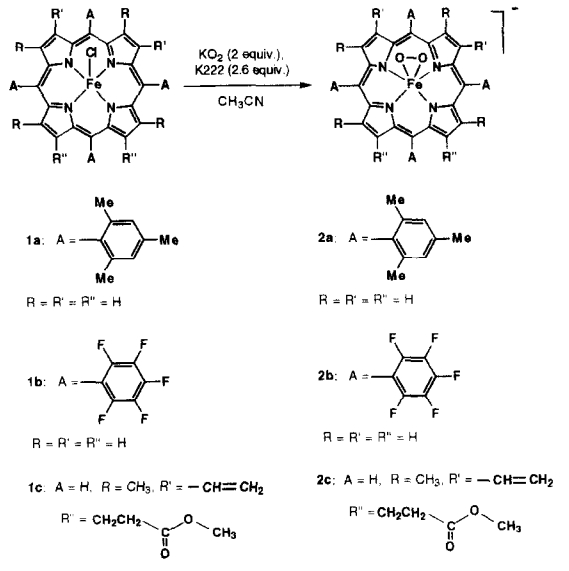
[a] In CH_3CN at room temperature.
[b] At 90 K in CH_3CN.
[c] See Ref. [26].
[d] KBr pellet.
[e] See Refs. [19,20].

purposes, we have also prepared $[Fe(III)(F_{20}TPP)(O_2)]^-$, the ferric peroxo complex of a particularly electron-poor porphyrin ligand [26].

Earlier reactivity studies of ferric peroxo complexes have established that they are not themselves electrophilic, i.e., they do not oxidize typical olefins such as cyclohexene or styrene and they do not hydroxylate hydrocarbons [20,24,27–29]. Furthermore, even upon addition of various proton sources, no electrophilic reactivity could be observed [20]. Instead, nucleophilic reactivity has been found to be most characteristic of these complexes: Groves et al. have observed reaction of the manganese analogues with acyl halides [30], and Miksztal and Valentine [23] have studied the reaction of the ferric porphyrin peroxo complexes with SO_2. We describe here our observation that the ferric heme peroxo complexes derived from electron-rich porphyrins show unique nucleophilic behavior in that they are able to transfer an oxygen atom to simple electron-poor olefins such as 2-cyclohexen-1-one. Comparison with the very different reactivity of the electron-poor peroxo complex $[Fe(III)(F_{20}TPP)(O_2)]^-$ (F_{20}TPP = 5,10,15,20-tetrakis-(pentafluorophenyl) porphyrin) yields further insight into the reactivity of the ferric heme peroxo intermediate in the cytochrome P-450 enzymes.

2.1.1. Synthesis and characterization of iron(III) porphyrin peroxo complexes

Iron(III) porphyrin chloride complexes react smoothly in dry acetonitrile with two equivalents of KO_2 in the presence of 2.6 eq. of either 18-Crown-6 or K222 to form the corresponding ferric porphyrin peroxo complexes [19–21,23]. The characteristic UV–vis. spectral properties for the ferric porphyrin peroxo complexes $[Fe^{III}(TMP)(O_2)]^-$ (**2a**), $[Fe^{III}(F_{20}TPP)(O_2)]^-$



Scheme 1. Preparation of iron(III) porphyrin peroxo complexes. Reprinted with permission from J. Am. Chem. Soc., 118 (8) (1995) 2010. Copyright © 1995 American Chemical Society.

(**2b**), and $[Fe^{III}(PPIX)(O_2)]^-$ (**2c**), as well as those of other previously prepared iron(III) porphyrin peroxo complexes are shown in Table 1. All peroxo complexes show low energy Soret bands and two maxima between 500 and 600 nm as well as two shoulders on the latter peaks.

