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New Mechanistic Insights in the Cytochrome P-450 Model Reactions: Direct Identification of the Reactive Intermediates

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Abstract In $CH_3CN–H_20$ medium the porphyrin Fe(IV)=O and porphyrin-C_6F_5IO adduct, and $t\mathrm{Bu}\dot{\mathrm{O}}/t\mathrm{BuO}\dot{\mathrm{O}}$ radicals have been spectroscopically identified. The involvement of multiple reactive intermediates in C_6F_5IO oxidizing system has also been demonstrated.

Keywords Catalysis · Hydroxylation · Epoxidation · Hydroperoxide

1 Introduction

The iron (III) porphyrin compounds have been extensively used in modeling reactions catalyzed by cytochrome P450 [1]. Among many reactions catalyzed by this monooxygenase family of enzymes, the epoxidation and hydroxylation of hydrocarbons have been very thoroughly studied [1–4]. There has been considerable success in epoxidation but selective hydroxylation of unactivated C–H bonds still remained very elusive specially where hydroperoxides and hydrogen peroxide were the terminal oxidants [5–10]. The mechanistic studies were then more emphasized and it has now been realized that depending upon the terminal oxidants more than one potential reactive intermediates such as **1–4** (Scheme 1) are concomitantly evolved in these oxidizing systems [11–14]. It was long been known that in case of iodosylarenes, the oxoiron(IV) porphyrin cation radical (**2**) was the only reactive intermediate responsible in the direct oxygen transfer step to a substrate [2–10]. However the Collman group has claimed for the first time that the catalyst-iodosylarene adduct (**1**) along with **2** to be the major reactive intermediates in this oxidizing system [15]. Secondly in the reactions of iron(III) porphyrins with *t*-BuOOH, the evolution of alkoxy radicals have been proposed over several decades [16–19]. Incidentally neither of this catalyst-iodosylarene adduct (**1**) nor the alkoxy radical intermediates have ever been directly generated and identified in solution. It is not important today to know how they are formed, but it is certainly important to stabilize them in solution because this would assist more efficient oxygenation of substrates by such terminal oxidants. Herein we report for the first time: the evolution, stabilization and definitive spectroscopic identification of these two reactive intermediates in solution. Secondly we also report that in case of iodosylarene the oxo-iron(IV) porphyrin (**3**) is also definitively formed in the reactions of the iron(III) porphyrin with an iodosylarene both in stoichiometric and in catalytic reagent concentrations. In order to demonstrate these results we have used F_{20}TPPFe(III)Cl (F_{20}TPP = *meso*-tetrakis (pentafluorophenyl) porphinato dianion) as the catalyst, C_6F_5IO and *t*-BuOOH as the terminal oxidants and $CH_3CN–H_2O$ (9.09%) as the solvent.

2 Experimental Section

2.1 Materials

Acetonitrile was distilled under argon from CaH_2. Cyclohexene was distilled under argon to remove the inhibitor and passed through a silica gel column prior to reaction. Cyclohexane, all the oxidation products, dodecane (internal standard) and *t*-BuOOH (as ~70% solution in water) were purchased from Aldrich and were used as received without further purification. The pentafluoroiodosylbenzene (explosive [20]) was prepared by the reported method [21]. The

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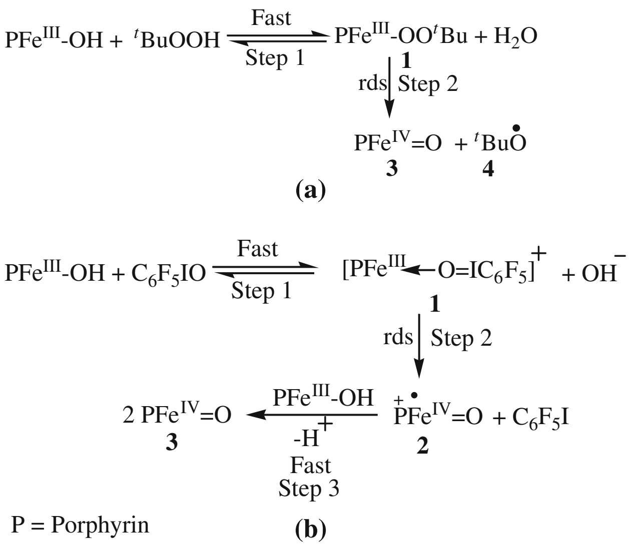

