CYTOCHROME P450 MODEL COMPOUND CATALYZED OXIDATION OF CYCLOHEXENE AND CYCLOHEXANE BY C_6F_5IO, ROOH AND H_2O_2: NATURE OF REACTIVE INTERMEDIATES

by

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in fulfillment of the requirements of the degree of Doctor of Philosophy

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Dedicated
To My
Beloved Parents

CERTIFICATE

This is to certify that the thesis entitled, **"CYTOCHROME P450 MODEL COMPOUND CATALYZED OXIDATION OF CYCLOHEXENE AND CYCLOHEXANE BY C_6F_5IO, ROOH AND H_2O_2: NATURE OF REACTIVE INTERMEDIATES"**, being submitted by **Mr. Arunava Agarwala** to the Indian Institute of Technology, Delhi for the award of the degree of **Doctor of Philosophy** in **Chemistry** is a record of bonafide research work carried out by him. **Mr. Arunava Agarwala** has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to my knowledge has reached the requisite standard.

The results contained in this dissertation are original and have not been submitted in part or full, to any other University or Institute for the award of any other degree or diploma.

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Abstract

Cytochrome P450 catalyzed monooxygenation of organic substrates has been a subject of intensive research for several decades. This enzyme catalyzes several reactions in biological systems for example: the hydrocarbon hydroxylation, olefin epoxidation, carbon-carbon bond cleavage reaction, heteroatom dealkylation, heteroatom oxidation. The active site of the enzyme contains an iron(III) porphyrin. Different metalloporphyrins are used as cytochrome P450 models. It has been established that electronegatively substituted iron(III) porphyrins are oxidatively robust and efficient catalyst for oxidation reactions. Peracids, iodosylbenzenes, hydroperoxides and hydrogen peroxide are the major terminal oxidants used in metalloporphyrin catalyzed oxidation reactions. The present study is focused on the iron (III) porphyrin catalyzed oxidation reactions by different terminal oxidants and identification of the reactive intermediates during reaction.

Chapter I: This chapter describes: (i) the known chemistry of a monooxygenase enzyme, cytochrome P450, (ii) known model compounds synthesized to mimic the reactions of cytochrome P450 and (iii) the updated understanding of the reactions of these model compounds in the oxidation of various organic substrates and the evolution of the work plan.

Chapter II: This chapter deals with: (i) purification methods of different solvents, and reagents, (ii) synthesis, purification and standardization of the oxidants such as *meta*-chloroperoxybenzoic acid and pentafluoroiodosylbenzene and (iii) synthesis and characterization of various porphyrins and their metallated derivatives.

Chapter III: This chapter has been divided into two parts. In the first part the reactions of four iron(III) porphyrin catalysts with *t*-BuOOH was described. In dry acetonitrile none of

these catalysts react with *t*-BuOOH and thus when these catalysts were reacted with *tert*-butylhydroperoxide in presence of excess of a hypersensitive probe 2,4,6-tri-*tert*-butylphenol (TTBP) there was no observable blue coloration of the solution due to the expected formation of stable 2,4,6-tri-*tert*-butylphenoxy radical (TTBP$^{\cdot}$ radical) in the medium. Interestingly when acetonitrile contaminated with water was the solvent, the activation of all the catalysts by *t*-BuOOH was observed and when the reactions were carried out in presence of H_2O smooth oxidation of TTBP to the corresponding radical was observed. In this study it was observed that ~9 % of water in acetonitrile to be the very good solvent for the activation of all the catalysts. The details of these are described here.

In the second part the results of the reaction of pentafluoroiodosylbenzene (C_6F_5IO) with *meso*-tetrakis(pentafluorophenyl)porphynato manganese(III)chloride (F_{20}TPPMnCl) in dichloromethane and acetonitrile solvent at 25±1 °C is described. The reactive intermediates formed in this reaction were quantitatively trapped by TTBP. It has been observed that the formation of TTBP$^{\cdot}$ radical in CH_2Cl_2 is distinctly biphasic indicating the presence of two reactive intermediates in this medium. However the same reaction became monophesic in acetonitrile solvent. The rate constants for the formation of TTBP$^{\cdot}$ radical in CH_2Cl_2 were dependent on the TTBP concentrations. However the rate constants of the formation of TTBP$^{\cdot}$ radical ($15\pm1\times10^{-3}$ s^{-1}) in CH_3CN solvent were independent of TTBP concentrations. These results along with probable structures of the reactive intermediates are described here.