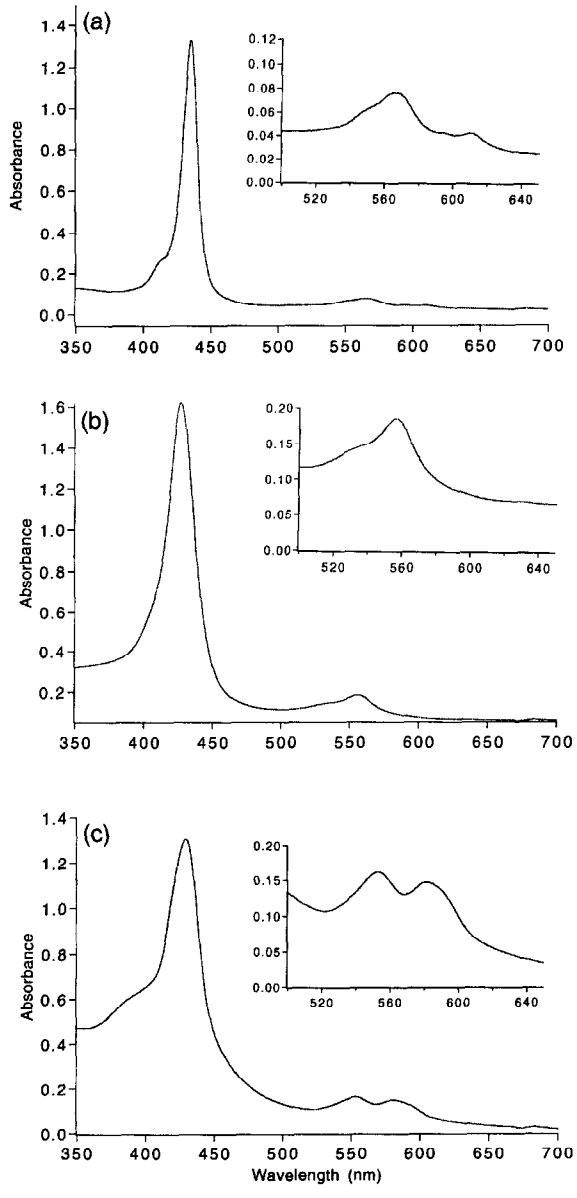


Fig. 1. UV–vis. spectra of three iron(III) porphyrin peroxo complexes in CH_3CN. (a) Complex **2a** (ca. 0.5 mM); (b) complex **2b** (ca. 0.5 mM); (c) complex **2c** (ca. 1 mM). Reprinted with permission from J. Am. Chem. Soc., 118 (8) (1995) 2010, Copyright © 1995 American Chemical Society.

The UV–vis. spectra of the peroxo complexes whose reactivity is described here are displayed in Fig. 1.

All of the ferric porphyrin peroxo complexes show a strong EPR signal between $g = 4.2$ and 4.3, indicating that these compounds are high-spin iron(III) complexes with a high degree of rhombicity. In the IR spectrum, the ν_{O-O} stretch of **2b** (Scheme 1) occurs at 804 cm^{-1}. During the decomposition of **2b** into the μ-oxo dimer and the iron(III) hydroxo complex (see below), this stretch disappears. The frequency of the ν_{O-O} stretch of **2b** is very similar to that of the complex peroxoiron(III) octaethylporphyrin at 806 cm^{-1} [19,20] and of the related ferric peroxo chlorin complexes where the ν_{O-O} stretch has been reported at 806 cm^{-1} [31].