Scheme 1 Proposed reactive intermediates: (**a**) for *t*-BuOOH and (**b**) for C_6F_5IO

exact active oxygen content of the oxidants were determined iodometrically prior to use. The iron-porphyrin catalyst was synthesized according to literature procedures [22–24].

2.2 Instruments

The UV-visible spectral measurements were performed with Perkin-Elmer Lambda (2S) spectrophotometer. The product analysis for cyclohexene and cyclohexane oxidation was performed on Perkin-Elmer AutoSystemXL gas chromatograph equipped with flame ionization detector (FID) and Perkin-Elmer carbowax capillary column of 30 m length. The EPR experiments were performed with Jeol-FA-100 series X-band spectrometer.

2.3 Catalytic Oxidation and Product Analysis

In a Typical reaction 15 ± 1 μM catalyst and 2.6–600 mM of substrate was prepared in 1.1 mL of argon saturated solvent mixture of CH_3CN–H_2O in a cuvette fitted with a silicone rubber septa. The oxidation was initiated by adding 1.2 mM of C_6F_5IO solution. After 10 min the standard solution of dodecane was added to the reaction mixture. The identification and quantitation of the products were done from response factors of standard products samples by GC analysis.z

3 Results and Discussion

It has been observed from our latest study that in the reaction of $F_{20}TPPFe(III)Cl$ with *t*-BuOOH, the $F_{20}PPFe(IV) = O$ (**3a**) was indeed formed and it is quite stable in CH_3CN–H_2O(9.09%) solvent system [19]. Interestingly when the same catalyst (15 ± 1 μM) was reacted with C_6F_5IO (0.6 mM) instead of *t*-BuOOH, the formation of **3a** was also noticed. This has been confirmed by reacting almost the same quantity of the catalyst with excess of *t*-BuOOH in the same solvent and then comparing these two spectra. The two UV-visible spectra shown in Fig. 1 are so remarkably superimposable to each other that if we conclude **3a** was formed from *t*-BuOOH then it was definitively formed from C_6F_5IO too [19, 25, 26]. The calculated extinction co-efficient(ε) of **3a** at 547 nm was 16,000 M^{-1} cm^{-1}. It has however been noted that **3a** formed from *t*-BuOOH was stable for more than 2 h, but that was formed from C_6F_5IO has stability of only up to 5 min and after that slow decay of the soret at 547 nm was observed. In the reaction medium of *t*-BuOOH when excess of C_6F_5I was added the stability of **3a** was nor perturbed. However it requires further investigation to address this issue.

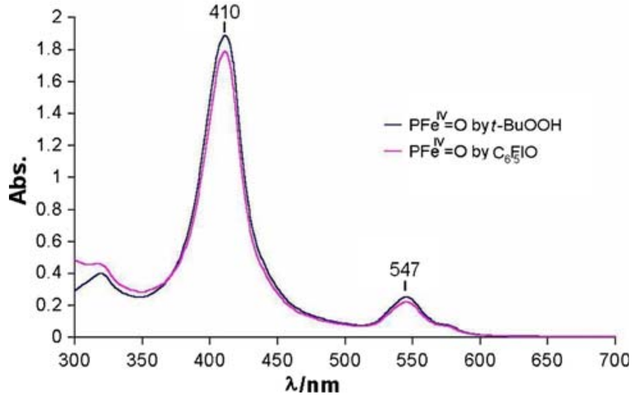

Fig. 1 UV-vis spectra of $F_{20}TPPFe(IV) = O$ in CH_3CN–H_2O (9.09%) at 25 ± 1 °C. Concentration of $F_{20}TPPFe(III)Cl$ = 15.4 μM, *t*-BuOOH = 2 mM in one set and concentration of $F_{20}TPPFe(III)Cl$ = 14.9 μM, C_6F_5IO = 0.6 mM in another set. Path length of the cell = 1 cm