Chapter IV: This chapter describes the iron (III) porphyrin catalyzed selective oxidation of cyclohexene and cyclohexane. When the oxidation of cyclohexene and cyclohexane was carried out under ambient conditions using F_{20}TPPFeCl as the catalyst and *t*-BuOOH as the

terminal oxidant in CH_3CN-H_2O medium, the exclusive products were 2-cyclohexen-1-ol and cyclohexanol respectively. In CH_2Cl_2-MeOH solvent cyclohexene oxide was the major product from cyclohexane oxidation. The rational explanation for these successful selective oxidation reactions is elaborated. The results observed with F_{16}TPPFeCl F_{12}TPPFeCl and F_8TPPFeCl and their comparative study with those of F_{20}TPPFeCl has also been described here.

Chapter V: In the F_{20}TPPFe(III)Cl catalyzed oxidation of cyclohexene by *t*-BuOOH, the 2-cyclohexen-1-ol was the only product when the reaction was conducted in CH_3CN-H_2O under argon, however the product profile was changed remarkably when the reaction was conducted under dioxygen. Almost exactly similar results were obtained when the terminal oxidant was cumene hydroperoxide. The details of the product analysis have supported the alkoxy radical derived reactive intermediates to be involved in the hydroxylation reactions by the hydroperoxides. In case H_2O_2 and C_6F_5IO were the terminal oxidants the major product was the epoxide rather than 2-cyclohexen-1-ol and in these latter reactions dioxygen has almost no role to play. These reactions when conducted with other two catalysts such as F_{16}TPPFe(III)Cl and F_8TPPFe(III)Cl similar results were observed in all cases. In order to address the role of solvents, several solvents systems were prepared and their polarity were measured (E_T^N value) and these solvents were used for the oxidation reaction. These studies have indicated that solvent polarity only is not the reason for the activation of the catalyst. The details are given in this chapter.

Chapter VI: The CH_3CN-H_2O (9.09%) was found to be the remarkable solvent system in stabilizing several reactive intermediates formed from the reaction of iron(III) porphyrins with iodosylarenes and hydroperoxides. In demonstrating this the reactions of

F_{20}TPPFe(III)Cl with C_6F_5IO and *t*-BuOOH were studied in details in this solvent at 25 ±1 °C. At higher catalyst (15±1μM): C_6F_5IO (0.6 mM) ratio (1:40) the definitive formation of the oxo-iron(IV) porphyrin has been observed. At relatively higher catalyst concentrations (~1.11 mM) but with lower catalyst: oxidant ratios (1:3 to 1:4) the formation of oxo-iron(IV) porphyrin was observed but interestingly the concomitant evolution of F_{20}TPP(III)-C_6F_5IO adduct has also been detected. The low temperature EPR spectra of the reaction mixture shows the expected spectral profile of a high spin iron(III) species with the g value of 5.6808. The presence of other hyper reactive intermediate, the oxo-iron(IV) porphyrin cation radical was though not detected but all these three reactive intermediates were almost fully trapped by 600 mM of cyclohexene and 96% of cyclohexene epoxide was the only product formed from this oxidizing system. Cyclohexane is less reactive with respect to cyclohexene with all these reactive intermediates; still with 600 mM of cyclohexane 58% of the reactive intermediate was trapped in the form of cyclohexanol. The competitive oxidation of these two substrates indicated that cyclohexene is ~100 times more reactive than cyclohexane. In this solvent system when the reaction of F_{20}TPPFe(III)Cl and *t*-BuOOH was performed the formation of the alkoxy radical was observed from the low temperature EPR measurements. These results are described in this chapter.

Table of Contents