2.1.2. Stability of the iron(III) peroxo complexes

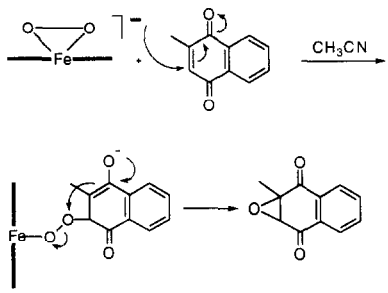
Except for the electron-poor complex peroxo-5,10,15,20-(tetrakis(pentafluorophenyl)-porphyrinato) iron(III), (**2b**), all ferric porphyrin peroxo complexes listed in Table 1 are very unstable to moisture. For example, complex **2a** is converted within minutes to the corresponding hydroxy complex [32], and CH_3CN solutions of **2c** are converted to the corresponding μ-oxo-dimer within minutes as well. However, the peroxoiron(III)-5,10,15,20-(tetrakis-(pentafluorophenyl) porphyrinato) complex, **2b**, is considerably more stable; it has a half-life in acetonitrile of ca. 30 min when the solution is exposed to air. The product obtained from the decomposition of **2b** in the presence of moisture is a mixture of the corresponding μ-oxo-dimer ($\lambda_{max} = 398$ (Soret)) and the corresponding hydroxy complex $Fe(F_{20}TPP)OH$ ($\lambda_{max} = 418$ (Soret)). The UV-vis. spectrum of this decomposition product mixture is identical to that of a mixture of $Fe(F_{20}TPP)OH$ and $Fe(F_{20}TPP)$–O–$Fe(F_{20}TPP)$ prepared from concentrated base and $Fe(F_{20}TPP)Cl$ [32]. Furthermore, acetonitrile solutions of **2b** can be stored under an inert atmosphere for several weeks without significant decomposition, whereas solutions of **2a**

and **2c** (as well as those of all other previously prepared iron(III) peroxoporphyrin complexes) decompose even under an inert atmosphere within 24 h.

2.1.3. *Reactivity of iron(III) peroxo complexes with electron-poor olefins*

Both the tetramesityl derivative **2a** and the protoporphyrin IX dimethylester derivative **2c** epoxidize electron-poor olefins [25,26]. The reaction of either **2a** or **2c** with 2-methyl-1,4-naphthaquinone (menadione) gives menadione epoxide in about 70% yield (determined by HPLC). The reaction products of **2a** and menadione have also been analyzed by ^{1}H-NMR. The peaks for the menadione epoxide were identical to those from a sample of menadione epoxide prepared from H_2O_2/NaOH, and the yield of epoxide determined by this method was also 70%. The reaction of **2a** and **2c** with the less electron-poor cyclohexeneone results in only about 25% yield of cyclohexenone epoxide. The epoxidation reaction and its proposed mechanism are depicted in Scheme 2.

In contrast to complexes **2a** and **2c**, the peroxoiron(III)-5,10,15,20-(tetrakis(pentafluorophenyl)porphyrinato) complex, **2b**, shows *no* interaction with electron-poor olefins. Addition of up to 20 eq. of menadione to an acetonitrile solution of **2b** did not result in any changes in the UV–vis spectrum (except for dilution effects). Also, no menadione epoxide was detected by HPLC.



Scheme 2. Epoxidation of an electron-poor olefin (2-methyl-1,4-naphthaquinone) with a ferric porphyrin peroxo complex.

2.1.4. *Reaction with triphenylphosphine and electron-rich olefins*

It has been previously reported that small amounts (13–30%) of triphenylphosphine oxide were obtained (determined by HPLC) upon addition of triphenylphosphine to peroxoiron(III) octaethylporphyrin. However, the triphenylphosphine did not appear to have been oxidized by direct reaction with the peroxo complex, since addition of triphenylphosphine did not result in any changes in the UV–vis. spectrum of the peroxo complex [20,24]. In the present study [26], we found that none of the peroxo complexes **2a–2c** oxidized triphenylphosphine: When 2–5 eq. of PPh_3 were added to a sample containing 1–5 mmol of the peroxo complex in CD_3CN and the sample mixture was subsequently sealed in the dry box, *no* formation of PPh_3O was observed by ^{31}P-NMR. When the peroxo complex was allowed to decompose under air in the presence of 2–5 eq. of PPh_3, however, ca. 40% (based on the Fe-complex) of PPh_3O was obtained (by ^{31}P-NMR). Addition of PPh_3 to solutions of complexes **2a–2c** did not result in changes in the UV–vis. spectra of the peroxo complexes; the fluorinated derivative **2b** was stable in the presence of 5 eq. of triphenylphosphine for two weeks! However, when a ^{31}P-NMR spectrum of PPh_3 in the presence of the fluorinated complex **2b** was taken, much of the phosphine 'disappeared' (i.e., the signal-to-noise ratio was much worse than for a spectrum of PPh_3 without the presence of **2b**) and the peak was dramatically broadened. The same effect could be observed for the fluorinated iron(III) chloro complex **1b**. Upon addition of pyridine to an acetonitrile solution of **2b** and PPh_3, the broadening of the PPh_3 resonance disappeared, and the signal-to-noise ratio greatly improved. The UV–vis. spectrum of the porphyrin complex obtained this way showed a Soret band at 420 nm, and peaks in the α, β-region that were identical with those of the complex Fe(III)(F_{20}TPP)$(py)_2$ prepared from Fe(III)(F_{20}TPP)Cl and pyridine. The exact amount of line-broadening of the phosphine ob-