Now a concentrated solution of the catalyst (1.11 mM) was titrated with C_6F_5IO (up to 4 equivalents) in CH_3CN–H_2O (9.09%) and the evolution of the 547 nm band due to **3a** was observed (Fig. 2). In case the oxidant concentration was above 3.5 equivalents, the peak height at 547 nm was not increased any more rather it was decreased. It has been noted that with this high concentration of the catalyst and only 3–3.5 equivalent of the oxidant, ~50–53% of **3a** was formed and it was stable only for about 20 s. This quantitative estimation of **3a** was performed from the known extinction co-efficient (ε) of **3a** in the same solvent system at 547 nm (16,000 M^{-1} cm^{-1}). The nearly clear isosbestic spectral change of the catalyst (Fig. 2) in this titrimetric experiment also indicated the probable existance of another reactive intermediate (besides **3a)** in the medium. In order to address this, another fresh solution of the catalyst

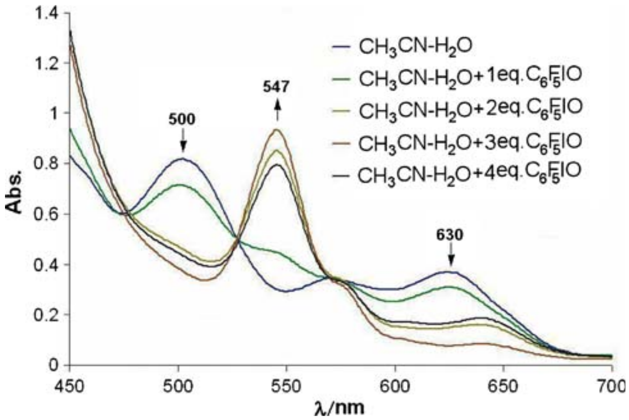

Fig. 2 Spectral change of $F_{20}TPPFe(III)Cl$ (1.11 mM) with the addition of C_6F_5IO in $CH_3CN–H_2O$(9.09%) at 25 ± 1 °C. Path length of the cell = 0.1 cm

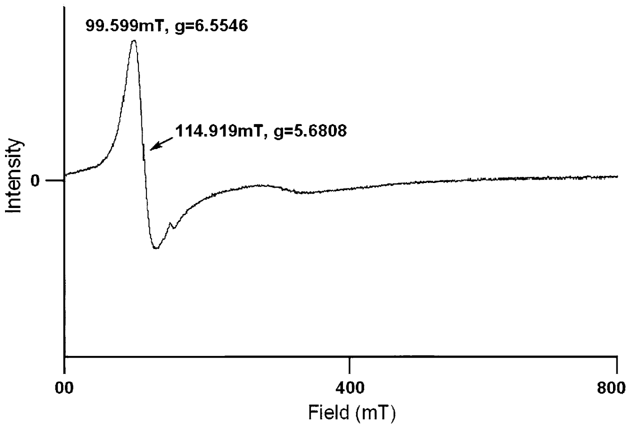

Fig. 3 EPR spectrum of $F_{20}TPPFe(III)Cl$ (4.6 mM) + C_6F_5IO (16 mM) in $CH_3CN–H_2O$ (9.09%) at −147 °C

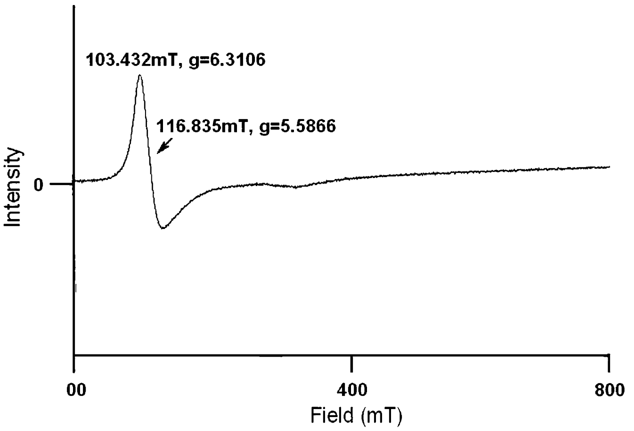

Fig. 4 EPR spectrum of $F_{20}TPPFe$ (III)Cl (4.6 mM)) in CH_3CN at −130 °C

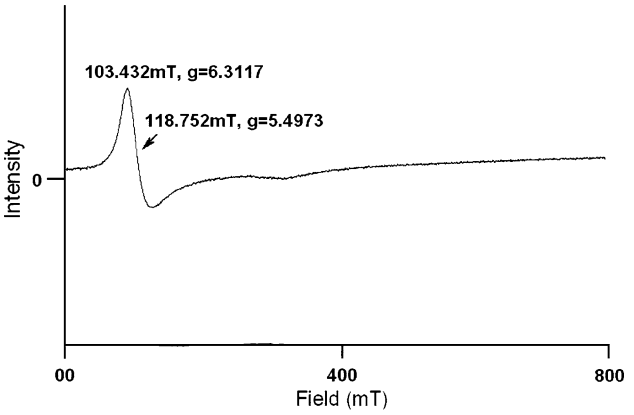

Fig. 5 EPR spectrum of $F_{20}TPPFe$ (III)Cl (4.6 mM)) in $CH_3CN–H_2O$ (9.09%) at −130 °C

(4.6 mM) with ~3.5 equivalent of C_6F_5IO (16 mM) was prepared in a long quartz tube and the solution was immediately frozen at −147 °C and its EPR spectrum was recorded. The spectrum (Fig. 3) signifies the presence of a new high spin iron (III) species with the g value of 5.6808. The g-values of high spin $F_{20}TPPFe(III)Cl$ and $F_{20}TPPFe(III)OH$ (Fig. 4 and Fig. 5) were observed at 5.5866 and 5.4973 respectively. This small change in g value is due to the change of axial ligands (Cl^- and OH^-). The g values of high spin iron (III) complexes with variable porphyrins normally appear in this region (g ~5–6) [27, 28]. These three EPR spectra are remarkably similar in shapes and g values. So the most rational explanation for the ERP spectrum of the new species could be due to $F_{20}TPPFe(III)–C_6F_5IO$ adduct (**1a**), because the other species (**3a**) present in the medium (~50%) is EPR silent [26]. Thus at higher catalyst concentrations, **1a** and **3a** do coexist and at lower catalyst concentrations **3a** was the only major identifiable reactive intermediate. It has been noted that at lower catalyst concentration the ratio of the catalyst: oxidant was well above stoichiometric (1:40). Thus we conclude that the active involvement of this **3a** along with other reactive intermediate(s) in the iron(III)porphyrin catalyzed oxidation reactions by iodisylarene reactions are indeed important.

Now a concentrated solution of $F_{20}TPPFe(III)Cl$ (1.1 mM) was reacted with *t*-BuOOH (70–80 mM) in $CH_3CN–H_20$ (9.09%) where **3a** was indeed formed, but in 50–55% yields and this reactive intermediate was stable definitively up to 30 s at 25 °C. At lower oxidant concentrations (<70 mM) less of **3a** was formed and at higher oxidant concentrations (>80 mM) it was less stable. Thus another fresh solution of the catalyst (1.56 mM) was reacted with *t*-BuOOH (109 mM) and the low temperature (−80 °C) EPR spectrum of the immediately frozen reaction mixture was recorded and a sharp signal at g = 2.0172 was detected (Fig. 6). The complete disappearance of the EPR signal near g = 5–6 signifies the absence of any high