served for both complexes **1b** and **2b** depended on both the concentration of the porphyrin complex and the amount of PPh_3 present; increases in the concentration of either substrate increased the amount of line-broadening. These results are consistent with very rapid formation of a weak bond between the iron complex and the phosphine. No such line broadening of the PPh_3 signal in the ^{31}P-NMR was observed for any other of the iron(III) porphyrin peroxo complexes or their precursors. Bonding of very basic phosphine ligands as axial ligands to iron porphyrin complexes has been reported previously by several authors [33–35]. However, the complexes obtained by these authors are all diamagnetic Fe(II) species. The interaction of triphenylphosphine with complex **2b**, on the other hand, did not lead to displacement of the axial peroxo ligand, and there were no visible changes in the electronic spectrum of **2b**.

It was also tested whether any of the peroxo complexes **2a–2c** are capable of oxidizing the electron-rich olefin tetramethylethylene (TME). In all cases, there was no interaction between the peroxo-complex and the TME.

All reactions of the peroxo-complexes **2a–2c** are summarized in Table 2.

2.2. *Oxidative dehydrogenation of a ferrous cyclam complex by dioxygen and ascorbic acid*

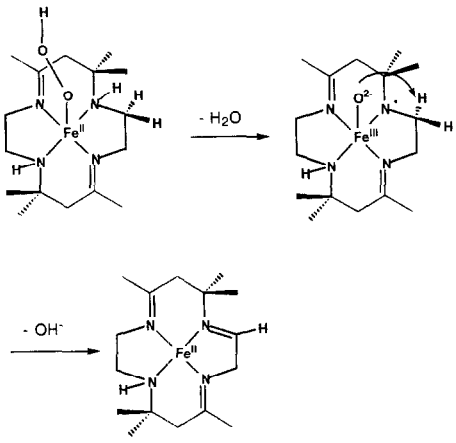
Ferrous cyclam complexes have been subject to several studies recently because of their ability to catalyze the epoxidation of olefins with single oxygen donors such as H_2O_2, MCPBA, and PhIO [3]. It is probable that the reactive intermediate in the reaction with H_2O_2 is the ferric hydroperoxide complex Fe(cyclam)OOH, while the reactions with MCPBA and PhIO proceed via a ferric–oxidant complex the exact nature of which is currently unknown. The latter reactions are, however, not particularly good monooxygenase models because such enzymes function by activating dioxygen rather than single oxygen atom donors.

The well-studied olefin epoxidation with Fe(bleomcycin) and dioxygen occurs in the presence of the reducing agent ascorbic acid [41,42]. We therefore attempted also to catalyze reactions of dioxygen with substrates by ferrous cyclam complexes in the presence of ascorbic acid. However, no epoxidation of cyclohexene was ever observed, regardless of whether the reactions were carried out in methanol or in a 3:1 mixture of methanol–acetonitrile. Dramatic color changes of the reaction mixture after addition of dioxygen indicated that the macrocyclic cyclam ligand was modified. These color changes were almost identical to those that occurred upon reaction of ferrous cyclam with hydrogen peroxide. We believe that oxidative dehydrogenation of the macrocycle is the most likely explanation for the color changes observed, since Goedken and Busch [43] have shown that the substituted cyclam derivative $Fe^{II}(Me_6[14]4,11\text{-diene}$ undergoes oxidative dehydrogenation in the presence of dioxygen, acid, and water leading to the ferrous trien, then to the ferrous tetraene, and finally to the destruction of the macrocycle. We thus propose Scheme 3 to explain our observations.

The initial step in this mechanism involves formation of a ferric superoxide complex by the binding of dioxygen to the ferrous cyclam. The

Table 2
Reaction of mononuclear iron(III) peroxo complexes

Compound	Menadione epoxide (% yield)	Cyclohexenone epoxide (% yield)	Reaction with triphenyl–phosphine	Tetramethyl–ethylene epoxide
Fe(III)(TMP)O_2^- **2a**	76	23	No reaction	0
Fe(III)(F_{20}TPP)O_2^- **2b**	0	0	Binds reversibly	0
Fe(III)(PPIXDME)O_2^- **2c**	70	20	No reaction	0

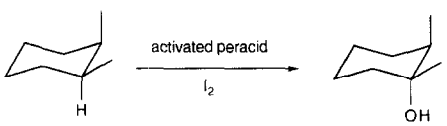


Scheme 3. Oxidative dehydration of a ferrous cyclam complex in the presence of dioxygen and ascorbic acid.

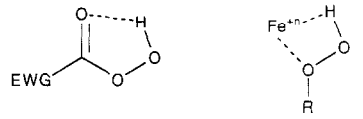
ascorbic acid acts as a reducing agent, leading to the generation of a ferrous hydroperoxide species via a two-electron-reduction. Protonation of the hydrogen peroxide followed by homolytic O–O bond cleavage and abstraction of a hydrogen atom from one of the secondary amines leads to formation of a ferric oxide complex and the elimination of water. Loss of OH^- finally generates the ferrous triene complex.

2.3. Hydroxylation of hydrocarbons in the presence of ferric non-heme complexes

One of the most interesting of the many reactions catalyzed by cytochrome P450 is the hydroxylation of alkanes. Extensive investigations of this reaction have led to the conclusion that the key intermediate in this process is the high valent iron porphyrin oxo species [1]. This process involves the formation of enzyme-bound free radicals. However, there is speculation that hydroxylation can also occur with intermediates other than the high valent oxo porphyrin that might function by a non-radical mechanism [2].



Scheme 4. Hydroxylation of an alkane with retention of configuration by an activated peracid. See Ref. [42].

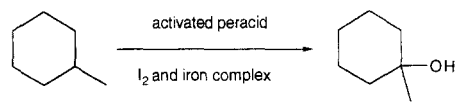


Scheme 5. Activation of C–H bonds by electron deficient peracids. (EWG = electron-withdrawing group).

There is evidence that alkanes can be hydroxylated via a non-radical mechanism if highly activated, i.e., if electron-deficient peracids are used. This reaction was shown to occur with retention of configuration in chiral alkanes, thus proving that the reaction indeed occurs via a non-radical pathway (Scheme 4). Furthermore, the formation of non-radical products was only possible upon by addition of I_2 [44].

These results are intriguing and of potential importance for the hydroxylation catalyzed by cytochrome P450 because the metal in the active site of this enzyme coordinates dioxygen. It might be possible that such coordinated dioxygen is highly activated and could be capable of insertion into carbon–hydrogen bonds without radical formation (Scheme 5).

Since the ferric porphyrin peroxo complexes **2a** and **2c** discussed in the first part of this paper are nucleophilic and thus are not expected to be able to undergo oxygen insertion into C–H bonds, we investigated instead the reactivity of iron compounds that are capable of acting as Lewis acids to the peracid MCPBA. The general reaction examined is shown in Scheme 6. $Fe(III)(acac)_3$, $[Fe_2OCl_6]^{2-}[Et_4N]_2^+$ and $Fe(III)(phenanthroline)_3$ were used as metal catalysts. In a typical experiment, 1 M of methylcyclohexane and 1 M of MCPBA were reacted in freshly distilled CH_3Cl in the presence of either a catalytic or stoichiometric amount of the iron complex. We found that the formation of the hydroxylated product, 1-methylcyclohe-