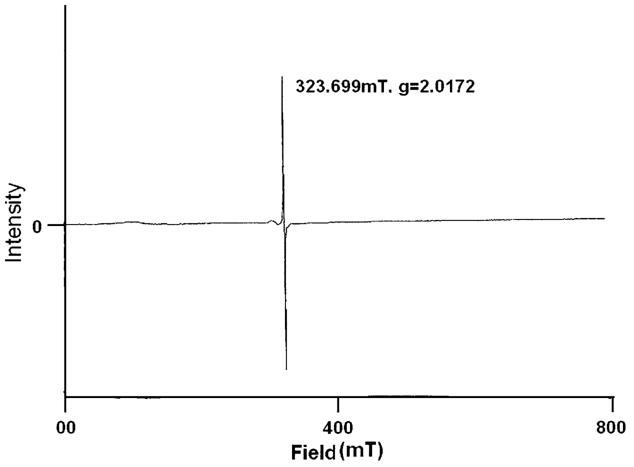

Fig. 6 EPR spectrum of an immediately frozen solution of F_{20}TPPFe(III)Cl (1.56 mM) + *t*-BuOOH (109 mM) in CH_3CN–H_2O (9.09%) at −80 °C

spin Fe(III) starting material or even the catalyst-oxidant adduct in the medium. To the best of our knowledge this is the first report of this radical detected in solution in this oxidizing system. This result indicated that in hydroperoxide oxidizing system **3a** and the alkoxy/alkylperoxy radicals are indeed formed in the reaction medium [29]. At the higher catalyst concentration the faster decay of some catalyst in absence of any substrate is probably taking place through the intermediacy of oxo-iron(IV) porphyrin cation radical (**2a**) by some self-oxidative pathway which is not known to us at the moment.

Now the F_{20}TPPFe(III)Cl catalyzed epoxidation of cyclohexene and the hydroxylation of cyclohexane by C_6F_5IO were performed in this oxidatively stable solvent system. In a typical experiment cyclohexene (600 mM) was reacted with C_6F_5IO (1.2 mM) in presence of F_{20}TPPFe(III)Cl (15 ± 1 μM) for only 10 min and cyclohexene epoxide (96%) was formed as the only product. In this reaction the turnover number and turnover frequency of the catalyst were 76.8 and 460.8 h^{-1} respectively. In this reaction when cyclohexane (600 mM) was the substrate, cyclohexanol was the only product formed in 58% yields in 10 min. The two reactions were then conducted with variable substrate concentrations. In both cases non-linear increase of the product formation with the increase of the substrate concentrations (Figs. 7 and 8) were observed. This observation supported the involvement of more than one reactive intermediates both in the epoxidation and hydroxylation reactions unlike that has been observed in the selective hydroxylation of cyclohexene by *t*-BuOOH [19].

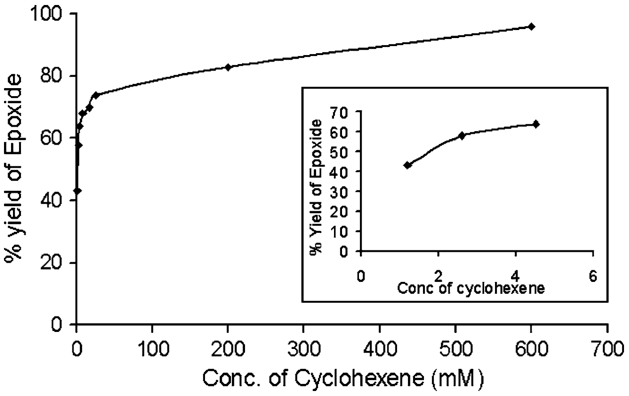

Fig. 7 Plot of Epoxide yields with cyclohexene concentrations. F_{20}TPPFe(III)Cl = 15 ± 1 μM; C_6F_5IO = 1.2 mM in all the experiments conducted in CH_3CN–H_2O (9.09%) at 25 ± 1 °C for 10 min. Yields are based on the total oxidant. Each data point is an average of duplicate sets of experiments. Inset: First three data points are given with amplified scale for clarity