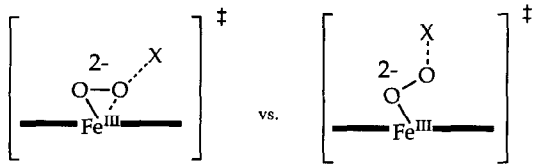
Scheme 6. Hydroxylation of alkanes with peracids and iron complexes.

xane-1-ol was indeed greatly enhanced by the iron complexes employed. Also, the reaction was considerably faster in the presence of the iron complexes than in the control experiments without the iron complexes (1–2 h compared to 16 h reaction time). However, a great number of additional products were also formed, apparently by a radical mechanism. Addition of various radical quenchers to the reaction mixture (I_2, $Sn(Bu)_3H$) did not significantly inhibit the formation of these side-products, even when large excesses of quencher were employed.

3. Discussion

3.1. Ferric porphyrin peroxo complexes as a model for direct oxygen transfer by cytochrome P-450 enzymes

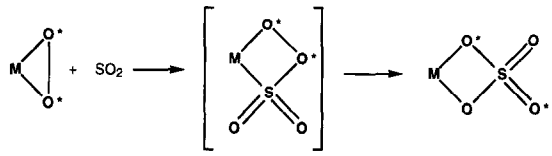
Cytochrome P450 arom converts androgen to estrogen via a series of transformations that involve hydroxylation followed by aromatization [7,14–16]. Graham-Lawrence et al. [16] have recently suggested that *direct nucleophilic attack by an open peroxo species* may indeed be the key step in the aromatization step catalyzed by cytochrome P450 arom. They hypothesize that during the hydroxylation step, O–O bond cleavage, rather than nucleophilic attack, occurs because of the availability of protons for the iron complex, while the direct nucleophilic attack in the aromatization step takes place because there are no accessible protons for the active site of the enzyme. The fact that the peroxo ferric heme complex **2c** is able to transfer an oxygen atom to electron-poor olefins is new and powerful evidence that such direct nucleophilic attacks can occur in biological systems.



Transition State A: Closed peroxo complex

Transition State B: Open peroxo complex

Scheme 7. Possible transition states for oxygen transfer of peroxo ferric heme complexes.



O* = ^{18}O; M = Ru Peroxy sulfite intermediate

Scheme 8. Formation of sulfato complexes from peroxo complexes and SO_2. To form the peroxysulfite intermediate, one of the metal–oxygen bonds must be broken.

While the perfluorinated complex **2b** is by far the most stable iron porphyrin peroxo complex prepared to date, its electronic spectrum as well as its O–O stretch in the IR spectrum are very similar to those of the more electron-rich porphyrin peroxo complexes. However, the reactivity of this complex is dramatically different, as evidenced by its failure to epoxidize electron-poor olefins. Based on the similar spectral properties and the different reactivity of **2b**, we hypothesize that the electron-withdrawing ligands affect the difference between the ground state energy and the transition state energy during the epoxidation reaction [26]. Two possible transition states are depicted in Scheme 7.

Transition state **A** resembles the closed triangular peroxo complex. The properties of this transition state would be expected to be very similar to those of the ground state. Specifically, if this were the transition state in the nucleophilic epoxidation reaction, the perfluorinated complex **2b** would likewise be expected to undergo this reaction, given that its ground state electronic properties appear to be very similar to those of all the other ferric porphyrin peroxo complexes. We therefore favor transition state **B**, involving an open peroxo complex (Scheme 8). Ring-opening of η^2 peroxo complexes has been suggested in a number of oxidation reactions by such complexes. For example, in the reaction of η^2 peroxo complexes with SO_2, ^{18}O labeling studies have conclusively shown that

prior ring-opening of the peroxo complex is a necessary step for the formation of the peroxysulfite intermediate [36,37].