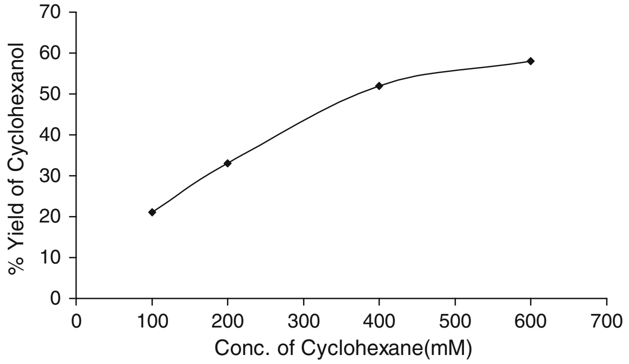

Fig. 8 Plot of cyclohexanol yields with cyclohexane concentrations. F_{20}TPPFe(III)Cl = 15 ± 1 μM; C_6F_5IO = 1.2 mM in all the experiments conducted in CH_3CN–H_2O (9.09%) at 25 ± 1° C for 10 min. Yields are based on the total oxidant. Each data point is an average of duplicate sets of experiments

These epoxidation and hydroxylation reactions stated above were all conducted with identical concentrations of the catalyst and the oxidant and the product yields were measured after a pre-determined fixed time (10 min). In these reactions it has been observed that the total yield of cyclohexanol was 58% (Fig. 8) when the cyclohexane concentration was 600 mM (the highest we could reach). However to reach to this much yield (58%) of the epoxide from same quantity of catalyst and oxidant, only 2.6 mM of cyclohexene was required (Fig. 7). This result indicated that almost all the efficient hydroxylating intermediates formed in this medium could be trapped by 2.6 mM of cyclohexene. On the basis of this observation a mixture of cyclohexene (2.6 mM), cyclohexane (600 mM) and catalyst (15 ± 1 μM) was when reacted with C_6F_5IO (1.2 mM) for only 10 min, the epoxide and cyclohexanol were formed in 21 and 38% yields respectively (total yield, 59%). This result indicated that the hydroxylating intermediates together are about 127 times more epoxidizing than hydroxylating. Thus keeping the cyclohexane

Table 1 Competitive oxidation of cyclohexene and cyclohexane by C_6F_5IO in $CH_3CN–H_2O$(9.09%) solvent at 25 ± 1 °C[a]

Entry	Substrate(mM)	Yield of cyclohexene oxide (%)[b]	Yield of cyclohexanol (%)[b]	Total yield (%)[b]
1	Cyclohexene(2.6) + Cyclohexane(600)	21	38	59
2	Cyclohexene(25) + Cyclohexane(600)	63	14	77
3	Cyclohexene(50) + Cyclohexane(600)	73	10	83

[a] Concentration of catalyst = 15 ± 1 μM, C_6F_5IO = 1.2 mM in all the reactions

[b] Yields were based on total oxidants. Averages of duplicate sets of experiments are given. In all the reactions the products were determined after 10 min of the reaction

concentration high (600 mM) when the concentrations of cyclohexene were increased in the competitive reaction mixtures, the yields of cyclohexanol were gone down and the yields of the epoxide were gone up as expected. These results are shown in Table 1. Considering all these results on competitive oxidations we may anticipate that the efficient hydroxylating intermediates are about 107 ± 20 times more epoxidizing than hydroxylating.

4 Conclusion

The $CH_3CN–H_2O$ (9.09%) was found to be a very interesting solvent system to stabilize almost all the reactive intermediates formed from C_6F_5IO and *t*-BuOOH except **2a**, which is hyper-reactive. In case of C_6F_5IO and *t*-BuOOH the the oxo-iron (IV) and the alkoxy radicals respectively have been spectroscopically identified for the first time. Cyclohexane is less reactive than cyclohexene with all these reactive intermediates, still with 600 mM of cyclohexane 58% of the reactive intermediates were trapped and the results (Fig. 8) showed that at some unreachably high cyclohexane concentration the formation of 100% of cyclohexanol is possible. We are inspired to evolve more comprehensive picture of the solution composition of various reactive intermediates formed from a variety of iron(III) porphyrins with hydrogen peroxide and other hydroperoxides also. The basic objective will be to understand the relative ability of various reactive intermediates to hydroxylate substrates of both academic and of industrial importance by these oxidants.

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