Lawson and Atwood have proposed an open transition state during the oxidation of small inorganic molecules like PPh_3 by some Vaska-like iridium peroxo complexes [38]. Also, it has recently been shown that during the reductive elimination of dioxygen from some peroxo complexes derived from group VIII transition metals, the oxygen is in the triplet state [39,40] which implies that the two metal–oxygen bonds of the peroxo complex are not broken simultaneously and that hence an open peroxo complex must exist as an intermediate in these reactions. All of the nucleophilic epoxidation reactions have been carried out in acetonitrile, a highly coordinating solvent. It is quite possible that a solvent molecule is bonded to the ferric heme peroxo complexes as a second axial ligand and that this ligand stabilizes the open transition state depicted in Scheme 7.

Some support for this hypotheses is derived from the fact that for the electron-poor perfluorinated peroxo complex **2b**, a second axial ligand can be directly observed; the interaction with phosphines, although weak, clearly demonstrates that this complex is able to coordinate another ligand in the axial position. The electron-withdrawing ligands increase not only the affinity of this complex for a second axial ligand, but also they are also expected to make the ring-opening step depicted in transition state **B** less favorable and hence decrease the reactivity. However, an 'Umpolung' (i.e., switch from nucleophilicity to electrophilicity) of the reactivity of the peroxo functionality cannot be accomplished even by such extremely electron-withdrawing ligands as the pentafluorophenyl groups of complex **2b**. Like all other iron(III) porphyrin peroxo complexes, complex **2b** does not oxidize electron-rich olefins or triphenylphosphine.

The peroxo complex of iron protoporphyrin IX dimethylester is a remarkably good nucleophil. It appears to be an excellent model for the nucleophilic attack by an open peroxo complex proposed as a potential pathway for certain oxidation reactions catalyzed by cytochrome P450. Furthermore, the likelihood of the existence of a second axial ligand and the evidence gained for an open transition state during the epoxidation reaction demonstrate how important insights that may be crucial for the understanding of enzymatic mechanisms can be gained from the study of chemical model systems.

The remarkable nucleophilicity of the ferric porphyrin peroxo complexes **2a** and **2c** also explains why all experiments that attempted to use these compounds for oxygen insertion into C–H bonds have been unsuccessful [20,27,28]. Iron non-heme complexes that are capable of acting as Lewis acids to the peracid MCPBA appear to be much more promising candidates to undergo this important biological and industrial reaction. Our results obtained with the iron non-heme complexes $Fe(III)(acac)_3$, $[Fe_2OCl_6]^{2-}[Et_4N]_2^+$ and Fe(III)(phenanthroline)$_3$ indicate that such complexes can indeed be catalysts for the hydroxylation of alkanes, but that at present it is not possible to determine whether the active intermediate is an activated iron peroxo or hydroperoxo species that is capable of directly hydroxylating the alkane or whether the reaction proceeds exclusively through a radical mechanism. Given the enormous biological — and industrial! — importance of hydroxylation of carbon–hydrogen bonds and the obvious advantages that a non-radical system would possess, further research using metal complexes and peracids seems to be a worthwhile undertaking.

4. Experimental

4.1. General

All reactions were carried out in an inert atmosphere chamber (Vacuum Atmospheres) under helium, except as noted otherwise. Solvents were rigorously dried before use. Acetoni-

trile was distilled from calcium hydride. It was then stirred over KO_2 (Aldrich) in the dry box for ca. 1 h and subsequently passed over Super I neutral alumina (Sigma). ^{1}H-NMR spectra were recorded on a Brucker 360 MHz spectrometer. ^{31}P-NMR spectra were recorded on the same instrument, using an external $P(OMe)_3$ standard. FTIR spectra were recorded on a Nicolet 510P spectrometer, and UV–vis. spectra on a Cary 3 UV–vis. spectrophotometer.

4.2. Preparation of the peroxo complexes 2a–2c

An acetonitrile solution of 2–2.5 mmol of KO_2 and 2.5–3 mmol of K222 or 18-Crown-6 was stirred for ca. 1 h. Any undissolved KO_2 was removed by filtration. 1 Mmol of complexes **1a–1c** was then added and the resulting black–red solution was stirred for several min. Solutions of the perfluorinated peroxo **2b** complex could also be prepared by employing much larger concentrations (ca. 50 mmol of the starting complex **1b**.

4.3. Spectroscopic analyses of complexes 2a–2c

4.3.1. UV–vis. spectroscopy

Sample of complexes **2a–2c**, prepared as described above, were transferred inside the dry box into a 0.1 mm pathlength cell (Starna Cells), which was sealed with a rubber septum before being taken out of the box.

4.3.2. EPR spectroscopy

Samples containing ca. 1 mmol of the complexes were transferred into EPR tubes inside the box. The spectra were measured on an IBM (Brucker) 200 D spectrometer at liquid nitrogen temperature. Four scans were accumulated per spectrum. The g value was determined at the zero crossing point of the derivative signal.

4.4. Reactivity studies of complexes 2a–2c

4.4.1. General

Before each study was carried out, formation of the peroxo complex was confirmed by the checking the UV–vis. spectrum of the solution.

4.4.2. NMR analyses of the reaction with menadione

To a 1–4 mmol sample of complex **2a–2c** prepared, as described above, was added a 2–3 fold excess of menadione (Aldrich). The sample was stirred for 5 min in a dry box; the solvent was then evaporated in the dry box and the residue was redissolved in $CDCl_3$. This solution was transferred to an NMR tube for analyses.

4.4.3. HPLC analyses of the reactions with menadione and cyclohexenone

To a 1–4 mmol sample of complex **2a–2c** was added a 2–3 fold excess of menadione or cyclohexenone (Aldrich). The mixture was stirred for ca. 5 min and then injected into the HPLC (Beckmann 114 M solvent delivery module, flow 1 ml/min, 65% acetonitrile/35% water, with a 165 variable wavelength detector set a 265 nm). Cyclohexenone and its epoxide were analyzed by GC–MS using decane as an internal standard. Epoxide yields were determined by comparison against standard curves.

4.4.4. NMR analyses of the reaction with PPh_3

To a 1–4 mmol sample of complex **2a–2c** in CD_3CN was added a 4–5 fold excess of triphenylphosphine. The sample was transferred into an NMR tube and sealed inside the dry box.

4.4.5. UV–vis. analyses of reactions of 2a–2c with PPh_3 and tetramethylethylene (TME)

1 Mmol acetonitrile solutions of **2a–2c** were transferred to a 0.1 mm UV–vis. cell in a dry box. The cells were sealed with a rubber septum. Solutions of PPh_3 or TME were added by syringe in portions varying from 0.5–2 eq. Up to 20 eq. of substrate was added to the peroxo-complex solution.

4.4.6. Control experiments

0.1 Mmol KO_2 was reacted with 2 eq. menadione for 10–15 min. The yield of epoxide was $8 \pm 1\%$. 3.4 mmol of KO_2 was reacted with 2 eq. of menadione for 2–4 min prior to analyses by GC–MS. Less than 3% of the epoxide was detected.

4.5. Reactions of ferrous cyclam complexes in the presence of ascorbic acid, dioxygen and olefins

The ferrous cyclam complexes were prepared by a literature method [45]. The reactivity studies were carried out in a 3:1 mixture of CH_3CN/CH_3OH. 0.02 mM of the cyclam complex, and 1 mM of cyclohexene were added to this mixture. Subsequently, an excess of ascorbic acid was added. A slow stream of O_2 was then bubbled through the mixture for 1 h. The reaction was followed by UV–vis., and the organic products were analyzed by GC–MS.

4.6. Hydroxylation of alkanes with iron complexes

All chemicals were obtained from Aldrich with the exception of Fe(III)$(acac)_3$ which was obtained from Strem. Fe(III)$(phenanthroline)_3$ was prepared as previously described [46]. Chloroform was freshly distilled prior to use in each reaction. A CH_3Cl solution of 1 M methylcyclohexane and MCPBA and 0.004 M in I_2 was refluxed at 60°C for 16–24 h. Products were analyzed by GC–MS. Reaction mixtures containing 0.01 M to 1 M iron complex were prepared as described above; however, the amount of I_2 used was increased to 0.04 M and the reaction was essentially complete after 2 h.

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